An investigation into the neurochemical and behavioural patterns of C57Bl6 mice exposed to "Sugars" and its constituents

By

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Submitted in fulfilment of the Degree of Doctor of Philosophy (Health Sciences)

In the

Discipline of Physiology

School of Laboratory Medicine and Medical Sciences

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2020

DECLARATION

I, **Yvette Yolanda Chetty**, declare that:

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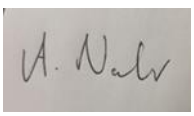

11/10/2020

Dr Anand Nadar — Date

__10.10.2020_

Prof Panjasaram Naidoo — Date

DEDICATION

During my academic journey, I have lost loved ones, but I have also gained more than I ever thought I could. I have learnt that family is anyone that loves you unconditionally and that biology is just science. I dedicate this work to my family.

For my parents (Mr. Krishnan Chetty and the late Mrs. Charmaine Chetty), for all that you have done to ensure that I had the best life had to offer and for supporting my academic ambitions. Thank you both of you for your guidance and love.

To my younger twin sisters (Odette and Odell Chetty), you have grown into exceptional young women and I am very proud of you both. Thank you for all the love and encouragement over the years and for being my number one fans.

For my stepmother (Mrs. Sharmla Chetty) and new siblings (Linell and Prelin Nair), thank you for all the support you have offered me and for making our house a home once again. Thanks also to my nephew, Zayhaan, for giving me company while I did my "homework".

To Professor Lelika Lazarus, the mother I found along this journey, thank you for pushing me to always strive for excellence and for having my best interests at heart.

ACKNOWLEDGEMENTS

"If I've learned anything important in life, it's that you should tell people how important they are to you. Not because they could leave at any moment, but because they're here now, and it's worth saying." – Unknown

I wish to place on record my sincere gratitude to the following individuals:

- My supervisor, Dr Anand Nadar, Department of Human Physiology (UKZN) and co-supervisor Professor Panjasaram Naidoo, Discipline of Pharmaceutical Sciences (UKZN), for providing expert academic advice while still giving me creative freedom with this research. I am grateful to have been under your guidance during this journey. The time and dedication that you have both put into my work and my growth as an academic is greatly appreciated.
- Mr. Sam Pillay and Ms. Shantal Bechoo (Chatsworth Anti-Drug Forum) for their assistance in acquiring the drug samples.
- Professor Thavendren Govender and Professor Tricia Naicker, Catalysis and Peptide Research Unit (UKZN) for their assistance with the drug analysis in my master's degree, which provided the foundation for this work.
- Professor Hans Peter Lipp, University of Zurich (Switzerland), for loaning us the use of his automated behavioural lab, the Intellicage®, and providing invaluable advice during my experimental protocol.
- My academic siblings, Chantel Sookoo and Sadhna Singh, the givers of advice, the finders of fool-proof recipes, and the source of many hilarious moments.
- Mr. Anand Munsamy, my other half, for being patient and understanding while I completed this task and for always motivating me to work harder to achieve my goals.
- Tashveer and Una Bodhi, the first friends I made when I relocated to Limpopo, who ended up becoming family along with Shanel Balraj. Thank you for everything you have done for me.
- Staff and postgraduate students at the Department of Human Physiology (UKZN) for their assistance with technical aspects of this work.
- My colleagues at the Department of Physiology and Environmental Health (University of Limpopo) who are also completing doctoral qualifications, thank you for sharing your advice.
- Mr Shiraz Mohamed, thank you for generously funding my undergraduate degree which started my journey along this path to postgraduate studies and academics.
- College of Health Sciences (UKZN) and the National Research Foundation (NRF) for the scholarships awarded, which enabled the successful completion of this work.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 3

Chapter 4

LIST OF TABLES

Chapter 5

LIST OF ABBREVIATIONS

ADF	Anti-Drug Forum
AREC	Animal Research Ethics Committee
BA	Basophils
BEE	Black Economic Empowerment
C	Control
CW	Control Withdrawal
ELISA	Enzyme Linked Immunosorbent Assay
EO	Eosinophils
HGB	Haemoglobin
LY	Lymphocytes
MO	Monocytes
MPV	Mean Platelet Volume
N	Noscapine
NE	Neutrophil
NW	Noscapine Withdrawal
N-W-E	North-West-East
P	Papaverine
PLT	Platelet
PW	Papaverine Withdrawal
SA(A)	“Sugars” Administration Group A
SA(B)	“Sugars” Administration Group B
SA(C)	“Sugars” Administration Group C
SEM	Standard Error of Mean
SW(A)	“Sugars” Withdrawal Group A
SW(B)	“Sugars” Withdrawal Group B
SW(C)	“Sugars” Withdrawal Group C
WBC	White Blood Cell

ABSTRACT

"Sugars" is an illicit drug cocktail that is a low-grade mixture of heroin and other opioids. The composition of this cocktail is highly varied as other ingredients are added during its manufacturing process to add bulk to the mixture and possibly increase profits of the suppliers. This highly addictive cocktail requires only a single use to initiate dependence and if not used timeously thereafter, severe withdrawal symptoms occur as soon as four hours after the last use. Due to the highly variable composition of this drug cocktail, it has been difficult to create a rehabilitation program with a low relapse rate as the physiological mechanisms of action of this cocktail have not been previously investigated.

This study therefore aimed to investigate the physiological effects of "Sugars" and its ingredients in an animal model. This would provide novel findings on the pharmacological actions of the components of "Sugars" in the body as well as the physiological changes that may result during administration and withdrawal of the drug.

This thesis is comprised of four manuscripts viz. one review paper that discusses the psychosocial issues of "Sugars" from an ethnographic standpoint and three experimental papers that focus on neurophysiology, behaviour, and immunology. The first experimental paper focuses on dopamine concentrations which were analysed using an ELISA assay and the sucrose preference test which can be used to assess the anhedonic behaviour in an animal model. The second paper focuses on the changes in memory function which was assessed using the Morris water maze and hippocampal mass and the third paper discusses changes in circulating immune cells following the analysis of blood samples with a heamotology analyser .

The major findings emanating from this study were that administration of "Sugars" resulted in substantial changes in the dopaminergic system, cognitive abilities and haematological parameters involved in immunity; however, it was also observed that these changes were potentially reversed following a withdrawal period of 10 days in a mouse model. The extent of the effects observed may have also been influenced by the ratio of the ingredients in the cocktail. These novel findings can therefore assist in the formation of a targeted rehabilitation program that factors in the changes in the various physiological systems as discussed in this thesis.

Key words: "Sugars", illicit drug cocktail, dopamine, heroin, hippocampus

CHAPTER ONE

INTRODUCTION

1.1 Background of study

"Sugars" was first reported in the media in 2005 as a designer drug that had become popular amongst school children in Durban[1]. Since then, the use of this illicit drug cocktail has spiralled out of control and has spread to other provinces as well[2]. The easy accessibility and affordability of this cocktail has allowed it to target middle to low income communities. It is thought that the poverty and lack of growth or personal development in these areas are a driving force towards substance abuse[3]. There are currently a few rehabilitation programs such as the Anti-Drug Forum (ADF) to treat "Sugars" addiction[4]; however, one of the many challenges posed to these organizations is the highly variable composition of this drug cocktail. This variable composition coupled with a lack of research conducted on the neurobiological and physiological changes that occur following the use of the drug makes it a difficult task to rehabilitate individuals. The painful withdrawal symptoms also contribute to high relapse rates[5]. Our laboratory has previously chemically characterized the cocktail into its components which include heroin, noscapine and papaverine amongst other substances[6]. These ingredients could exert independent or synergistic effects; thus, it was necessary to investigate the physiological actions of this cocktail and its individual ingredients on the brain and behaviour to better understand the mechanisms of action of this cocktail.

1.2 Significance of the research problem

The issue of drug addiction is not only an individual problem as it affects the family of the individual, their community, and the country. This drug cocktail has grown in popularity since 2008 and has become widely known for its easy accessibility and its affordability. Currently, there are non-profit organisations such as the Anti-Drug Forum (ADF) in Chatsworth that have been trying to rehabilitate individuals. The drug addiction itself is difficult to treat as the exact composition varies; therefore, developing a targeted treatment protocol that focuses on the active ingredients is difficult. Currently, ADF is working on a holistic method which combines drug management with exercise and counselling to improve the recovery rate of recovering addicts[4]. The current withdrawal treatment focusses on heroin being the active ingredient; however, there have been no studies done to corroborate this.

1.3 Review of relevant literature

1.3.1 "Sugars"

"Sugars" is a highly addictive illicit drug cocktail that has become popular amongst the youth in South African communities[5]. It is easily accessible and is prevalent in low income areas due to its affordability. It is usually sold in pieces of plastic straw sealed on both ends and costs between R10-35[7]. It is used by addicts by lighting the mixture on a piece of foil and inhaling the fumes through an empty pen or other hollow tube to experience the euphoric high of the drug[3]. This method of drug administration is

commonly used by heroin addicts and is known as 'chasing the dragon'. Psychosocial research conducted on addicts has described the 'roster' or withdrawal as comprising of symptoms such as runny nose and watery eyes, hot flushes, cold sweats, severe body pain such as joint pain, abdominal cramps, constipation, cravings and decreased concentration[5]. These symptoms are evident after every 4 hours if the drug is not smoked timeously[5]. This drug has recently been chemically characterised in our laboratory and found to contain heroin, papaverine and noscapine in addition to other substances[6]. It is thought that common household substances are added to the cocktail to add bulk to the mixture which may have also complicated our analysis to accurately identify all the compounds included in this cocktail.

This highly varied composition of the cocktail plays a role in the success of the rehabilitation program as the addition of random substances to the cocktail results in several possible combinations of ingredients, which makes it difficult to narrow down the compound to target in order to successfully rehabilitate the addict. In 2010, a drop-out rate of approximately 40 percent was reported by a rehabilitation centre in KwaZulu-Natal which used a medication called Subutex® to treat "Sugars" addiction[8]. Subutex® and Suboxone® which contain buprenorphine and a buprenorphine naloxone combination respectively, are two types of medication used to treat "Sugars" addiction since it prevents withdrawal symptoms of heroin and other opiates[8]. The introduction of Subuxone® in the market place was also to prevent the diversion of Subuxone® for the intravenous or nasal abuse of buprenorphine [9]. Another treatment drug called methadone was previously used to treat "Sugars" addiction; however, addicts developed dependence on this medication[4]. Naltrexone is another medication that blocks the opioid receptors in the brain and spinal cord[4]. These treatments focus largely on treating heroin dependence; however, it does not factor in that other opioids and alkaloids also exist in the cocktail. This could be due to the fact that this cocktail was only recently chemically characterised in our laboratory and the effects of these other compounds have not yet been researched. It was, therefore, important to research these drugs in an animal model to investigate the mechanisms of action of these compounds which can then be used to understand the effects exerted on humans by "Sugars".

1.3.2 Neurophysiology

Memory and hippocampal weight

Addiction is a habit that is reinforced by repetitive actions[10] and by encountering stimuli that are associated with the drug[11]. It has been shown that the neural pathways that serve reward-based learning may also help in reinforcing addictive behaviours[12-14]. One of the most important areas in the brain which deals with learning and memory is the hippocampus. The hippocampus is responsible for both emotional and cognitive aspects of memory, and may possibly be implicated in maintaining the addictive cycle which is initiated by the exposure of a drug and leads to its misuse, abuse and the

consequences thereof [15, 16]. The use of opiates has been shown to decrease learning and memory[17] ; however, following a prolonged withdrawal period it was noted that cognitive abilities increased[18]. Studies on other drugs suggest that there may be a relationship between drug use and hippocampal volume[19]. This suggests that drugs affect both the cognitive function and physically alter the hippocampus; however, there is currently a lack of literature that adequately investigates the relationships between the two factors with regard to the use of illicit drugs.

Dopamine and sucrose preference

The dopaminergic system has been reported to be associated with addiction and the reward system[20]. The effects of opiates and other drugs are reinforced by increased dopamine activity in the mesolimbic region[21]. This effect is also reciprocated as the repetitive use of drugs of abuse have been reported to decrease the response to non-drug related rewards whilst sensitizing the individual to drug cues by eliciting long term changes in the neuroplasticity of glutamatergic inputs of dopamine neurons[22]. Noscapine, which a non-narcotic alkaloid derived from the opium poppy, has been shown to inhibit the biosynthesis of dopamine when administered to PC12 cell line[23]. There are conflicting reports on the effects of papaverine on dopamine concentration as one study suggests that it does not significantly affect the dopaminergic activity[24] whereas another study suggests that papaverine does in fact decrease the presence of dopamine in specific brain regions[25]. The sucrose preference test is a well-established method to assess anhedonia[26] and sucrose or sucrose containing items are also considered as rewards[27]. Opiates have been found to influence the taste perception and appeal of food to its users[28, 29]. Sucrose preference has been shown to be higher in opiate users as compared to the control and opiate antagonist groups[30].

Sucrose preference correlates with feelings of wellbeing[27] such as living in an enriched environment which has been shown to increase sucrose preference[31]. This could be explained by literature that suggests that sugar itself is a drug[10] and the stressed early lifestyle possibly promoted drug seeking behaviour. The general trend; however, as discussed above is that sucrose preference is an indicator of wellbeing and the ability to enjoy pleasurable activities. Dopamine has also been linked to feelings of wellbeing ; however, it has been shown to be responsible for incentive salience which denotes wanting a reward but does not guarantee the enjoyment of that reward[32]. The dopamine dysfunction theory suggests that dysregulation in the dopamine system can lead to variation in motivational anhedonia[33]. There are studies that argue that dopamine and sucrose preference are interlinked[32, 33]; however, there are others that insist that there is no relationship between the two parameters[34, 35]. The effects of "Sugars" and its ingredients on sucrose preference and dopamine have not previously been investigated and will add new knowledge to the understanding of how "Sugars" exerts its effects on dopamine concentrations.

1.3.3 Immunological blood parameters

"Sugars" addicts use the drug in a method known as "chasing the dragon"[5] which involves inhaling the fumes through a hollow instrument. This method provides the advantage of almost immediate onset of effects of the drug due to rapid absorption via the rich intranasal vascular network, bypassing the intestinal and hepatic metabolism[36]. This can also be a contributing factor to the "Sugars" withdrawal that occurs every four hours as instant gratification is closely linked to addiction and resulting repeated use of drugs[36]. The repeated use of "Sugars" which, if an addict successfully satiates their craving every four hours, raises the concern that blood cells themselves may be affected as the drug in absorbed directly into the bloodstream via the nasal vasculature. The symptoms of withdrawal such as runny eyes and nose, hot flushes and cold sweats[3] suggest changes in the immune system as the symptoms are like common cold or flu symptoms. Another symptom of withdrawal, bone pain, suggest irregularities in the bone itself which may impact the normal levels of blood cells which are produced in the bone. This study therefore combined the above factors to investigate the changes caused by "Sugars" and its ingredients on haematological parameters. Studies suggest that opioids do play a role in the immune system; however, their mode of action is still under investigation[37]. The investigation of these parameters is important as current research is focusing on vaccines to treat addiction, one of which has been successful at the preclinical stage[38]. It would therefore be beneficial to investigate if "Sugars" causes changes in blood parameters, so more information is available to treat addicts of this highly addictive cocktail.

1.4 Problem statement

There is currently a lack of empirical evidence on the effects of the drug cocktail as well as uncertainty on which ingredient is responsible for perpetuating the severe withdrawal symptoms. It is, therefore, necessary to investigate the individual and potentially synergistic effects of the individual components of the "Sugars" cocktail in an animal model to observe the physiological effects of the drug *in vivo*. This study will possibly explain the unusual symptoms exhibited by addicts as reported by psychosocial studies as well as to determine which ingredients in the cocktail elicits the symptoms.

1.5 Research question

The research questions for this study were:

- ✓ Does the administration of "Sugars" affect the memory of the mice and is this effect improved or exacerbated by the withdrawal?
- ✓ Do the changes in memory have any relation to hippocampal mass?
- ✓ Does the dopamine concentration in the prefrontal cortex of the mice increase following administration of "Sugars" and decrease following withdrawal as observed in studies investigating other opioids?
- ✓ Will the sucrose preference results correlate with the concentration of dopamine?

- ✓ Does “Sugars” create fluctuations in the blood immunological factors to support or intensify the withdrawal symptoms?
- ✓ Are the effects of the drug cocktail ingredients due to synergistic activity, or is there specific ingredients that are responsible for the major symptoms experienced by addicts?

1.6 Aims and objectives

This study aimed to investigate the physiological effects of “Sugars” and a selection of its key ingredients in a mouse model.

The objectives of this study were to:

i. To assess the potential effects of “Sugars” and its main components on cognitive function, behaviour and haematological parameters associated with immunity using a combination of biochemical and physical experimental protocols.
ii. To determine if the potential changes observed in the above factors were altered in any way following a withdrawal period from drug administration.
iii. To evaluate if any of the ingredient/s in this illicit drug cocktail may be responsible for symptoms reported by addicts.

1.7 Methodology

1.7.1 Animals

Seventy-two nulliparous female C57Bl6 mice (mass 20-22g) aged 6 to 8 weeks, were randomly divided into either administration or withdrawal groups as outlined by the diagram below (Figure 1). One mouse was removed from each “Sugars” withdrawal group due to unrelated illnesses. All mice were housed in the Intellicage®, a behavioural laboratory that incorporates automated behavioural testing, at the Biomedical Resource Unit, University of Kwa-Zulu Natal, Westville Campus. Mice were anaesthetised before the experimental protocol, and the Intellicage® transponder was injected subcutaneously in the interscapular region. This transponder enabled the Intellicage® system to record the data of each mouse. The functionality of the transponder was checked before and after placing it into the mice. A 12-hour day/ night cycle was maintained, and all animals had access to food and water *ad libitum*. All handling and testing of animals were conducted during the active phase of the day: night cycle. Ethical approval was awarded by the Animal Research Ethics Committee (AREC) at the University of KwaZulu-Natal (AREC/024/017D) and was valid throughout the protocol. A permit to handle scheduled drugs for research purposes was obtained from South African National Department of Health (POS 345/2017/2018).

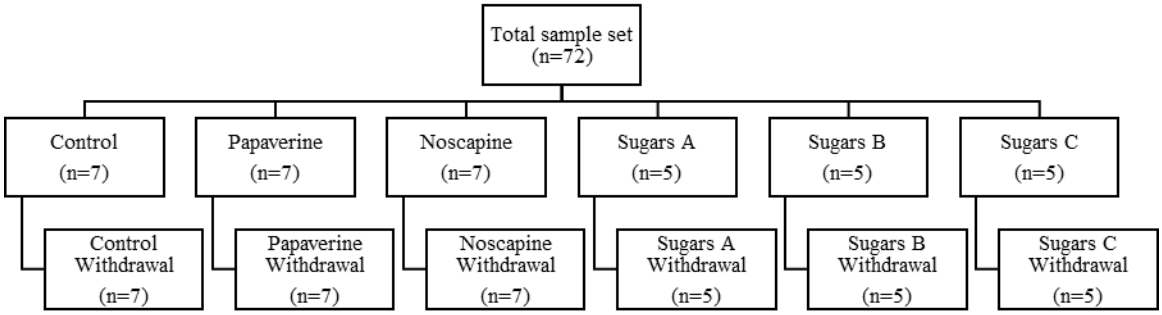

Figure 1. Graphical representation of the organisation of the sample size

1.7.2 Administration of the drug

Samples of the drug cocktail were obtained via a local rehabilitation centre. Papaverine and noscapine were obtained from Capital Labs Supplies CC (Catalog no.s P3510 and N1300000 respectively). The drugs were administered via a specially designed smoke chamber. The chamber consisted of a Perspex box with an fitted lid which contained a tripod stand for the burning of the drug sample and an area for mice to be placed to inhale fumes. The administration groups were treated with one of three samples of the 'Sugars' cocktail (A, B & C), noscapine or papaverine. The drug was burnt on a piece of foil in a chamber to simulate the way addicts use the drug. Mice were introduced to the chamber for 5 minutes to get exposure to the fumes. The mice were exposed to the drug for the addictive phase of the study which lasted 12 days; thereafter, they experienced a withdrawal of 10 days. The doses for "Sugars", noscapine and papaverine were 72mg, 72mg and 2.5mg, respectively. These doses were based on daily human consumption of "Sugars" as per data from the Anti-Drug Forum as well as the percentage composition that each compound was detected in the cocktail which was then extrapolated per gram body weight of the mice. Behavioural tests were conducted in the last week of the addiction phase and the last week of the withdrawal phase. Mice were sacrificed by decapitation at the end of either the administration or withdrawal phase depending on their group.

1.7.3 Behavioural and biochemical analysis

Blood samples and brain tissue were collected for further biochemical analysis. The blood samples were analysed to assess the varying concentrations of immunological factors in the blood to provide clarity on how the drug affects the immune system. The brain was harvested immediately post sacrifice and dissected on ice to extract the hippocampus and prefrontal cortex. The mass of the hippocampus was recorded and was compared with the cognitive performance from the Morris Water Maze. The Morris Water Maze test assessed the ability of the mice to use memory to find a hidden platform within a specified time frame. The homogenates of the prefrontal cortex tissue samples were used in an enzyme-linked immunosorbent assay (ELISA) to detect the dopamine concentration region. The dopamine

concentration was compared to the sucrose preference test which assesses anhedonic behaviour in mice. The protocols of all behavioural and biochemical assays are described in detail in their respective manuscripts.

1.7.4 Statistical analysis

All results were analysed using GraphPad Prism 5 software. Comparisons were made between the administration groups and the control group as well as their corresponding withdrawal groups using the Mann-Whitney test. Significance was set at a p-value of less than 0.05.

1.8 Thesis outline

This thesis is presented in the manuscript format and contains five chapters. Each chapter that contains a manuscript has linking text which connects it to the next chapter to ensure a logical presentation of the findings of this research. This thesis contains one review manuscript and three experimental manuscripts, as outlined below.

Chapter one provides a short background of the research topic and states the aim and objectives of conducting this research. It includes a discussion of relevant literature on the effects of the drug and its ingredients on the physiological parameters focussed on in this thesis and puts forward pertinent research questions. This chapter also provides a brief methodology that was used to achieve the objectives of the study.

Chapter 2 is a review paper and expands on Chapter one. It includes one review manuscript on the history of "Sugars" in Chatsworth, a township in KwaZulu-Natal, South Africa written in an ethnographic style titled *"Tracing my roots: An ethnographic review of "Sugars" addiction"*. It provides a unique perspective in understanding how the drug became popular in the community by including the authors own experiences.

Chapter 3 contains an experimental manuscript titled *"The relationship between sucrose preference and dopamine concentrations following administration of "Sugars" and a selection of its ingredients"*. This manuscript discusses the relationship between anhedonic tendencies in mice and the dopamine concentrations in the prefrontal cortex.

Chapter 4 contains the second experimental paper in the thesis titled *"Changes in hippocampal form and function associated with chronic administration of an illicit low-grade heroin cocktail"*. This manuscript presents findings on the impact of "Sugars" and its ingredients on the cognitive function and hippocampal mass of mice exposed to "Sugars".

Chapter 5 is composed of a manuscript titled "*"Sugars" and its effects on immunological blood markers following prolonged exposure and withdrawal period*". This manuscript presents changes in leukocytes,

monocytes and other blood immunity factors following either administration or withdrawal of “Sugars” and its ingredients.

Chapter 6 is the final chapter of the thesis. It is a synthesis of the chapters that preceded it and provides a succinct summary of the main findings of each manuscript and suggests how the findings address the objectives of the thesis. It also includes the limitations experienced during the experimental protocol and offers recommendations for future studies.

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INTERFACE

Chapter 1 provides a background and literature review of the “Sugars” cocktail. It presents a concise methodology that is designed to achieve all objectives outlined and concludes with a detailed layout of the thesis. Chapter 2 is a review paper which is an extension of the literature review presented in Chapter 1. This review paper uses autoethnography as a tool to merge the science discussed in Chapter 1 with the author’s own experience of the socioeconomic factors that play a role in the addiction of “Sugar”.

CHAPTER TWO

Tracing my roots: An ethnographic review of "Sugars" addiction

Presented in publication format of THE ORIENTAL ANTHROPOLOGIST

Submitted to the Editor on 20 June 2019

Accepted for June 2020 issue on 8 November 2019

Published online: 9 April 2020

Article

Tracing My Roots: An Ethnographic Review of "Sugars" Addiction

The Oriental Anthropologist
1–10

DOI: 10.1177/0972558X20913958
journals.sagepub.com/home/oan

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Abstract

"Sugars" is an illicit drug cocktail that has gained popularity in Chatsworth, KwaZulu-Natal, which was prehistorically classified as an Indian township. This area has now become interracial but is still predominantly inhabited by South Africans of Indian descent. "Sugars" is a mixture of various ingredients including low-grade heroin, and its cycle of addiction has proven difficult to rehabilitate in affected individuals. This is possibly due to the variation in its composition, which may lead to ineffective treatment. Addiction is thought to be influenced by both genetic and environmental cues; therefore, this review provides an introspective view on how the living conditions in Chatsworth may have played a role in making its community susceptible to drug use. This review is therefore a reflexive ethnography, which uses the first authors' childhood experiences of growing up in Chatsworth as a foundation to understand the challenges and experiences that the local community has been exposed to over the years. This review also focuses on the marketing tactics used in the illegal drug trade, the scientific background of "Sugars" and addiction, and possible suggestions on ameliorating the drug problem in South Africa. The combination of science and ethnographical viewpoints in this review article provides an interesting and informative perspective of the "Sugars" issue in Chatsworth, Durban, South Africa.

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Keywords

"Sugars," heroin, Chatsworth, addiction, autoethnography

Introduction

As a postgraduate student in medical sciences, almost every aspect of life burns under the gaze of our own scientific scrutiny. In one such instance, I found myself pondering over decisions and more specifically decisions in research. How do we end up researching the topic that intrigues us? How much of our interest is personal and is it beneficial to our work? Of course, if a close relative is battling through stage 4 cancer, it may push a researcher to work harder to find a cure for the illness and therefore personal interest may be beneficial but will not necessarily add context to the research. My research topic is addiction, addiction to "Sugars" to be precise. This topic was not initially my field of interest, I had wanted to find a better treatment for diabetes (my grandmother's condition motivated this); however, I have realized that sometimes our research topic chooses us instead. "Sugars" addiction is a devastating problem in Chatsworth, a predominantly Indian community in KwaZulu-Natal, my hometown.

Scientific experiments are based on clearly defined parameters and the quantification of variables to provide clarity on a given subject. There are some instances, however, where there is an overlap between the social and scientific realms of a research topic. As a scientist, it is easy to get caught up in the laboratory experiments that can provide detailed chemical results; however, this can be described as the "street light effect," where we tend to search where the "light" is or where it is easiest to look. To avoid this in the current study, this article provides an autoethnographic review of "Sugars" addiction.

Ethnography can be defined as a branch of anthropology that studies and describes different cultures (Oxford English Dictionary, 2008, May 30). Autoethnography was developed as a research method to allow researchers to place emphasis on their interactions with the culture that they are researching (Holt, 2003). Ellis and Bochner (2000) describe this style of writing as a multi-consciousness approach that links the personal identity to the cultural identity. Although all autoethnographic studies incorporate the researcher's voice, the degree to which the researcher plays a role varies. There are several types of autoethnography, such as indigenous/native, narrative, reflexive, dyadic interviews, reflexive ethnographies, layered accounts, interactive interviews, co-constructed interviews, and personal narratives. This review falls under the scope of a reflexive ethnography, which usually serves to illustrate how a researcher has developed due to the research. Although that is not the focus of this study, this type of ethnography best relates as it deals with reflective or narrative sharing of the author's life and thereafter moves into the ethnographic comparison of their own life with the lives of fellow cultural members (Ellis et al., 2011). The result is therefore an emerging growth from a single research focus and individual impact to one of a community with extrapolated thoughts and feelings.

This review is formulated in accordance to the criteria by Anderson (2006), in which he provided guidelines to create a more analytical product of autoethnography. He suggested three criteria to promote this: that the researcher is a member of the group or setting being researched, is seen as such in published material, and is devoted to generating an understanding of social phenomena from a theoretical perspective. This method is rich in its benefits such as the diverse and evocative data obtained and the easy availability of such data, as the researcher is part of the research phenomenon. A major benefit of autoethnography is giving the author the ability to have a voice in the text and become fully encompassed by the research (Hitchcock & Hughes, 1995), which can lead to a more humanistic and evocative text that will engage readers and possibly invoke a sense of introspection (Méndez, 2012). This method is not without limitations which may include the unpredictability of the readers' response to the emotional narrative (Bochner & Ellis, 2006) and the full disclosure of the researchers' experiences which should be honest and willingly disclosed.

There are also ethical considerations of this method such as the inclusion of names of individuals that play a role in the researcher's narrative; however, this can be avoided by taking precaution in how close relations are referred to in the narrative (Wall, 2008). Since autoethnography places a substantial amount of emphasis on the self, it is the duty of the researcher to be as ethical and honest in their narrative and to allow for data triangulation to limit bias. This current review focuses on a common social phenomenon of addiction and will include aspects of personal narrative to create a thought-provoking text to create an understanding of the scourge of drug abuse in local South African communities with a spotlight cast on "Sugars" addiction and the Indian community of Chatsworth.

Methodology

Autoethnography is a method of writing which analyzes (-graphy) the personal experiences (auto) to give understanding to cultural experience (ethno-) (Ellis, 2004). Although several studies have discussed the psychosocial aspects of "Sugars," this review serves as an autoethnographic portrayal of "Sugars" addiction from a cultural perspective. In this article, the author aims to provide a clear review of the introduction of "Sugars" into society, the factors that enable it to thrive, treatment strategies, and future direction. The inclusion of personal narrative in this review may allow for a holistic approach rather than purely academic and will allow the reader to engage with the subject matter on a deeper level.

Chasing the Dragon: "Sugars" Then and Now

In March 2005, Independent Online (IOL) News published an article on a new craze that had become popular in some communities in the Durban South region of KwaZulu-Natal (Hunsewraj, 2005). A so-called designer drug, thought to

contain heroin and cocaine, was being sold for approximately R10 per use. The drug, known as "Sugars," was packaged in green plastic, possibly refuse bag plastic, tied to form a loop. The light brown colored mixture was initially taken with marijuana or dagga or as a pill, but some users chose to smoke the drug in a manner known as "chasing the dragon," a common practice of heroin users which consists of inhaling the odorless vapor through a hollow instrument while burning the drug on a piece of foil (Jagganath, 2012). About a year later, another article suggested that the drug was being bulked with additives such as rat poison or baby powder and that the drug produced severe withdrawal symptoms such as body pain, stomach cramps, constipation, goose bumps, cravings, and loss of concentration (Tolsi, 2006). By June 2006, the use of this drug had spread to other provinces (Kuppan, 2006) and was being labeled as a "pain" drug rather than a "crave" drug, possibly due to the severe withdrawal. "Sugars" abuse was still popular in 2010 when the drug was supposedly marketed as "Whoonga" to confuse legal authorities (2010).Presently this drug is still easily available and affordable and is thought to be marketed as "Sugars," "Whoonga," or "Nyaope" depending on the location; however, there has not been any scientific analysis that has been conducted to verify these theories.

Co-incidental Marketing or Not

> *In primary school, we played the 'telephone game' where something was said to a person at the start of a line of people and the message had to be relayed one by one till the end of the line. The message was sometimes distorted so badly that it had even lost context. Could it be that these various names for the cocktail came about out of mere misunderstanding? Are "Sugars" and "Whoonga" the same drug? Do these names have any relevance to the drug itself?* (personal reflections of first author)

There has recently been an increase in the affordability of heroin in South Africa in the form of adulterated low-grade mixtures and this may be due to the increased production of heroin in Afghanistan (Van Schoor, 2015). The street names for heroin in South Africa are "Sugars" (Durban), "Unga" (Western Cape), "Nyaope" (Gauteng) and "Woonga" (Durban) (Njuho & Davids, 2010). It is well known that sugar is often found as an adulterant in heroin seized from eastern continents; this may be a possible reason for the name "Sugars." The phonetics of "Whoonga," "Woonga," and "Unga" are quite similar and may be varieties with different spelling. It was initially thought that the word "Whoonga" was Tanzanian; however, there is no Google translation from Swahili (language of Tanzania) to English. Interestingly, "Unga" translates to "powder" from both Swahili and isiZulu, possibly explaining why the name "Whoonga" became so popular and relatable in KwaZulu-Natal considering the large isiZulu-speaking population. Traditionally, symbolism is important in the isiZulu culture (Berglund, 1976) and a translation of "Nyaope" (Swahili/isiZulu) is "the net." A "net" as defined by the Oxford Dictionary is "a system or procedure for catching or entrapping a person

or persons" (Oxford English Dictionary, 2008, May 30). The above definitions suggest that the names of the drug are indeed related to either its appearance ("Unga") or its effects ("Nyaope"); however, this alone does not imply that all the names may be possibly describing one drug. In our lab, we found that the "Sugars" samples analyzed primarily contained heroin, papaverine, and noscapine (Chetty, 2017). Noscapine and papaverine are two compounds that are removed from the final heroin product as waste (Zerell et al., 2005), which may explain the affordability of "Sugars." An unpublished analysis of "Whoonga" by Prof T. Govender of the University of KwaZulu-Natal showed that the sample contained heroin, strychnine, and morphine (Health-e News, 2011). This suggests that "Whoonga" and "Sugars" are two chemically different drug mixtures that have heroin as a common ingredient. It is important to note that the bulking of the drug cocktail may be a negative factor when attempting to draw similarities between batches of the drug either with the same street name or when comparing two batches with different names, as each supplier may use adulterants that are easily available to him and therefore each batch has a unique chemical composition. This highly variable nature plays a significant role in the designing of a treatment protocol for rehabilitation.

Home "Sweet" Home

> *Indians were brought to South Africa to cut the grass and grow the cane. Nowadays, they smoke the grass and drink the cane.* (quote taken from Singh, 2012)

The above statement refers cynically to the first arrival of Indians into South Africa in 1860, when they were brought to work in the sugar cane fields. In later years, the political climate of the country would lead to the forced displacement of the descendants of these early settlers into selected areas. Chatsworth is a predominantly Indian community that was initially developed during Apartheid (a government policy of segregation based on race), for Indians to live following the Group Areas Act in 1950. The area is currently home to various races, although still largely inhabited by Indian residents. Chatsworth is geographically divided into several units. In some units, houses were built as semi-detached houses (also known as "attached" houses) and the other units consisted of owner-built houses which were usually inhabited by the so-called well-to-do (higher socioeconomic level) residents. Some of the challenges I faced were the affordability and availability of extracurricular activities that one wished to pursue, which resulted in either pursuing limited sport offered at school or reading or watching television programs. This also contributed to lower rate of fitness in the youth as well as little or no outlet for dealing with stress. Another difficulty was the process of deciding what to pursue following secondary school as there was little guidance on the matter other than common occupations and very few career talks by select individuals, which did not give a realistic portrayal of everything that was available after school life. The massive cost associated with tertiary education

was also an intimidating factor for school leavers. Due to the economic conditions of Chatsworth and the low income of many households, tertiary education was and continues to be a limited opportunity. I was fortunate to be sponsored for my undergraduate degree, which set my foundation to follow this postgraduate career path. Unfortunately, not everyone is as lucky, and there are still school leavers that do not further their studies and try to find a job to help support the family.

Once I had finished my degree, I faced newfound challenges in a democratic South Africa trying to right the wrongs of the past government. The Black Economic Empowerment (BEE) policy became a thorn in the sides of many Indians, as they were considered for jobs based on the color of their skin instead of their qualifications. A similar approach was used in promotions and left many older Indians stuck in their current positions as they did not meet the racial requirements for a higher rank. These changes in the work environment also impacted the tertiary education sector and it became more difficult for Indian students to obtain bursaries and to get acceptance into certain courses. These new factors heightened the economic despair in some Chatsworth households and left the older members of the community feeling stuck and trapped in their jobs with little prospect of growth. This decrease in viable employment options led many Indians (including myself) to leave Chatsworth and the province in search of other opportunities. Not everyone is able to do this, and Pattundeen (2008) discusses the phenomenon of missing out on this opportunity to migrate to other parts of the country. She suggests that this lack of opportunity combined with other socioeconomic factors are likely contributors to the growing substance abuse problem in Chatsworth. She suggests that the factors that have allowed substance abuse to grow may also be the changes in the socio-economy, the pre-exposure to substance abuse from family members, and the easy accessibility of drugs, owing to the widespread distribution via taxis and the drug dealers that are also residents of the community.

Poverty, education, and family background all play a role in setting the foundation for drug abuse. These factors can almost be likened to a vicious cycle, as poverty leads to a lack of resources available to an individual, leading in turn to poor grades and disrupted schooling, which results in decreased opportunity for employment and the cycle continues. It is not always poverty, however, that sets people on the path to substance abuse, and it could also be boredom and the lack of activities to occupy individuals or mere curiosity and rebellion of parental beliefs. These are all stirring factors that can lead to experimentation with addictive substances and thereafter a possible lifetime of addiction.

Treatment Strategies

Opioids are substances that act on the mu-opioid receptors and can either be synthetic or natural. Opiates are the derivatives of these compounds such as morphine or heroin. The use of these substances has implications in medicine, for example, morphine is administered to patients with extreme pain; however, many use these drugs for recreational use, and this may lead to either abuse or

dependence. The *Diagnostic and Statistical Manual of Mental Disorders*, 5th revision defines abuse as "a maladaptive pattern of substance use manifested by recurrent and significant adverse consequences related to the repeated use of substances." (American Psychiatric Association, 2013), which may lead to difficulties in completing daily responsibilities. Substance dependence, however, is the use of substances despite the consequences of its use and is usually associated with developing tolerance, withdrawal when not used and a compulsive pattern of substance use (American Psychiatric Association, 2013).

Opioids can control the physiological or psychological responses of an individual (Evans & Cahill, 2016). In acute doses, opioids induce feelings of analgesia and euphoria; however, when opioids are administered chronically, there are corresponding changes in neural circuits which facilitate a drug-dependent state (Evans & Cahill, 2016). If the drug is not administered, the individual experiences withdrawal symptoms that can last from a few days to months and range in severity from common flu-like symptoms such as fever and sweating to more intense feelings such as insomnia and anxiety. "Sugars" gives users a similar feeling of euphoria; however, the withdrawal symptoms occur after approximately 4 hours. These withdrawal symptoms, also known as a "roster," typically include runny eyes and nose, hot flushes and cold sweats, severe body pain especially joint pain, abdominal cramps, constipation, goose-bumps, cravings, and decreased concentration (Pattundeen, 2008). The severity of these withdrawal symptoms is often a motivating factor that promotes relapse and drug use. There have been several treatment drugs that have been administered to individuals to assist in their rehabilitation. Weich et al. (2008) have outlined the stages of rehabilitation as (a) identifying the problem and a motivation to change, (b) detoxification, (c) the management of co-morbid and mental medical issues, and (d) the prevention of relapse. The detoxification consists of using a substitute, which can either be a full or partial antagonist, agonist, or a non-substitute chemical.

Currently, drugs such as Methadone, Subutex®, naloxone, and Naltrexone are used in the rehabilitation of "Sugars" addicts. Methadone (full agonist) is a pain reliever and reduces withdrawal symptoms without the sense of euphoria usually experienced by the addicts. It may potentially have some side effects on the cardiac system. Subutex is the branded name for buprenorphine (partial agonist) and does not give patients the euphoria of street drugs; however, there is a potential for abuse as it has some narcotic effects being a partial agonist, while naloxone is an antagonist at the mu receptor thereby blocking opioid effects, hence used for long-term treatment of addiction. Naltrexone is used to block cravings for both alcohol and narcotics and has a longer acting effect. It is important to note that this chemical is different from naloxone, which acts rapidly to reverse the effects of an overdose but will not be useful to rehabilitate individuals due to its shorter half-life (Bloom, 2018). The treatment of addiction cannot be complete with only chemical detoxification, as this will solve only one aspect of drug abuse. As discussed above, there are several social aspects of living that may render an individual more susceptible to drug experimentation; therefore, rehabilitation programs need to offer a holistic approach to treatment. In the 1970s, two researchers conducted separate experiments on the administration of morphine to

rats. Woods (1978) showed that rats that were isolated and addicted to a narcotic substance would self-administer this substance, often with disregard for food or water until a point of death. The development of "Rat Park" by another researcher sought to understand the social aspect of drug addiction by placing addicted rats in a comfortable cohabiting environment with free choice to food, water, and mates to promote normal living conditions. These rats, when given the option to choose between morphine-laced water, chose to drink normal water despite their withdrawal symptoms and even the attempt to sweeten the morphine solution to make it more enticing did not tempt them (Alexander et al., 1978; Hadaway et al., 1979). Recent studies have also suggested that an enriched environment would offer protection against relapse in an animal model (Solinas et al., 2008). The environment, in which an individual is placed after rehabilitation should therefore be carefully considered and, if possible, improved to assist in preventing relapse. Due to the various other factors that may also affect the relapse rate and initial draw of drug experimentation, my research also focuses on the impacts of social isolation and interaction from a genetic perspective to investigate if the findings of "Rat Park" may be genetically influenced. This will therefore add to the strength of developing more inclusive rehabilitation programs.

Future Directions

> *I watch my father as he ties a straight stick to a flimsy branch on one of our trees. When I had asked why he had done that, he pointed at the flimsy branch and explained that it needed support to force it to grow straight and firm, but this must be done when the branch is still young and malleable, or it will be difficult later...* (personal reflections of first author)

The above practice in gardening can be adapted to improving the drug problem that is slowly consuming our community. First, the core of any rehabilitation program should be to offer support to the individual and their families as well as for the families to offer their support to the individual. Second, support at a later stage or adult life is not as effective as providing support during the formative years in an individual's life. Like the young branch, children are more impressionable than adults and it is in guiding them that the future of our communities can improve. The Anti-drug forum has already developed a "Smart Club" aimed at promoting knowledge of drug abuse in schools, thus helping to prevent juvenile drug experimentation (Jagganath, 2015). This type of community outreach needs to be well promoted and all schools, with the help of rehabilitation centers and tertiary researchers, should develop programs that educate learners on the dangers of drugs. Rehabilitated individuals should also be encouraged to participate in such ventures to provide authentic experiences that can be relatable to learners. Future scientific research should focus on the development of a treatment drug that has minimal side effects and potential reduction in relapse rate.

Conclusion

In this review, I have reflected on my experience growing up in Chatsworth and the challenges facing the community living in the area, as well as the impact of "Sugars" addiction and how it has been treated thus far. My work on "Sugars" addiction has given me a chance to draw on my ethnic background to provide an authentic story of how addiction can develop in a community and the choices we can make in the future to curb drug addiction. Although there are currently measures in place for treatment, our research provides new information to medically treat "Sugars" addiction. Due to the complexity of the drug problem, a holistic approach is needed and therefore lifestyle changes and family support are necessary to aid in preventing relapse after rehabilitation. The statement, "prevention is better than cure" is also relevant, as a lot of effort is needed to positively influence the youth in South Africa to deter them from drug use. More bursaries for studying and career opportunities are needed to expose the youth to different ideas and promote employment and accessibility to tertiary education.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors would like to acknowledge the University of KwaZulu-Natal, College of Health Sciences and the National Research Foundation (NRF) for the awarding of PhD scholarships to the main author which assisted in funding this research.

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INTERFACE

Chapter 2 contained a detailed review paper that merged science and sociology and provided a holistic outlook on the scourge of drug abuse in South African communities. A key point that emanated from this paper is that there is a challenge to create a targeted rehabilitation regime which stems from the lack of literature or studies conducted on the effect of "Sugars "in the body. Chapter 3 therefore provides clarity on the effects of "Sugars" and its ingredients on dopamine concentrations in the prefrontal cortex as well as anhedonic behavioural changes using the sucrose preference test as a diagnostic tool. Changes in dopamine play a role in many factors of physiological wellbeing including the reward system which motivates individuals to strive toward a given purpose expecting a positive outcome.

CHAPTER THREE

The relationship between sucrose preference and dopamine concentrations following administration of "Sugars" and a selection of its ingredients

Presented according to author guidelines of PHARMACOLOGY, BIOCHEMISTRY AND BEHAVIOR

Submitted to the editor on 5 June 2020

Manuscript ID: ADDICTBEH-D-20-00162

Status: Under review

Scientific Manuscript 1

Title: The relationship between sucrose preference and dopamine concentrations following administration of "Sugars" and a selection of its ingredients

Short title: The effects of "Sugars" on sucrose preference and dopamine concentrations

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Abstract

Introduction: “Sugars” is an illicit South African drug cocktail which is popular amongst the youth because of its easily accessible and affordable nature. It contains noscapine, papaverine and traces of heroin as primary components. There is a lack of literature on the effects of this cocktail on dopamine concentration and anhedonic behaviour, therefore this study sought to investigate if there are any effects of this cocktail or its ingredients on dopamine and sucrose preference and if these two factors are interlinked.

Methods: Seventy-two female C57Bl6 mice were treated with either papaverine, noscapine or the “Sugars” cocktail for 12 days, followed by a 10-day withdrawal. Sucrose preference was performed in the last 3 days of either the administration or withdrawal period. Dopamine enzyme-linked immunosorbent assay (ELISA) was conducted on prefrontal cortex samples.

Results: Papaverine and noscapine administration group had significantly lower dopamine concentrations compared to their corresponding withdrawal groups. “Sugars” administration groups (A) and (B) had significantly higher concentrations than their withdrawal groups. Noscapine had a significantly higher percentage of visits and nose-pokes than its withdrawal group.

Discussion: The results suggest that noscapine and papaverine reduce the dopamine concentration but does not impact the preference for sucrose; however, almost all the “Sugars” administration groups presented with contradictory results.

Conclusion: Based on the findings of this study, it can be concluded that there is a relationship between dopamine and preference for sucrose ; however, the nature of this relationship seems to be dependent on whether the ingredients are administered individually or as a cocktail.

Key words: “Sugars”, sucrose preference, dopamine, noscapine, papaverine

Introduction

"Sugars" is an illicit drug cocktail that has become popular amongst South African youth, especially those from low income communities[1]. The drug cocktail is alluring due to its easy accessibility and affordability ; however, it is highly addictive once used[2]. The drug itself is a mixture of several compounds and has been shown to contain heroin, papaverine and noscapine[3]. The chemical composition of this cocktail is highly variable which adds to the difficulty in rehabilitation and prevention of future relapse. The drug is administered via inhalation and if not used timeously, withdrawal symptoms can occur as little as 4 hours after the last use. The withdrawal symptoms include bone and body pain, runny nose and watering of the eyes, hot flushes, cold sweats and lack of concentration[2]. The severity of the withdrawal symptoms coupled with a potentially inadequate rehabilitation drug, are thought to be influential factors in the high relapse rate of "Sugars" addicts. Although the withdrawal symptoms may be linked to the high relapse rate, it may be possible that the drug itself could initiate neurobiological changes to reinforce addiction to the drug.

The administration of noscapine to PC12 cells has been shown to inhibit dopamine biosynthesis; however, the implications of this finding was not further investigated *in vivo*[4]. Very early research suggests that papaverine may be a dopamine receptor blocker ; however, the overall conclusion was that papaverine administration does not affect dopaminergic activity significantly[5]. Another study reported that the administration of papaverine increased the loss of dopamine in the caudate nucleus and tuberculum accumbens[6]. This indicates that the ingredients of "Sugars" could play a role in the dopamine balance in the body which is understandable given that the dopaminergic system has been previously linked to addiction and the brain reward system[7]. The chronic use of drugs of abuse have been shown to cause long-lasting changes in the plasticity of glutamatergic inputs to dopamine neurons in the striatum and midbrain which decreases the sensitivity of non-drug rewards while increasing the response to drug related cues[8]. Although it has been shown that enhanced dopamine transmission in the mesolimbic region can also reinforce the effects of opiates and other drugs of abuse, there are also non-dopamine related systems that contribute to the reinforcement of the use of these drugs which suggests that many factors play a role in reinforcing drug abuse[9].

A test commonly used to assess drug related behavioural changes is the sucrose preference test as sweet solutions are commonly viewed as rewards[10]. Green and colleagues[11] showed that opiate users found sucrose more palatable than the control and opiate antagonist group which correlates with other studies showing that opiate users have a preference for processed sugar intake and that opiates play a role in taste perception and appeal of food in drug users[12, 13]. It has also been shown that the living environment also plays a role in the preference of sucrose as sucrose naïve mice that had access to an enriched environment showed a higher sucrose preference[14]. This indicates that increased sucrose preference correlates with feelings of wellbeing and a decreased sucrose preference can be seen as the inability to

enjoy pleasurable activities such as the consumption of sucrose solution[10]. This is not consistent with a study on maternal separation in early development in mice which showed that mice that experienced maternal separation had a preference for sucrose or aspartame solution[15]. This differs from the general trend that attraction to sucrose solution predominantly occurs in non-stressed or non-depressed animals. Dopamine is also known to play a role in feelings of wellbeing. Berridge and Robinson suggest that dopamine systems are responsible for incentive salience which can be explained as wanting a reward but not necessarily enjoying the reward or learning what is enjoyable or not to the individual[16]. A recent theory known as the dopamine dysfunction theory suggests that a dysregulation of the dopamine system can lead to changes in motivational anhedonia [17],which is consistent with the concept of incentive salience. It can be observed that drugs of abuse have definite effects on both dopamine and taste perceptions in drug users; however, the relationship between these two factors is still under investigation. Several studies[18, 19] show that there is no relationship between the two parameters ; however, there is lack of literature regarding effects of the "Sugars" cocktail on dopamine concentration and sucrose preference. It is thus necessary to investigate the effects of the cocktail and its ingredients on the dopamine concentration and if this has any impact on the sucrose preference as well as if these potential changes are ameliorated after a withdrawal period.

Materials and Methods

Animals

Seventy-two female C57Bl6 mice (mass 20-22g) aged between 6 to 8 weeks, were randomly divided into either administration or withdrawal groups. A 12-hour day/ night cycle was maintained, and all animals had access to food and water *ad libitum.* Animals were treated with either the cocktail or an ingredient of the cocktail for 5 minutes daily over 12 days followed by a withdrawal period of 10 days using a smoke inhalation chamber. The doses for "Sugars", noscapine and papaverine were 72mg, 72mg and 2.5mg, respectively. These doses were based on daily human consumption of "Sugars" as per data from the Anti-Drug Forum as well as the percentage composition that each compound was detected in the cocktail which was then extrapolated per gram body weight of the mice. The administration animals were euthanised immediately after the administration phase of the study and the withdrawal group immediately after the 10-day withdrawal period. The use of a Control withdrawal group was to ensure that there was an accurate account of any changes that may occur due to age of animals despite the duration between the administration and withdrawal endpoints being minimal. Ethical approval was awarded by the Animal Research Ethics Committee (AREC) at the University of KwaZulu-Natal (AREC/024/017D) and was valid throughout the duration of the protocol. A permit to handle scheduled drugs for research purposes was obtained from South African National Department of Health (POS 345/2017/2018).

Tissue collection

All mice were euthanized by decapitation. The cranium was removed using surgical scissors and the brain was carefully extracted using forceps and immediately placed in 10% saline solution for further dissection. The brain was placed on a petri dish directly on ice to maintain its structural integrity. The prefrontal cortex was dissected out of the brain for this analysis and other areas of the brain were harvested for further research. All brain regions harvested were snap frozen in an Eppendorf tube using liquid nitrogen and were then stored at -80°C until analysis.

Dopamine ELISA

Brain tissue samples were weighed and rinsed in PBS to remove excess blood from the sample. Tissues were homogenised using ice-cold PBS (0.01M, pH=7.4). The homogenates were then centrifuged for 5 minutes at 5000xg. The supernatant was collected and used in further analysis. The reagents for the ELISA assay were prepared as per Elabscience® user manual for dopamine (Catalog no. E-EL-0046, Elabscience®).

The standard or sample (50µL) was added to each well and 50µL of Biotinylated Detection Ab was added immediately to each well. The plate was incubated for 45 minutes at 37°C. The contents of the plate were aspirated, and the plate was washed 3 times with detergent solution. HRP Conjugate (100µL) was added to each well and the plate was incubated for a further 30 minutes at 37°C. The contents were aspirated, and the plate was washed 5 times using detergent solution. Substrate reagent (90µL) was added to each well and the plate was incubated for 15 minutes at 37°C. After the 15-minute incubation, 50µL of Stop solution was added to each well and the plate was read immediately at 450nm. The concentrations of dopamine present in each sample were extrapolated using a standard curve. All standards and sample were tested in duplicate.

Sucrose preference test

All animals were housed in an automated behavioural cage called the Intellicage®, that was interfaced to a laptop to record data[20]. Each mouse was injected in the intrascapular region with a transponder that was functional with the cage sensors. The four corners of the cage each contained two drinking bottles and a computerised sensor at the opening to each of the bottles. The sensor recognised the transponder on each mouse allowing entry to the drinking bottle and recording accurately which mouse drank. The sensor detected the number of nose-pokes, which is the number of times the animals nose passed the sensor indicating an intention to drink as well as how many times the animal visited a corner indicating preference for that corner. The teat of each bottle was placed into a hollow tube that functioned as a lickometer and recorded the number of times a mouse licked the bottle in the process of water consumption. The sucrose preference test was used to evaluate the anhedonic behaviour of the mice.

Two sets of bottles were made available to the mice in opposing corners of the cage, one containing normal drinking water and the other containing a 10% sucrose solution. The mice were exposed to the bottles for 3 hours daily over 3 days. Each day the position of the bottles was swopped to avoid bias. The amount of sucrose consumed was expressed as a percentage of the total amount of liquid consumed during that period. The method used was adapted from the sucrose test used by Thomson and colleagues[21].

Statistical analysis

All results were analysed using GraphPad Prism 5 software. Comparisons were made between the administration groups and the control group as well as their corresponding withdrawal groups using the Mann-Whitney test. Significance was set at a p-value of less than 0.05.

Results

Sucrose preference

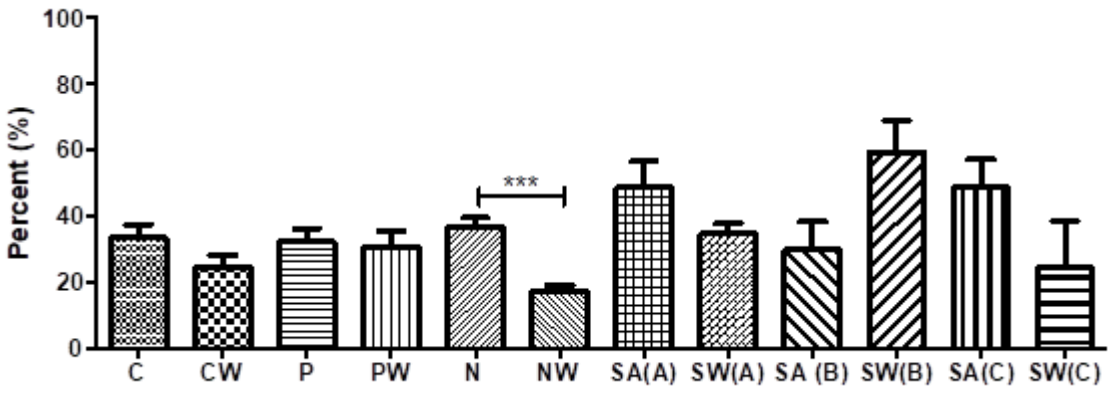


Figure 2. The mean percentage ±SEM of visits recorded to the sucrose containing bottle. ***p<0.001, N vs. NW, Mann-Whitney test

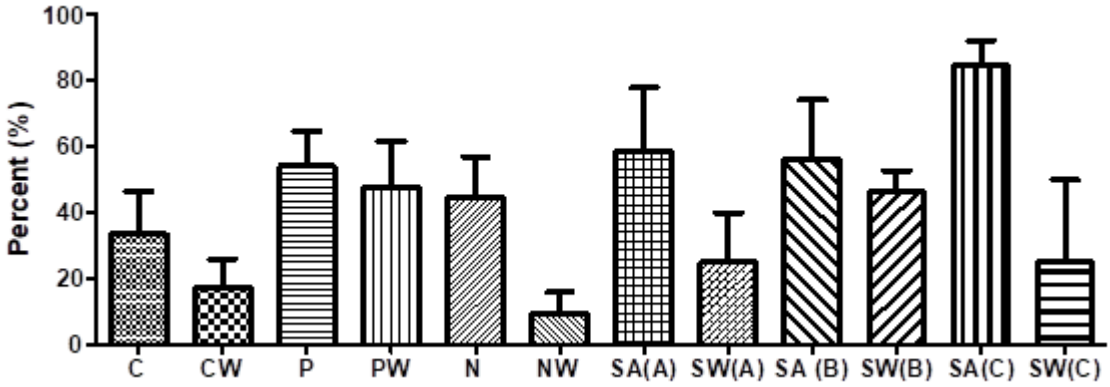


Figure 3. The mean percentage ±SEM of licks recorded to the sucrose containing bottle.

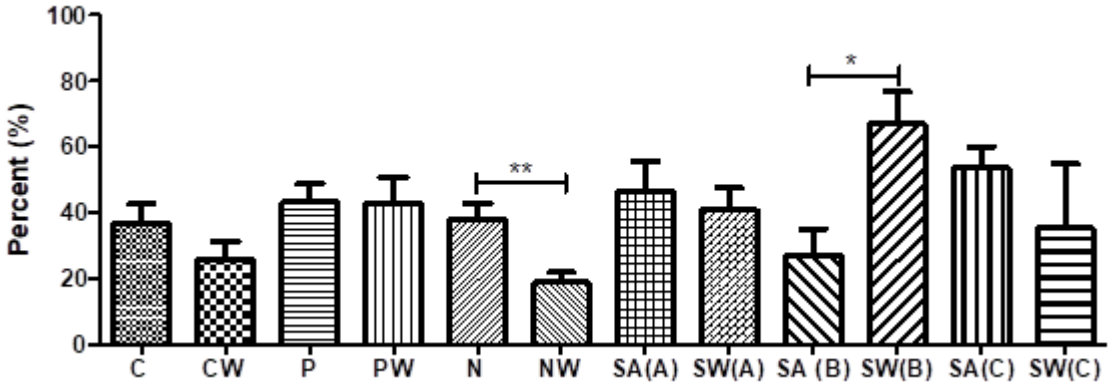
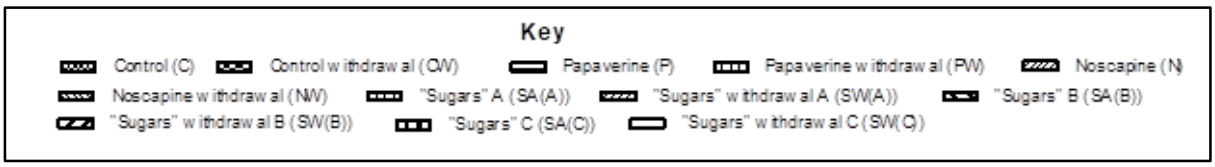


Figure 4. The mean percentage ±SEM of Nose-pokes recorded to the sucrose containing bottle. *p<0.05, SA(B) vs. SW(B), Mann-Whitney test; **p<0.05, N vs. NW, Mann-Whitney test



The noscapine withdrawal group visited the corner containing the sucrose bottles significantly fewer times than the noscapine administration group. There were no significant differences observed between the mean and SEM of other administration and withdrawal groups; however, it was interesting to observe that the "Sugars" groups had varying responses. The withdrawal groups of "Sugars" administration group (A) and (C) had a higher number of visits (mean±SEM) than their withdrawal group; however, "Sugars" administration group (B) was the opposite. There were no significant differences observed between any groups for the percentage of licks; however, it was noticed that all administration groups had a higher lick percentage (mean±SEM) than their corresponding withdrawal groups. There was a significant difference observed for the noscapine administration and withdrawal groups with the noscapine administration having a higher number of nose-pokes (mean±SEM). It was

also noted that "Sugars" administration group (B) had significantly fewer nose-pokes than its withdrawal group.

Dopamine levels

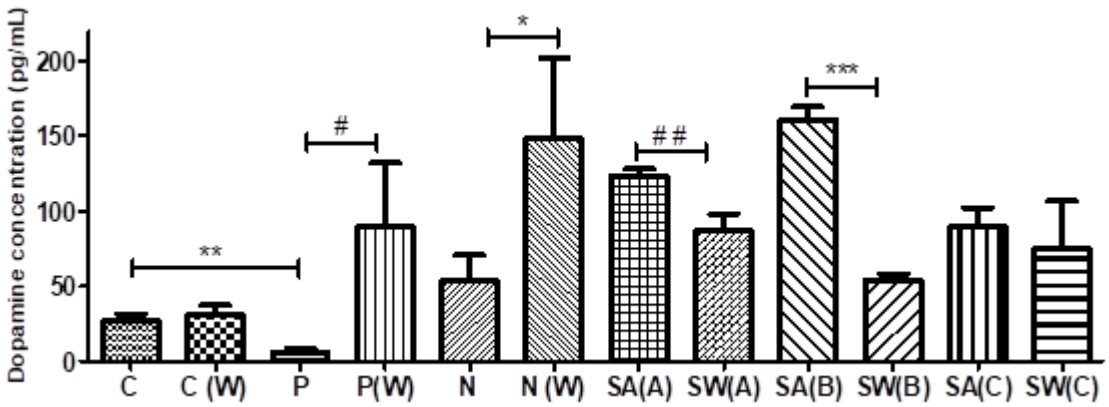


Figure 5. The mean percentage ±SEM dopamine concentration. **p<0.01, C vs. P, Mann-Whitney test; #p<0.05, P vs. PW, Mann-Whitney test; *p<0.05, N vs. NW, Mann-Whitney test; ##p<0.05, SA(A) vs. SW(A), Mann-Whitney test;***p<0.05, SA(B) vs. SW(B), Mann-Whitney test

There were several significant associations observed for the dopamine concentrations. The papaverine administration group had a significantly lower mean±SEM than both the control group and its corresponding withdrawal group. The noscapine administration group had a significantly lower dopamine concentration (mean±SEM) than its corresponding withdrawal group and the "Sugars" administration groups (A) and (B) had significantly higher dopamine concentrations than their corresponding withdrawal groups.

Discussion

The Intellicage® behavioural laboratory recorded the nose-pokes, licks and visits to the corner containing the sucrose bottles. The nose-pokes and visits are indicative of sucrose seeking behaviour and the licks are the actual consumption of the sucrose solution. The results for the licks recorded did not show any statistical significance between the groups; however, it was observed that the papaverine and noscapine administration groups had a higher preference for sucrose than the control group. This was also the case with all sugar's administration groups and all administration groups had higher sucrose consumption than both the control and their corresponding withdrawal groups. This is similar to findings by Green and colleagues that opiate users find sucrose more preferable than non-users or those who are on opiate antagonists[11]. The influence of opiates on taste perception[12, 13] may explain the findings in this paper; however, it may also be impacted by a common addictive circuit in the brain. Behavioural addictions such as gambling, overeating and internet addiction can indirectly affect neurotransmitter levels in the brain as compared to drug use which affects the neurotransmitters

directly[22, 23]. Orzack suggests that individuals afflicted with behavioural addictions are likely to also have other addictions[24]. This co-existance of addictions imply that common circuitry may be involved in initiating new addictive patterns and reinforcing addictive trends[25]. Another plausible reason for high sucrose preference as discussed by Holgate and colleagues[14], was the use of the Intellicage® as an enriched living environment. This may also explain the higher sucrose preference in this study since all animals were housed in the Intellicage® for the duration of the protocol.

The noscapine administration group had a significantly higher visiting and nose-poke percentage to the sucrose corner than its withdrawal group. This can be explained using the same suggestions above as these patterns correlate with the trends observed for noscapine in the lick results. The "Sugars" administration group (B) was the only administration group to have a significantly lower nose-poke percentage than its corresponding withdrawal group. This trend was also observed for the visits percentage for "Sugars" administration group (B) but was opposite to the trend observed for the same group for licks. This demonstrates that although this group visited or attempted to gain access to the drinking bottle fewer times, their duration of time spent in the sucrose corner was most likely longer therefore their sucrose consumption was still high.

The papaverine administration group was found to have a lower dopamine concentration than both the control and its corresponding withdrawal group. Papaverine has previously been shown to reduce the dopamine content in specific areas of the brain[6]. The higher concentration of dopamine in the papaverine withdrawal group suggests that although papaverine may have reduced the dopamine content, the change may be reversible; however, the dopamine levels of the papaverine withdrawal group were also higher than those of the control group. This implies that during withdrawal of papaverine, there is an overcompensation for the reduced dopamine levels during administration to above normal levels. Supraphysiological levels of dopamine have been implicated in schizophrenia which is characterised by aggressiveness, paranoia, hallucinations and bizarre thoughts[26]. These symptoms may explain some of the violent behaviour, mood swings and insentient actions of "Sugars" addicts during their withdrawal state as described by psychosocial studies[1]. Noscapine administration group was also significantly lower than its corresponding withdrawal group. The administration of noscapine to PC12 cells showed an inhibition of dopamine biosynthesis[4] which relates well to the findings in this study and the higher dopamine content for the noscapine withdrawal group suggests that this inhibition of dopamine biosynthesis can be returned to normal. The "Sugars" administration groups (A) and (B) had significantly higher dopamine concentrations than their withdrawal groups which was opposite to the individual effects of noscapine and papaverine which suggests that the ingredients possibly exerted a synergistic effect which resulted in an increased dopamine concentration.

In conclusion, the results of this study show that the administration of noscapine and papaverine results in an inverse association between dopamine concentration and sucrose preference. The sugars

administration sucrose preference parameters; however, correlated directly with dopamine concentration except for “Sugars” administration group (B) which was not consistent for all parameters of sucrose preference. This could be due to longer visits to the corner containing sucrose solution since the licks showed a direct relationship with dopamine concentration. Whilst some studies suggest that dopamine plays a role a salience and the reward system, which includes preference to sucrose[16, 17]; other studies argue that there is no correlation between the two factors[18, 19]. The results of this study speak more to the former view of the above conundrum as the individual administration of noscapine and papaverine showed an inversely proportional association between sucrose preference and dopamine concentrations. The results of the “Sugars” administration groups; however, suggest that the combined effects of the ingredients of the cocktail may result in a directly proportional association between sucrose preference and dopamine concentrations. Our study therefore demonstrates that the nature of the association between sucrose preference and dopamine concentrations are influenced by the variation of the ingredients of the “Sugars” cocktail. This poses severe treatment challenges as these varying compositions of the cocktail would yield different symptoms therefore making it extremely challenging to rehabilitate individuals.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgements

The authors would like to acknowledge the University of KwaZulu-Natal, College of Health Sciences, and the National Research Foundation (NRF) for the awarding of PhD scholarships to the main author which assisted in funding this research.

The authors would also like to place on record their gratitude to Professor Hans Lipp (Institute of Anatomy, University of Zürich), for allowing them the use of his automated behavioural lab, the Intellicage® as well as providing his expert advice during this protocol.

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INTERFACE

Chapter 3 discussed the behavioural changes that were brought about during the administration and withdrawal of “Sugars” and its ingredients. There were fluctuations in both dopamine concentrations and sucrose preference following the use of “Sugars or its ingredients however it was also seen that some of these changes were reversed during the withdrawal period. Reichenbach and colleagues have shown that altered signaling of dopamine receptors may play a role in some aspects of memory consolidation. Chapter 4 explores the effects of “Sugars” and its ingredients on memory and hippocampal mass. Chapter 4 also assesses the relationship between the hippocampal mass and memory to evaluate if “Sugars” and its ingredients are responsible for morphological changes that result in alterations in memory.

CHAPTER FOUR

Changes in hippocampal mass and function associated with chronic administration of an illicit low-grade heroin cocktail

Presented according to author guidelines of NEUROBIOLOGY OF LEARNING AND MEMORY

Submitted to the editor on 2 June 2020

Manuscript ID: NLM-20-163

Status: Under review

Scientific Manuscript 2

Title: Changes in hippocampal mass and function associated with chronic administration of an illicit low-grade heroin cocktail

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Key words: "Sugars", heroin, illicit cocktail, South African, addiction

Abstract

"Sugars" is a highly addictive and is easily available illicit South African drug cocktail. Our laboratory has shown that it primarily contains heroin, papaverine and noscapine. It is prevalent in low income areas due to its affordability. There is a lack of empirical data to explain the effects of this cocktail, therefore this study investigates the effects of this drug cocktail on hippocampal weight and memory in a mouse model. Sixty-nine, female C57Bl6 mice were housed in the Intellicage®, an automated behavioural lab, and had access to food and water *ad libitum*. Animals were allocated to either an administration group or a withdrawal group. Both groups were treated with either the drug cocktail, noscapine or papaverine for 12 days, thereafter only the withdrawal group underwent a 10-day withdrawal phase. All groups performed the Morris Water Maze test. The hippocampus was removed posthumously, and its weight was recorded. There was a significant difference between the Noscapine (administration) and Noscapine (withdrawal) groups for the Morris Water Maze test. The papaverine and noscapine administration groups had higher hippocampal weights than the control group. There was a significant difference in hippocampal weights between the papaverine administration and papaverine withdrawal groups as well as between the control and control withdrawal groups. The changes in mass that were evident in the hippocampus; coupled with altered memory patterns suggest that there are changes in the reward pathway which need to be further investigated.

Introduction

Addiction can be described as an action or habit that occurs at the expense of all other actions and is reinforced by repetition of this behaviour [1]. This suggests that an individual may be psychologically conditioned to act against intrinsic survival mechanisms to satisfy an addiction. The patterns of drug use may start from the first use as drug seeking behaviours. These behaviours are learnt and include behaviours such as knowing where to purchase the drug or the usual environment of use which suggests the availability of the drug. These behaviours are reinforced by other stimuli that occur concurrently with drug use, e.g. the paraphernalia used, and effects experienced after drug use[2]. As an individual seeks out and takes the drugs, these behaviours are reinforced by the same neural pathways that maintain reward-based learning [3-5]. The hippocampus may play a significant role in maintaining the addictive cycle [6] as it is responsible for semantic, episodic and spatial memory which encompasses both the cognitive and emotional aspects of memory[7, 8].

Drugs of abuse have varying effects on hippocampal memory. Stimulant drugs, cannabis and alcohol enhance hippocampal dependent learning and memory whereas abuse of opiate has been shown to cause a deficit [9]. Opiate withdrawal (prolonged); however, results in enhanced cognitive function in the hippocampus [10]. It is possible that the use of drugs of abuse also cause morphological changes in the

hippocampus. Xu, Kober [11] suggests that there is a relationship between drug use and hippocampus size. Cocaine users showed a positive correlation between the number of days of drug use and the hippocampal volume; however, a negative correlation was observed between the hippocampal volumes and days of withdrawal from cocaine[11]. These patterns between hippocampal volume and drug use and between drug use and memory suggest that drugs of abuse may exert its effect on hippocampal learning and memory by altering the physical properties of the hippocampus. The effects of drug cocktails on the hippocampus and memory have not previously been reported in the literature. Further research is needed to investigate the effects of combined drugs given the potential combined or synergistic effects which may explain the severe and unusual withdrawal symptoms.

"Sugars" is a highly addictive illicit drug cocktail that has recently become popular in South Africa. The cocktail is both easily accessible and affordable which has allowed it to become a popular drug in low income communities [12]. Our laboratory has shown that the cocktail primarily contains heroin, papaverine and noscapine which suggests that it is a waste product of the illicit heroin manufacturing process [13]. The users experience euphoria after smoking the drug; however, this high only lasts approximately 4 hours before the withdrawal symptoms are felt. Psychosocial research conducted on addicts has described the withdrawal ('roster') as comprising of symptoms such as runny eyes, hot flushes and cold sweats, severe body pain especially joint pain, abdominal cramps, as well as constipation, goose-bumps, cravings and decreased concentration[12]. The severity of the withdrawal symptoms is a possible reason for the high relapse rate of "Sugars" addicts. These harsh symptoms vary between users and this may, in part, be attributed to the inconsistent composition of the drug and the presence of adulterants which are to bulk up the cocktail. This drug is therefore a low-grade mixture of heroin. There is currently a lack of empirical evidence on the effects of "Sugars" on cognitive function. The aim of this study was therefore to assess the effects of "Sugars" and a selection of its constituents on memory and to determine if there are any corresponding changes in hippocampal morphology.

Material and methods

Animals

Sixty-nine female C57Bl6 mice (20-22g), aged between 6 to 8 weeks, were housed in the Intellicage®, an automated behavioural cage. Animals were exposed to a 12-hour day/night cycle and had access to food and water *ad libitum*. The mice were divided into 4 administration groups viz. Control (C; n=7), Papaverine (P; n=7), Noscapine (N; n=7), "Sugars" (SA; n=12) and 4 corresponding withdrawal groups (CW, PW, NW, SW). The "Sugars" administration and withdrawal groups were further divided into 3 sub-groups each as the drug was sourced from three independent sources. All mice were housed in the Biomedical Resource Unit and this protocol was approved by the animal committee at the University of KwaZulu-Natal (AREC/024/017D).

Drug administration

All groups, except the control and control withdrawal groups, were exposed to the drug that was burnt on aluminium foil in a special smoke chamber for 5 minutes daily over 12 days. The administration groups performed the Morris water maze test (Days 8-11) and were thereafter euthanized after the administration phase on Day 12. The withdrawal groups underwent a 10-day withdrawal period during which they performed the Morris water maze test (Days 6-9) and were euthanized thereafter on Day 10 of the withdrawal phase. The doses for "Sugars", noscapine and papaverine were 72mg, 72mg and 2.5mg, respectively. These doses were based on daily human consumption of "Sugars" as per data from the Anti-Drug Forum as well as the percentage composition that each compound was detected in the cocktail which was then extrapolated per gram body weight of the mice[13]. A permit to handle scheduled drugs for research purposes was obtained from South African National Department of Health (POS 345/2017/2018).

Morris Water Maze Test

The Morris water maze consisted of a circular pool (150 cm diameter) with a transparent escape platform (5cm X 10cm) in the southern quadrant of the pool; filled with tap water to the level of the transparent escape platform. Testing was conducted on four consecutive days and each mouse was subjected to three training trials, each timed until either the mouse found the hidden platform or a cut-off time of 1 minute and 15 sec passes. Start positions were North – West – East (N-W-E) for all mice tested.

Euthanization and hippocampal dissection

All mice were euthanised by decapitation and the brain was dissected to remove the hippocampus. The hippocampus was weighed and then immediately snap frozen in liquid nitrogen and stored at -80C for future biochemical analysis.

Statistical analysis

Data was analysed using GraphPad Prism 5 software and a p-value of less than 0.05 was considered significant. Mann Whitney tests were used to determine significance.

Results

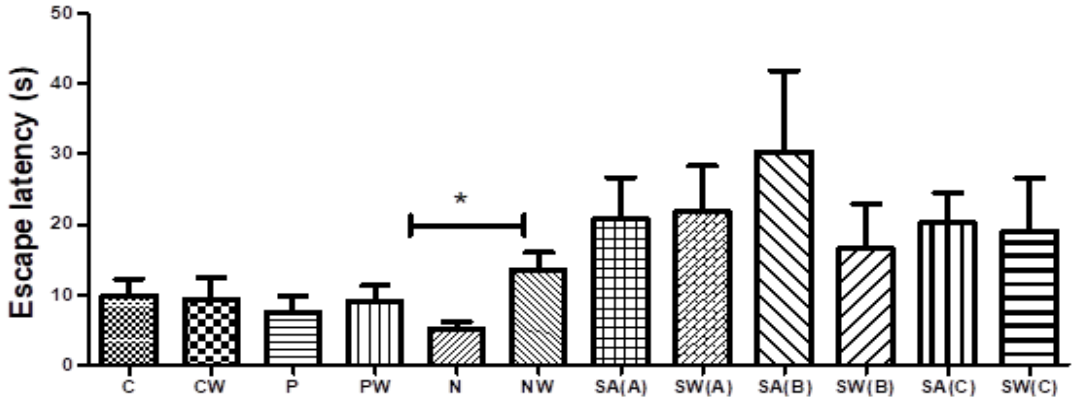


Figure 1. Time taken to find platform in Morris Water Maze test. *p<0.05, N vs. NW, Mann Whitney test

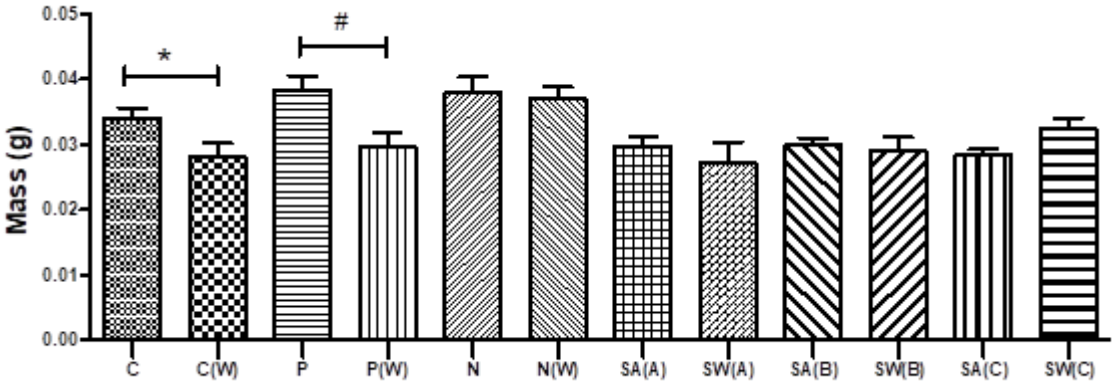


Figure 2. Hippocampal weights *p<0.05, C vs. CW, Mann Whitney test; #p<0.05, C vs. CW, Mann Whitney test

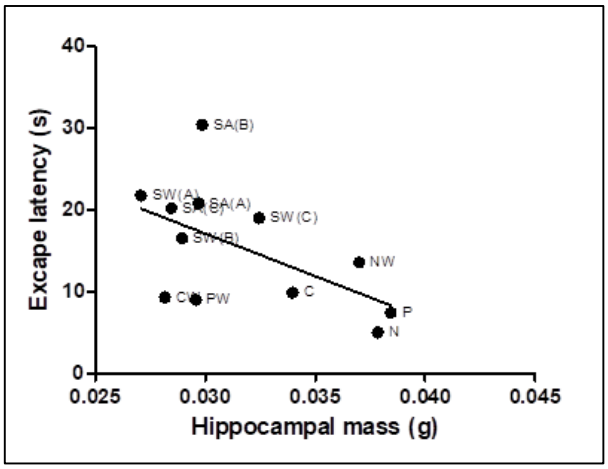
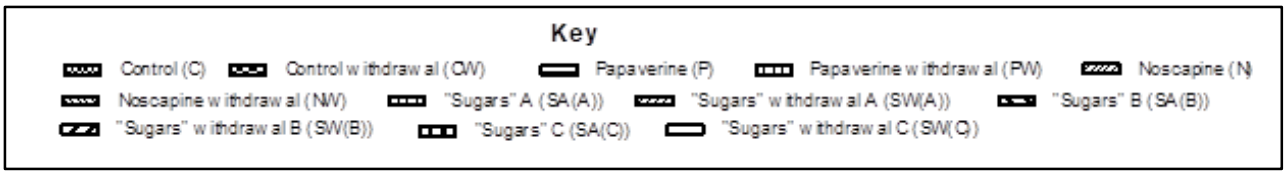


Figure 3. Hippocampus mass vs. time taken to find the hidden platform (r^2=0.32)



Morris water maze test

There was no significant difference between the control and control withdrawal groups as expected; however, it was observed that the papaverine and noscapine administration groups showed a decrease in the escape latency (time taken to find the hidden platform). This suggests that these drugs improved memory. The corresponding withdrawal groups of the above drugs had an increased escape latency which could imply that the withdrawal of these substances negatively alter memory. This can be substantiated by the statistically significant difference between the noscapine administration and withdrawal groups as shown in Figure 1 (Mann-Whitney test; $p<0.05$). "Sugars" administration group A had a reduced escape latency; however, the withdrawal groups of "Sugars" B and C had increased escape latency. These results suggest that the composition of the cocktail was highly variable.

Hippocampal weights

The control withdrawal and papaverine withdrawal groups showed significantly lower hippocampal weights than their corresponding administration groups (Mann-Whitney test; $p<0.05$). All administration groups, except for "Sugars" administration group C, had higher mean hippocampal masses than their corresponding withdrawal groups.

Correlation between hippocampal mass and time taken to find the hidden platform

The average time taken to find the platform for each group was plotted in relation to the average hippocampal weight, to investigate if hippocampal mass had any relationship to cognitive function. Although the relationship between time and hippocampal weight was not statistically significant, there was a negative trend observed between the time taken to find the hidden platform and the mass of the hippocampus i.e. the greater the mass of the hippocampus, the less time taken to find the hidden platform.

Discussion

The results of this study suggest the administration and withdrawal of "Sugars" results in changes in both cognitive function and hippocampal mass. Noscapine administration showed a pronounced increase in memory whereas the administration of papaverine resulted in a significant increase in hippocampal mass. Previous studies that have examined the effects of acute administration of noscapine

and papaverine on memory, have shown that these drugs either worsen or have no effect on memory [14, 15]; however, the chronic administration of papaverine, improved cognitive function in an animal model of Huntington's disease [16]. It is possible that the improved memory associated with chronic administration of noscapine and papaverine as observed in this study, may be attributed to the common neural pathways shared by drug abuse and learning and memory [17]. The decline in cognitive function associated with withdrawal of these substances have also been observed in the withdrawal stages of other addictions such as cannabis, cocaine, alcohol, and nicotine. The "Sugars" administration groups B and C showed typical cognitive decline associated with opiate abuse with improvement following withdrawal of the drug. "Sugars" administration group A had the opposite effect which could be attributed to the highly varied composition of the drug cocktail and the possibility that this sample could contain a higher concentration of a substance with stimulant properties. This substance could possibly be noscapine or papaverine as these substances were present in all samples of the drug in varying concentrations and showed stimulant effects when administered individually. "Sugars" administration groups B and C are likely to have higher concentrations of heroin as the effects observed reflect opiate abuse associated changes in cognitive function.

The effects of papaverine and noscapine on hippocampal weight are not well documented ; however, it has been shown that heroin decreases the number of proliferating cells in the hippocampus [18]. This could be an explanation for the lower hippocampal weights in the "Sugars" administration groups B and C as decreased cell numbers may possibly result in lower hippocampal weight. Adult hippocampal neurogenesis is an important function of the hippocampus in which new neurons are developed in the dentate gyrus of the hippocampus, some of which become fully functional and become part of the hippocampal circuitry. Several studies have established that adult hippocampal neurogenesis is essential to interpret declarative memory during hippocampal dependent behavioural assessments; however, a decrease in this form of neurogenesis may hinder the performance in these tests[2, 19]. This type of neurogenesis thus serves as an augmenter of hippocampal efficiency [20]. This highly plastic nature of the hippocampus allows it to be an integral component of the addictive cycle as its memory functions may assist in forming drug induced hippocampal changes [9]. Recent studies on adult hippocampal neurogenesis in heroin addicts have shown that the growth rate of neurons is slower than in non-addicted controls. The addicted cohort also presented with less neural precursor cells, fewer dendritic trees and did not show normal increases in the differentiating cell number with age [21]. This fluctuation in cell quality and quantity may lead to changes in the cognitive functions of the hippocampus with corresponding changes in mass of the hippocampus as observed in the Figure 3 which shows an inverse trend between the time taken to find the hidden platform and the mass of the hippocampus.

Conclusion

The results of this study highlights the differences between the effects of individual drugs of abuse versus drug cocktails and provided clarity on the possible mechanisms underlying the varied effects of "Sugars". The data obtained in this study suggests that the composition of the drug cocktail, possibly the ratio of the primary ingredients plays an important role in the effect of "Sugars" on cognitive function. A negative trend was observed between escape latency and the mass of the hippocampus and we postulate that changes in hippocampal mass can be attributed to the state of neurogenesis that the drug of abuse induces in the hippocampus. Further research should investigate the concentration of apoptotic proteins such as *Fas*, *FasL* and *Bad* which play a role in neurotoxicity and cell death to determine if the changes in hippocampal morphology and cognitive function are associated with those pathways. These tests should be conducted in conjunction with hippocampal dependent behavioural assessments to verify the link between drugs of abuse, memory, and hippocampal mass.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgements

The authors would like to acknowledge the University of KwaZulu-Natal, College of Health Sciences, and the National Research Foundation (NRF) for the awarding of PhD scholarships to the main author which assisted in funding this research.

The authors would also like to place on record their gratitude to Professor Hans Lipp (Institute of Anatomy, University of Zürich), for allowing them the use of his automated behavioural lab, the Intellicage® as well as providing his expert advice during this protocol.

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INTERFACE

Chapter 4 focused on the effects of "Sugars" and its ingredients on changes in the hippocampus and memory following administration and during withdrawal. It was shown that there are definite changes in memory as well as morphological changes in the hippocampus following drug administration. Marin and colleagues suggest that there is a strong link between the immune system and the brain. They show that disruptions in the immune system can result in impaired cognition and neurogenesis. Chapter 5 thus examines the effects of "Sugars" and its ingredients on the immune system with a specific focus on blood immune cells. Since the drug cocktail is administered via inhalation, it is rapidly absorbed into the bloodstream. This may allow it to exert changes in the concentrations of immune cells in the blood which may explain the flu-like withdrawal symptoms associated with this cocktail.

CHAPTER FIVE

“Sugars” and its effects on haematological markers following prolonged exposure and withdrawal periods

Presented according to author guidelines of JOURNAL OF KERMAN UNIVERSITY OF MEDICAL SCIENCE

Submitted to the Editor on 21 January 2020

Manuscript ID: JKMU-2001-2162

Status: Under review

Scientific Manuscript 3

Title: "Sugars" and its effects onhaematological markers following prolonged exposure and withdrawal periods

Short title: The impact of "Sugars" on select haematological parameters

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Abstract

Introduction: “Sugars” is an illicit drug cocktail that is easily available and affordable to youth in low income South African communities. The drug cocktail is highly addictive with a varying composition and has been found to contain heroin, noscapine and papaverine. Addicts experience severe withdrawal symptoms which include pain and other symptoms that are commonly associated with the cold or flu which prompted investigation into the effects of “Sugars” and its ingredients on the blood cells that play a role in the immune response.

Methodology: The drug cocktail and its ingredients were administered via smoke inhalation for 5 minutes daily to C56Bl6 female mice for a period of 12 days, followed by a withdrawal period of 10 days to assess changes in blood markers. Blood was collected and analysed used a Beckman Coulter apparatus.

Results: It was observed that papaverine and noscapine administration resulted in a significantly lower white blood cell count (WBC) than the control group; however, the WBC was significantly lower in the noscapine administration than its corresponding withdrawal group. Papaverine administration has a significantly lower mean platelet volume (MPV) than the control group but this improved during the withdrawal phase. The “Sugars” administration groups had varying results which were indicative of their varying composition.

Conclusion: The results suggest that there is a clear influence of the “Sugars” cocktail and its ingredients on the blood immunology markers and may potentially have an aetiological role in the withdrawal symptoms experienced.

Key words: “Sugars”, opioids, immunology, haematology, heroin

Introduction

"Sugars" is a low-grade illicit drug cocktail that has recently become popular in South African communities[1]. It is easily accessible and affordable making it a drug of choice for youth especially those from low income communities[1]. It was previously thought to be a heroin-based cocktail but has been shown to contain heroin, papaverine and noscapine amongst other substances[2]. The unidentified substances may possibly be bulking agents added by suppliers of the drug thus making its composition highly variable[2]. The drug itself is highly addictive and addicts experience withdrawal symptoms as soon as 4 hours after their last inhalation which enforces the relapse and further use of the cocktail[3]. The symptoms of withdrawal can be likened to flu-like symptoms such as runny nose, watery eyes, hot flushes, cold sweats, severe body pain especially joint pain, abdominal cramps, as well as constipation, goose-bumps, cravings and decreased concentration[3].

Addicts use the drug by burning the drug powder on a piece of aluminium foil and inhaling the fumes through a hollow column like object such as a straw or an empty ball point pen body[3]. This method is known as 'chasing the dragon' and is popular amongst heroin users as well[3]. Pharmacologically, there are several advantages and disadvantages of using this method of administration. The advantages of this method are that the drug fumes are rapidly absorbed into the rich vascular supply of the respiratory system thereby allowing a rapid onset of effect (7-10 seconds) by avoiding the intestinal and hepatic metabolism of the drug[4]. The disadvantages ; however, are that there is an addictiveness associated with this method due to the instant gratification experienced and that the drug has a shorter half-life, which can lead to the need to re-use the substance within a short space of time [4].

The disadvantages and advantages as explained above can be used to describe how the drug cocktail became highly addictive merely due to its chosen route of administration. However, it does raise questions on whether the blood system is affected by repeated exposure of the body to the drug cocktail. The withdrawal symptoms suggest that the blood system may be influenced as the users experience joint or bone pain which may imply adverse changes in the normal physiological function of bone and a consequential alteration in cellular composition of the blood. Fluctuations in blood cell counts can in turn affect the functioning of the body and immune system which may provide an explanation for the flu-like symptoms experienced by addicts during withdrawal. Recent studies have shown that opioids can potentially influence the immune system, but their exact mechanism of action is not totally understood[5], ; however, there is currently research into creating vaccines to effectively treat addiction which has been successful at the preclinical trial phase[6]. Given these facts, it is necessary to investigate the impact of "Sugars" and its ingredients on the blood with specific reference to blood markers which play a role in the immune response.

Materials and methods

Animals

Seventy-two female C57Bl6 mice (mass 20-22g), aged between 6 to 8 weeks, were randomly divided into one of twelve administration or withdrawal groups. Three samples of the drug cocktail were chemically characterized in our laboratory and individual ingredients were extracted and purified[2]. The three sample cocktails as well as the two ingredients that were identified, were each assigned an administration group. Each administration group had a corresponding withdrawal group. A 12-hour day/ night cycle was maintained, and all animals had access to food and water *ad libitum*. Animals were treated with either the cocktail or an ingredient of the cocktail over 12 days followed by a withdrawal period of 10 days. The drug was administered using a specialised smoke apparatus and each animal had 5 minutes of exposure daily. The doses for "Sugars", noscapine and papaverine were 72mg, 72mg and 2.5mg, respectively. These doses were based on daily human consumption of "Sugars" as per data from the Anti-Drug Forum as well as the percentage composition that each compound was detected in the cocktail which was then extrapolated per gram body weight of the mice. The administration group animals were euthanised immediately after the administration phase of the study and the withdrawal group immediately after the 10-day withdrawal period. The use of a control withdrawal group was to ensure that there was an accurate account of any changes that may occur due to the age of the animals despite the duration between the administration and withdrawal endpoints being minimal. Ethical approval was awarded by the Animal Research Ethics Committee (AREC) at the University of KwaZulu-Natal (AREC/024/017D) and was valid throughout the duration of the protocol. A permit to handle scheduled drugs for research purposes was obtained from South African National Department of Health (POS 345/2017/2018).

Sample collection

Blood samples (2mL) were collected following decapitation. Blood samples were stored in standard EDTA blood plasma collection tubes in ice and tested within 8 h of collection. Each blood tube was removed from the ice bath and warmed to room temperature prior to analysis. The blood was then analysed using a Coulter AcT Diff Haematology analyser (Beckman Coulter Inc., California, United States). The machine tested several parameters; however, data was extracted on white blood cell count (WBC), haemoglobin (HGB), platelet count (PLT), mean platelet volume (MPV), neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO) and basophils (BA) for the purposes of this study.

Statistical analysis

The data was analysed using GraphPad Prism 5 software and a p-value of less than 0.05 was considered significant. Student t-tests (Mann-Whitney) were used to test for significance between the

administration groups and their respective control or withdrawal groups. Results were depicted in tables with the standard error of mean (SEM).

Results

Table 1. Summary of blood parameters for the control groups, papaverine, noscapine and their respective withdrawal groups.

	Mean (SEM) per group					
	Control	**Control (W)**	**Papaverine**	**Papaverine (W)**	**Noscapine**	**Noscapine (W)**
	(n=7)	**(n=7)**	**(n=7)**	**(n=7)**	**(n=7)**	**(n=7)**
WBC	12.07 (2.06)	6.14 (1.61)	3.36 (0.47) ***	4.86 (1.27)	2.43 (0.27) *** #	6.13 (0.83)
(10^3/uL)						
HGB	12.69 (0.72)	11.09 (0.88)	13.37 (0.67)	10.91 (2.24)	2.76 (0.5) **##	14.59 (0.28)
(g/dL)						
PLT	733.7 (308.6)	569.6 (97.77)	279.3 (35.01)	418.9 (76.27)	21.0 (10.37) *##	791.7 (177.7)
(10^3/uL)						
MPV	8.80 (1.47)	8.20 (1.11)	5.24 (0.21) *	6.50 (1.20)	6.52 (0.54)	5.70 (0.12)
(fL)						
NE	12.10 (0.45) #	14.50 (0.83)	16.40 (1.69) *	16.65 (1.21)	16.67 (0.90) **	16.33 (2.17)
(%)						
LY	61.80 (3.15)	65.59 (3.71)	57.46 (4.73)	61.60 (4.0)	77.20 (0.80) ***	77.70 (2.34)
(%)						
MO	2.26 (0.34)	2.47 (0.26)	1.91 (0.33)	2.25 (0.25)	3.94 (0.38) *#	2.64 (0.38)
(%)						
EO	12.57 (2.00)	9.21 (2.09)	12.04 (3.01)	9.65 (2.65)	0.99 (0.17) ***	1.33 (0.30)
(%)						
BA	11.27 (1.24)	8.23 (1.69)	12.19 (1.79)	9.85 (2.03)	1.20 (0.14) ***	2.00 (0.57)
(%)						

**: p<0.05, **: p<0.01, ***: p<0.001, vs. its respective control group, #: p<0.05, ##: p<0.001 vs. its respective withdrawal group*

Table 2. Summary of blood parameters for the control groups, the "Sugars" administration groups, and their respective withdrawal groups.

	Mean (SEM) per group							
	Control	**Control (W)**	**"Sugars" (A)**	**SWA**	**"Sugars" (B)**	**SWB**	**"Sugars" (C)**	**SWC**
	(n=7)	**(n=7)**	**(n=5)**	**(n=4)**	**(n=5)**	**(n=4)**	**(n=5)**	**(n=4)**
WBC	12.07 (2.06)	6.14 (1.61)	3.36 (0.81)	6.60 (1.27)	5.26 (1.13)	3.10 (0.52)	5.78 (1.04)	3.25 (0.38)
(10^3/uL)								
HGB	12.69 (0.72)	11.09 (0.88)	4.66 (3.89)	14.55 (0.69)	7.74 (4.75)	13.75 (1.23)	16.70 (4.54)	16.25 (1.68)
(g/dL)								
PLT	733.7 (308.6)	569.6 (97.77)	580.0 (189.0)	805.5 (67.66)	1021 (42.03)	887.0 (13.93)	826.0 (220.3)	909.3 (44.71)
(10^3/uL)								
MPV	8.80 (1.47)	8.20 (1.11)	4.40 (1.10)	5.58 (0.12)	5.50 (0.04)	5.65 (0.09)	5.66 (0.22)	5.65 (0.12)
(fL)								
NE	12.10 (0.45)	14.50 (0.83)	20.24 (1.32)	17.55 (5.90)	17.36 (1.09) #	25.98 (2.72)	19.58 (1.77)	23.85 (3.12)
(%)								
LY	61.80 (3.15)	65.59 (3.71)	73.16 (1.29)	63.93 (5.84)	77.12 (1.45) #	66.80 (2.50)	73.02 (1.02)	67.40 (3.68)
(%)								
MO	2.26 (0.34)	2.47 (0.26)	4.24 (0.56)	3.75 (1.41)	3.76 (0.44)	5.15 (1.04)	5.10 (0.98)	6.98 (2.13)
(%)								
EO	12.57 (2.00)	9.21 (2.09)	1.02 (0.14)	1.15 (0.19)	0.78 (0.10)	1.03 (0.31)	1.04 (0.20)	1.05 (0.29)
(%)								
BA	11.27 (1.24)	8.23 (1.69)	1.34 (0.19) #	0.70 (0.19)	0.98 (0.15) **	1.05 (0.06)	1.26 (0.29)	0.73 (0.11)
(%)								

**: p<0.05, **: p<0.01, vs. its respective control group*

There were only two instances where significant differences were observed between the control administration and control withdrawal groups for WBC (p=0.0262) and NE (0.0348). The most significant results were observed in the WBC data. The control group had a significantly higher WBC compared to papaverine and noscapine administration groups. It was also noted that the noscapine

withdrawal group was significantly higher than the noscapine administration group which indicates that withdrawal from noscapine resulted in an increased WBC. Although "Sugars" administration groups (B) and (C) had slightly higher WBC than their withdrawal groups, they were still lower than the control group. The noscapine administration groups showed significantly lower HGB level than the control and its respective withdrawal group. The papaverine administration group had a higher HGB level than its corresponding withdrawal group and was above the control level although not significantly. The noscapine administration group was significantly lower than the control group and its corresponding withdrawal group for PLT however the control group had a significantly higher MPV than the papaverine administration group. The "Sugars" administration group (B) had a higher PLT level than the control and their withdrawal group whereas noscapine and "Sugars" administration (C) groups had lower MPV than the control group but higher levels than their respective withdrawal groups.

The higher numbers of NE in the papaverine and noscapine administration group, compared to the control group, were statistically significant. It was also observed that "Sugars" administration group (B) had a significantly lower NE level than its corresponding withdrawal group. For MO, it was observed that the noscapine administration group had significantly higher values than the control group and its respective withdrawal group. The noscapine administration group was significantly higher than its corresponding control group for LY and it was also shown that all "Sugars" administration groups showed higher LY levels than both the control group and their respective withdrawal groups. The control group was significantly higher than noscapine administration group for EO and papaverine was shown to have a higher EO value than the control and its withdrawal group, but this was not statistically significant. The "Sugars" administration group (A) was significantly lower than its corresponding withdrawal group for BA however it was observed that the papaverine had a higher BA level than the control and its respective withdrawal group, whereas noscapine has a significantly lower BA level than the control group.

Discussion

Papaverine and noscapine administration groups had a significantly higher WBC when compared to the control. This differed to findings by Haghpanah and colleagues which stated that their opioid dependent group had a significant increase in WBC[7]. It is possible that the combined opiates used in the study by Haghpanah and colleagues, may have exerted their effects synergistically therefore resulting in a different result compared to the pure administration of either papaverine or noscapine. This can also be observed by the diverse effects obtained following administration of the "Sugars" cocktails which had varying opiate concentrations. There was also a significant improvement in WBC during the withdrawal phase for the noscapine withdrawal group. "Sugars" administration group (A) had a similar phenomenon as noscapine which could indicate that "Sugars" administration group (A) could have a

high noscapine content which may explain the similar results. The other "Sugars" administration groups had the opposite effects on WBC, possibly indicating a lower noscapine content. A recent study by Zhu and colleagues showed that white blood cells have the potential to produce small amounts of morphine which appear to be physiologically significant[8]. This morphine can either be produced by the CYP2D6 enzyme in white blood cells, which can produce morphine from tyramine, norlaudanosoline and codeine as well as a second pathway involving L-dopa[8]. It can therefore be deduced that white blood cells and morphine have an interlinked relationship and may influence each other's concentration in the body. We speculate that papaverine and noscapine which are opioids much like morphine may affect white blood cell balance as well. It is suggested that papaverine and noscapine may have exerted a similar physiological effect to morphine which reduced the demand for *in vivo* morphine synthesis and a possible negative feedback loop resulted in a reduction in the white blood cell concentration. This would explain the resulting significant improvement in white blood cell concentration following withdrawal in the noscapine group. There was a significant difference between the control and control withdrawal groups for WBC and NE. This was not anticipated as neither of these groups were exposed to the drug cocktails or its ingredients. It has been shown however, that WBC can decline with age[9] however our findings for NE levels does not concur with available literature that suggests a decline with age[10]. Since no significant difference was observed between these two groups in any other factors, it is suggested that there are no vital age-related effects on blood marker indices in this experiment.

The noscapine administration groups showed a significantly lower HGB level than the control and its respective withdrawal group. Papaverine and "Sugars" administration group (C) showed a higher HGB level than the control and their respective withdrawal groups. This suggests that "Sugars" administration group (C) may have a high papaverine content. It has been shown that papaverine decreases the oxygen affinity of haemoglobin[11]. This suggests that the increased HGB levels in the papaverine administration group could be a compensatory mechanism to provide the body with its required oxygen demand due to decreased oxygen affinity of cells. The decreased HGB levels following withdrawal could be attributed to the improvement in oxygen affinity of haemoglobin in the absence of papaverine administration indicating that the changes are reversible. This argument is further strengthened by a similar study that also showed increased HGB levels in their opium and heroin withdrawal groups which suggests that HGB levels increased to compensate for low levels during drug administration[7]. There was no relevant literature on the effects of noscapine on haemoglobin levels.

The noscapine administration group was significantly lower than the control group and its corresponding withdrawal group for PLT. It has been shown that a chemical analogue of noscapine causes a decline in the expression of cancer development regulators including a factor derived from platelets[12] which may possibly account for the lower levels of platelets in the noscapine administration group however further research is needed to add strength to this theory. The control group had a significantly higher MPV than the papaverine administration group and it was observed that the

papaverine administration group also had a lower PLT level than the control group. It has been shown previously that papaverine inhibits platelet aggregation[13] and is a potent vasodilator[11]. Based on these basic functions of papaverine, a possible relationship between platelet aggregation, vasodilation and MPV levels can be inferred such that increased diameter of a blood vessel increases the volume of blood in a given section of a blood vessel however the inhibition of platelet aggregation would result in fewer platelets being present in a given section of a blood vessel which implies that the MPV per a given area of blood vessel would thus be reduced. This theory can be supported by a study by Korniluk and colleagues which states that an elevated MPV correlates with increased platelet aggregation[14]. Furthermore, Wang and colleagues' stated that increased MPV is an index of arterial stiffness which indicates that due to the lack of pliability of the vessel leading to a reduced volume, there is a greater number of platelets at a given section of a vessel resulting in a higher MPV[15].

The noscapine administration group showed significantly higher NE and MO levels than the control group. The papaverine administration group also had significantly higher NE levels than the control group. This can be likened to another study in which the opium dependent group showed significantly higher neutrophil and monocyte levels[7]. The noscapine administration group also showed a significantly higher LY level than the control group and all "Sugars" administration groups had higher LY levels than both control and their respective withdrawal groups although not statistically significant except in the case of "Sugars" administration group (B) which was significantly higher than its corresponding withdrawal group. Noscapine is an anti-tussive agent that is used commonly in cough mixtures and studies have shown that *in vitro* exposure to noscapine resulted in polyploidy and aneuploidy of lymphocytes[16, 17]. The above studies were both conducted using histological analysis or non-automated assays which may be more accurate to detect abnormalities in lymphocytes however since these were conducted *in vitro,* it does not suggest the effects on the lymphocyte concentration in the blood. It may be possible that increased lymphocyte count following noscapine administration may be due to a physiological response to boost immunity due to the increase of non-functional aneuploidal and polyploidal lymphocytes. These mutagenic changes exerted on lymphocytes may also have an impact on fertility and the viability of offspring in addicts.

The control group had a significantly higher EO level than the noscapine administration group however there was no relevant literature that could explain the effect of noscapine on eosinophil levels. Eosinophils play a role in the immune response process, inclusive of tissue repair[18]. Based on our findings, it is therefore expected that the noscapine administration group would have an impaired immune response. The noscapine administration group had a significantly lower BA level than the control group and "Sugars" administration group (B) was significantly lower than its corresponding withdrawal group however there is no relevant literature discussing the effects of noscapine on basophil concentrations.

In conclusion, it can be inferred from the results that there are definite effects on the blood cells by both noscapine and papaverine. It is therefore possible to presume that these changes in blood markers can greatly influence the general functioning of the immune system and make the body more susceptible to the effects of the drugs. The effects of the drug cocktails could be due to the main ingredient contained in cases where effects were mirrored between either papaverine or noscapine and the "Sugars" administration groups. Another explanation would be the synergistic or combined effects of the different ingredients as seen in instances where the "Sugars" administration groups which had contradictory results. We suggest that the drug cocktail, "Sugars", and its individual ingredients exert strong effects on the blood parameters that are closely linked to the immune system. This may play a role in some of the withdrawal symptoms especially those related to pain and bone pain due to the changes in WBC, the morphine balance and the potential hematopoietic damage due to fluctuations in blood cell concentrations respectively.

Another factor that may play a role in both the concentrations of blood cells is that opium addicts have been shown to be more susceptible to bone loss than non-addicted individuals[19]. In most cases in this study, the drug administration groups showed better haematological parameters than the control and withdrawal groups thus suggesting that in the absence of the drug, the lack of euphoria encourages relapse in order to potentially escape the withdrawal symptoms. This study therefore concluded that "Sugars" and its ingredients affect the blood cells involved in the blood immune response which can possibly influence the withdrawal symptoms experienced by addicts during withdrawal and may therefore be a driving force for relapse.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgements

The authors would like to acknowledge the University of KwaZulu-Natal, College of Health Sciences, and the National Research Foundation (NRF) for the awarding of PhD scholarships to the main author which assisted in funding this research.

The authors would also like to place on record their gratitude to Professor Hans Lipp (Institute of Anatomy, University of Zürich), for allowing them the use of his automated behavioural lab, the Intellicage® as well as providing his expert advice during this protocol.

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INTERFACE

Chapters 3, 4 and 5 focused on the physiological changes that occur following administration of “Sugars” and its ingredients. It was shown that withdrawal of the cocktail either ameliorated or exacerbated the effects observed during administration. The findings presented in the previous chapters can potentially be classified under the field of neuroimmunology as they extend over both the nervous and immune systems. These findings therefore contribute to this new emerging research field and the relationship of these two systems in addiction. Chapter 6 consequently creates a concise synthesis of the findings presented in this thesis taking into consideration the relationship between the nervous and immune systems in “Sugars” addiction.

CHAPTER SIX

SYNTHESIS AND CONCLUSION

6.1 Synthesis

"Sugars" is a recently popularized drug, and as such, not much research has been conducted on this cocktail previously. It has been previously chemically characterized in our laboratory to show that it contains noscapine, papaverine as well as traces of heroin, amongst other substances that add bulk[1]. This bulking up of the drug, usually with common household substances, makes it difficult to pinpoint an accurate chemical composition of the cocktail. This thesis, therefore, focused on investigating the effects of the drugs that were easily detectable in the cocktail. There are several studies on the psychosocial aspects of "Sugars" addiction[2-4]; however, there is a lack of research on its physiological effects and underlying principles of action. The effects of this cocktail are numerous and span several focus areas. The present study focuses on two main areas of physiology viz. neurophysiology and immunology. This research focused on the effects of "Sugars" administration and withdrawal on dopamine concentration, sucrose preference, cognitive function, and hippocampal mass to assess neurophysiological changes. The analysis of haematological immune factors was conducted following the administration and withdrawal of "Sugars" to investigate the immunology aspect.

6.1.1 The relationship between hippocampal mass and memory

There is a potential inverse relationship between hippocampal mass and escape latency, as discussed in Chapter 4. It was shown that the greater the mass of the hippocampus, the lesser the time taken by the mice to find the hidden escape platform in the Morris water maze test. An example of this was papaverine treated mice having significantly higher hippocampal masses than its withdrawal group; however, having a lower escape latency in the MWM test. Early studies suggest that acute administration of either papaverine or noscapine does not affect memory[5, 6] which suggests that perhaps the frequency of dosage may play a role in eliciting effects. This theory can be supported by another study that showed that the treatment of an animal model of Huntington's disease with papaverine improved their cognitive function[7]. It is well established that drugs of abuse play a role in learning and memory as a means to establish an addictive pattern[8] however, this may also be applied in this study as the reinforcing effects of papaverine and noscapine administration might potentially lead to drug-induced memory improvement. There were varied changes observed following the administration of the "Sugars" cocktail. In Group A, there was an improvement in cognitive function whereas in Groups B and C, there was a decline in cognitive function which could be attributed to either the combined effect of the drugs as well as the ratio of each ingredient. It has been shown that manipulations in the dopamine neurotransmissions in a parkinsonian animal model showed that certain dopamine receptors might affect the consolidation of memory[9]. Blocking specific dopamine receptors in the prefrontal cortex hampered the ability to learn from new stimuli[10].

6.1.2 The fluctuation of dopamine concentrations in relation to sucrose preference

Cognitive areas of the brain such as the prefrontal cortex and hippocampus are richly innervated by serotonergic and dopaminergic nerve fibres[11]. It has emerged in recent studies that dysregulations in the serotonin and dopamine balance and cellular changes that result in cognitive impairment may be closely linked as discussed above[11]. The present study provided a diverse range of parameters to compare sucrose preference. The licks are representative of the actual consumption of sucrose, whereas the visits or nose pokes indicate the sucrose seeking behaviour, as shown in Chapter 3. Although the papaverine and noscapine had a higher overall preference for sucrose, there was a lack of literature that specifically discussed the effects of these chemicals on sucrose preference. Green and colleagues suggest that opiate users have a higher preference for sucrose than non-users or those on opiate antagonists[12]. Further research indicates that opiates do in fact, change taste preferences which may explain the higher sucrose preference observed in almost all administration groups of this study[13, 14]. Another factor that may have influenced sucrose preference in this study was the enriched living conditions which may result in higher sucrose preference[15].

Although there are conflicting findings on whether dopamine concentration affects sucrose preference[13, 14, 16, 17], the findings of this study suggest that there is a potential inverse relationship between dopamine concentration and sucrose preference. These findings correlate with literature that suggests that dopamine affects salience and the reward system, which is inclusive of sucrose preference[13, 14]. Dopamine has been shown to have diverse applications and is associated with physical and mental conditions[18]. Research on an animal model of Parkinson's disease which was created by 6-OHDA lesions in the substantia nigra, showed that treatment with dopamine lowered WBC, RBC and HGB[19]. Autoimmune conditions such as rheumatoid arthritis are also thought to have a lack of dopamine and have been successfully treated with dopamine analogs[20]. Dopamine is believed to have a protective role in the immune system such as the release of large amounts of dopamine from T-cells that inhibits their suppressive activity[20].

6.1.3 Changes in heamatological factors relating to withdrawal symptoms

Noscapine and papaverine elicited changes in several haematological factors such as WBC's, which influence the immune systems, as shown in Chapter 5. Noscapine had a lower number of WBC's than both the control and withdrawal groups. WBC has also been shown to produce small amounts of morphine[21]. A possible reason for the reduced WBC is that noscapine and papaverine act as opioids thereby reducing the need for the synthesis of morphine to maintain normal homeostatic balance. This reduced need for morphine may have reduced the amount of circulating WBC. Papaverine is a well-established vasodilator[22] and inhibits platelet aggregation[23]. The lower MPV levels can validate these changes in the papaverine administration group. These results indicate that the chronic administration of "Sugars" and its ingredients affected the blood immune cells negatively which can explain the flu-

like symptoms experienced by addicts every four hours. It was shown that the changes in some factors could be reversed; however, this was after a prolonged withdrawal period which may not always be possible in recovering addicts. It is thus necessary to incorporate immune boosting methods into rehabilitation programs to quicken the process.

6.2 Conclusion

The changes induced differed between the three "Sugars" cocktails, which collaborates with reports that the composition of "Sugars" is highly variable[1]. The degree in which these changes occur may be influenced by the ratio of papaverine and noscapine present in the cocktail. The effects observed for the cocktail which differed to the effects observed for the individual administration of papaverine and noscapine could be due to the combined effect of these compounds. The results obtained showed some relationship between each other such as groups with low dopamine content had a higher sucrose preference, higher hippocampal weight, and improved memory. The results for blood immune factors were varied but the common pattern was that the effects observed may be the reason why withdrawal symptoms present as flu-like symptoms. The overall conclusion of the thesis is that there are definite changes exerted by each compound as outlined in each manuscript and that a combination of the ingredients may present different results based on the ratio of ingredients. Further biochemical analysis is required to add depth to these novel and initial findings on cognitive function, blood immunology, dopamine, and anhedonia.

6.3 Recommendations

Although promising research findings emerged from this thesis, the molecular aspects of certain areas of the experimental design did not allow further investigation into the finer details of the observations made in this study. Further research should focus on testing the content of apoptotic proteins such as *Fas*, *FasL* and *Bad* which may be an underlying contributing factor to hippocampal shrinkage or growth in cognitive patterns. Other research needs to be conducted on circulating dopamine to determine a more accurate relationship between anhedonia and dopamine. The research on the blood immunology factors could also be diversified by investigating other blood cell concentrations as well as blood regulatory proteins and chemicals that maintain blood immune homeostasis. The phenomenon of morphine synthesis by WBC is novel and this can also be further probed by assessing the relationship between pain, pain receptors and WBC concentration which may explain the severe pain related withdrawal symptoms in greater detail. A limitation of this study which can be improved upon in in further research was the inability to adequately quantify the amount of heroin, noscapine and papaverine in the "Sugars" cocktails. The accurate quantification of the drug cocktail in future research could result in more pronounced and reproducible findings in future research.

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APPENDIX A

health
Department:
Health
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH
Private Bag X828
PRETORIA, 0001
Republic of South Africa

UMNYANGO WEZEMPILO
LEFAPHA LA MAPHELO

PERMIT IN TERMS OF SECTION 22A(9)(a)(i) OF THE MEDICINES AND RELATED SUBSTANCES ACT, 1965 (ACT 101 OF 1965) TO, POSSESS AND CONDUCT RESEARCH ON PLANT WHICH IS INCLUDED WITHIN SCHEDULE 6 TO THIS ACT.

Date of Issue: 06 October 2017	Expiry Date: 05 October 2018	Permit No: POS 345/2017/2018

Authority is hereby granted in terms of Section 22A(9)(a)(i) of the above-mentioned Act to **Ms Y Y Chetty** of **34 Twinkle Terrace, Arena Park, Chatsworth, 4092** to possess and conduct research on plant which is included within schedule 6 to this act. in respect of which the quoted quantity should not be exceeded during the period **06 October 2017** to **05 October 2018.**

Name of Scheduled Substance(s)	Schedule	Total quantity of substance(s) and/or preparation(s) allocated per calendar year
Heroin	Schedule-7	2 g [two grams]
Morphine	Schedule-7	300 g [three hundred milligrams]

Total Items: **02**

The acquisition, possess, use or supply of the relevant substances are subject to the following conditions:

1. The substances shall be used for **Scientific Research Purposes** only.
2. The control over the substances shall be the responsibility of:

   Full Name & Surname: **Ms Y Y Chetty**

   ID Number: **920131 0142 083**
3. Complete details of the substances acquired and used shall be recorded in registers designed specifically for this purpose in accordance with the provisions of the relevant regulations to the Medicines and Related Substance Act, 1965.
4. Orders for the substances shall be signed for by:

   Full Name & Surname: **Ms Y Y Chetty**

   ID Number: **920131 0142 083**
5. When the substances are acquired, the name and address of the supplier, the date supplied, the quantity supplied and the number of the relevant invoice shall be recorded **on this permit**.
6. The register referred to in paragraph 3, as well as copies of orders and invoices pertaining to the supply of the substances, shall be available at the offices of the 34 **Twinkle Terrace, Arena Park, Chatsworth, 4092** for a period of at least three years and shall be subject to inspection by Inspectors appointed in terms of the Medicines and Related Substances Act, 1965.
7. This permit expires on **05 October 2018** and shall on expiry be returned to the Department of Health for cancellation and shall be accompanied by a statement reflecting the quantity of substances on stock at expiry.

DIRECTOR-GENERAL:
DEPARTMENT OF HEALTH

DATE: 06/10/2017

APPENDIX B

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

08 September 2017

Ms Yvette Yolanda Chetty (210503478)
School of Laboratory Medicine and Medical Sciences
Westville Campus

Dear Ms Chetty,

Protocol reference number: AREC/024/017D
Project title: An investigation into the neurochemical and behavioural patterns of mouse strains exposed to "Sugars" and its constituents

Full Approval – Research Application

With regards to your revised application received on 30 June and 03 August 2017. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Please note: Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person. Please forward SAVC authorization once received.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 08 September 2018.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

.....
Professor S Islam, PhD
Chair: Animal Research Ethics Committee

/ms

Cc Supervisor: Dr Anand Nadar
Cc Academic Leader Research: Dr Michelle Gordon
Cc NSPCA
Cc Registrar: Mr Simon Mokoena
Cc BRU – Dr L Bester

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