SUB.ACUTE EFFECTS OF ORAL CONSUMPTION OF SODIUM NITRATE SALT ON HISTOLOGY OF LIVER AND KIDNEY OF ALBINOWISTER RATS

BY

OSARETIN INFLUENCE ABILITY

BMS1601855

DEPARTMENT OF MEDICAL LABORATORY SCIENCE
SCHOOL OF BASIC MEDICAL SCIENCE
UNIVERSITY OF BENIN
BENIN CITY

SUPERVISED BY
DR. B.E. OGEYEMHE

NOVEMBER, 2022

CERTIFICATION

This is to certify that this project work was carried out by OSARETIN INFLUENCE ABILITY with matriculation number BMS1601855 in the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) degree.

_____ _____

DR. B.E. OGEYEMHE DATE
Supervisor

_____ _____

PROF. (MRS) E.O. OSIME DATE
Head of Department

_____ _____

External Examiner DATE

DEDICATION

I dedicate this project to the Almighty God my creator, my source of inspiration, wisdom, knowledge and understanding. To my loving family, may God continue to bless you all

ACKNOWLEDGEMENT

Firstly, my thanks goes to God Almighty the creator; for the success of this project work, for blessing me with life, strength and the understanding throughout the course of this work. I also want to appreciate my supervisor, DR. B.E. OGEYEMHE, for his assistance and support towards me in ensuring the progress, completion and success of this project work.

To the head of department; PROF. (MRS) E. O OSIME, MLS lecturers and staffs, I really appreciate you all. I also appreciate my friends, my classmates and roommates for their encouragements and contributions. I say thank you and God bless you all.

My gratitude goes to my Parents Mr and Mrs. Osaretin, and my Aunt Mrs. Mitchell Carol for their support financially, spiritually, emotionally and all round as well as my siblings for always being there for me. I pray that you all continue to grow strong in health, wealth, strength, knowledge, wisdom and life. God bless you all.

ABSTRACT

Sodium nitrate is used as a preservative in processed meats and poultry products across the country. Though, production of nitric oxide and nitrite may prevent various types of cardiovascular disease including hypertension, atherosclerosis, and stroke. However,health concerns relating to cancer andleukaemia have not been ruled out. Therefore, this project was to examine histological changes inliver and kidney of white rats treated withsodium nitrate salt. Other objectives were to determine its effects on liver enzymes, and electrolytes and urea.Twenty (20) in-bred white rats of both sexes, aged: 2-4 months and weighed 160-200g were randomly picked to form five (5) groups (n=4) labeled A to D as test while E served as control. Rats were housed in plastic cages with saw dust as beddings and acclimatize for 2 weeksat temperature (25±5ºC), humidity (54-59%) and periodicity (12:12hrs) in a clean environment while Standard top feed® and water were provided regularly. Five gram (5g) of nitrate salt was measured with a weighing balance into a sterile conical flask while 1L of distilled water was added with a standard measuring cylinder and agitated vigorously with the GFL shaker for 5minutes until all dissolved. Rats in groups A to D were treated orally for 30days at 2days interval in this order: 5mg/kg, 10mg/kg, 15mg/kg and 20mg/kg body weight. Each rat was picked with a hand towel and administeredadequate dosages using the oro-gastric tube. After experimentation, animals were sacrificed by cervical dislocation while 4mL of blood was collected from the cardiac region for liver and kidney function test. The corresponding parameters were assayed with a spectrophotometer at varying wavelengths. The liver and kidney were excised, grossedand processed histologically.Sections were cut at 3-5um and stained according to H&E method. Sections were examined using Swift(R) binocular microscope with an in built light system and photographed with an Olympus photomicroscope. Data were presented as Means ± SD and analyzed with one way ANOVA and Duncan post hoc test whiletest of significance was set at $p < 0.05$ withSPSS version 16. All animals showed signs of partial anorexia, dullness and developed reduced activities but were pronounced in high dose treated rats (C and D) for the 30 days treatment. High dose treated ratslost more weight than those on lesser treatment regime while the control gained more weight. All parameters (K, Na, CL, HC03, urea and creatinine) for renal profile were within normal range. They were significantly expressed ($P \leq 0.003$) across all groupsincluding liver function test (TB, CB, TP, ALB, AST, ALT and ALP)indices ($P \leq 0.05$). No variation in colouration by gross examination while and histopathology findings were in keeping with normal histology of the liver and kidney. From the results so far,sodium nitrate has no deleterious effects on histology of the visceral organs studied but daily intake abuse of the salt particularly in a large concentration may be injurious to human health.

TABLE OF CONTENT

PAGES

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Sodium nitrate is used as a preservative in processed meats, helping to delay spoilage and preserve colour. At the turn of the 20th century, it was discovered that a particular type of salt containing high levels of nitrate preserved meat's pink colour (Romans *et al*., 2001). Somewhat later it was discovered that nitrate was changed to nitrite by bacteria during processing, and that it was in fact nitrite that was responsible for the pink colour, so the meat industry began using sodium nitrite directly. However, whether added to meat as nitrate or nitrite, the end product is the same (Romans *et al*., 2001).

Meat and poultry curing is one of the oldest forms of food preservation still in use today. Before the advent of refrigeration, fish and meat were preserved by methods found effective to control spoilage after animal harvest and to extend food supplies during times of scarcity. Although lost in antiquity, the curing process for meats is believed to have derived from preservation methods with salt as early as 3,000 B.C. (Romans *et al*., 2001). Over time, the realization that salt contaminated with saltpetre (potassium nitrate) was responsible for curing, would unknowingly provide the basis for the beginnings of unravelling the mystery of curing. With the development of refrigeration and food packaging technologies, the original purpose of curing highly perishable foods for preservation purposes has been widely replaced with creating convenience and variety for consumers (Pegg, 2004). The meat and poultry industry has greatly benefited from the use of sodium nitrite by allowing for the production of

products with improved food safety and an extended shelf-life with excellent storage stability (Pegg and Shahidi, 2000). Many of today's processed meat and meat products that are most enjoyed by consumers contain sodium nitrite. Sodium nitrite allows for the existence of meat and poultry products with unique colours, textures, and flavours which cannot be recreated by any other ingredient (Sebranek, 1979).

1.2 Statement of the Problem

The use of sodium nitrite for curing has not been without controversy. Due to a strong debate in the 1970s surrounding certain nitroso compounds with potential for carcinogenic nitrosamines, the use of nitrite for curing was nearly banned (Cassens, 1990, 1997a). As a result, several steps were taken by both industry and government to significantly reduce the risk of nitrosamine formation and alleviate potential human health concerns. Since then, health concerns involving risks relating to cancer and leukaemia, believed to be directly related to the consumption of nitrite cured meat and poultry products, have periodically resurfaced. Each of these occurrences has been addressed scientifically reassuring the public of the safety of nitrite usage in cured meats. Research conducted since the mid-1980s has suggested that nitrite is a significant molecule important for human health. However, new scientific discoveries are now providing a better understanding of the profound and important roles nitrite play in normal body functions. Dietary nitrates from vegetable consumption, for example, have been shown to serve as significant sources for the endogenous production of nitrite and nitric oxide in the body systems (Hunault *et al*., 2009).

1.3 Justification

Recent research has clearly shown that nitric oxide can be produced directly from nitrite and is involved in controlling blood flow in cardiac muscle and potentially other tissues (Bryan *et al*., 2007; Bryan and Hord, 2010). Normal production of nitric oxide and nitrite may prevent various types of cardiovascular disease including hypertension, atherosclerosis, and stroke (Bryan *et al*., 2007; Hunault *et al*., 2009). Despite that the aforementioned may serve as the basis for the consumption of nitrite. However, the effect on viscera organs (liver and kidney) has not been fully investigated. Recall that liver is the primary site for drug metabolism while the kidney serves a supporting role, and majorly for excretion of waste from the system. Therefore, there are no enough data particularly on these vital organs exposed to nitrate salts solution.

1.4 Significance of the Study

Meat and meat product's consumers stand to benefit from the outcomes of the present research, including producers and policy makers will be in the know of the actions of nitrate salt solution on the liver and kidney of animal model which may relate similarly to humans under same circumstances.

1.5 AIM AND OBJECTIVES

The present study was to examine histological changes in select visceral organs of white rats exposed to sodium nitrate salt solution considered as preservative for meat widely consumed by humans. Other objectives were to determine liver and kidney function via liver enzymes, and electrolytes and urea in rat's serum.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sodium nitrate

This is a chemical compound with the formula $NaNO_3$. This salt is also known as Chile saltpetre or Peru saltpetre (due to the large deposits found in each country) to distinguish it from ordinary saltpetre, potassium nitrate (Romans *et al*., 2001). Sodium nitrate is a white solid which is very soluble in water. It is a readily available source of the nitrate anion (NO_3^-), which is useful in several reactions carried out on industrial scales for the production of fertilizers, pyrotechnics and smoke bombs, glass and pottery enamels, food preservatives, and solid rocket propellant. It has been mined extensively for these purposes.

2.2 Applications

Sodium nitrate was used extensively as a fertilizer and a raw material for the manufacture of gunpowder in the late 19th century. It can be combined with iron hydroxide to make a synthetic resin (Hunault *et al.,* 2009). Sodium nitrate can be used in the production of nitric acid by combining it with sulphuric acid and subsequent separation through fractional distillation of the nitric acid, leaving behind a residue of sodium bisulphate. Hobbyist gold refiners use sodium nitrate to make a hybrid aqua regia that dissolves gold and other metals (Stevens, 2009). Sodium nitrate is also a food additive used as a preservative and colour fixative in cured meats and poultry; it is listed under its INS number 251 or E number E251. Therefore, sodium nitrate should not be confused with sodium nitrite, which is also a common food additive and preservative used for example, in daily meats (Huffman and Bryan, 2010).

Nonetheless, common applications include as an oxidizer in fireworks replacing potassium nitrate commonly found in black powder and as a component in instant cold packs. Sodium nitrate is used together with potassium nitrate and calcium nitrate for heat storage and, more recently, for heat transfer in solar power plants. A mixture of sodium nitrate, calcium nitrate and potassium nitrate is used as energy storage material in prototype plants, like the Andasol Solar Power Station and Archimede project (Milkowski *et al.,* 2010). It is also used in the wastewater industry for facultative microorganism respiration. Nitrosomonas, a genus of microorganisms, consumes nitrate in preference to oxygen, enabling it to grow more rapidly in the wastewater to be treated (Milkowski *et al.,* 2010). Sodium Nitrate is also sometimes used by marine aquarists who utilize carbon dosing techniques. It is used to increase nitrate levels in the water and promote bacterial growth (Hunault *et al.,* 2009).

2.3 Health concerns

Like sodium nitrite, sodium nitrate used in foods forms small amounts of nitrosamines (N-nitroso compounds or NOCs), some of which are human carcinogens known to cause DNA damage and increased cellular degeneration (De la Monte, 2009). Studies have shown a link between increased levels of nitrates and increased deaths from certain diseases including Alzheimer's, diabetes mellitus and Parkinson's, possibly through the damaging effect of nitrosamines on DNA, however, little is done to control for other possible causes in the epistemological results. Nitrosamines, formed in cured meats containing sodium nitrate and nitrite, have been linked to gastric cancer and oesophageal cancer (Hunault *et al.,* 2009). Sodium nitrate and nitrite are

associated with a higher risk of colorectal cancer. World Cancer Research Fund UK, states that one of the reasons that processed meat increases the risk of colon cancer is its content of nitrate. A small amount of the nitrate added to meat as a preservative breaks down into nitrite, in addition to any nitrite that may also be added. The nitrite then reacts with protein-rich foods (such as meat) to produce NOCs. NOCs can be formed either when meat is cured or in the body as meat is digested (De la Monte, 2009).

In addition to heart disease, Hord *et al*., (2009) reported that sodium nitrate has been linked to other menacing health conditions. A 2005 study of 200,000 men and women conducted over seven years at the University of Hawaii examined the relationship between processed meat consumption and cancer. The study revealed that people who consumed processed meat were 67% more likely to get pancreatic cancer than those who ate little or no meat products at all. While, the research did not pinpoint sodium nitrate as the culprit, it was noted that the preservative is converted in the body to nitrosamines, which are toxic to humans and promote the growth of cancer cells (Hord *et al*., 2009). Since the potential carcinogenic danger of sodium nitrate was brought to light in the 1970s, a battle has been waged between lawmakers and the meat processing industry over how much, if any, sodium nitrate should be allowed. Butler and Feelish, (2008); noted that measures have been taken to diminish the impact of sodium nitrate on human health by reducing allowable amounts and by buffering the chemical with ascorbic acid, or vitamin C, which appears to inhibit the formation of nitrosamines. Nitrate and nitrite are considered hazardous, and there are legal limits to their concentration in food and drinking water.

Nitrate from fertilizer accumulates in vegetables and fruit, and large-scale livestock production yields huge amounts of manure rich in nitrate that seeps into groundwater. Therefore, keeping nitrate concentrations below legal limits is a struggle for farmers. In this issue, Hord *et al*., (2009) challenges these limits. Other authors have already pointed out that the evidence for adverse effects of nitrate is inconsistent and that nitrate may actually be beneficial (Butler and Feelish, 2008). Furthermore, Hord *et al*., (2009), claim that nitrate and nitrite should be considered as nutrients and stated that food components are beneficial at low and harmful at high intakes. Meanwhile, World Health Organization first set an upper limit for nitrate in food in 1962 (EFSA, 2008). It was based on a brief report from the US Food and Drug Administration which calculated daily intakes of ≤500 mg of sodium nitrate/kg body weight from an experiment concluded that the prescribed daily intakes were harmless to rats and dogs. This figure was divided by 100 to yield an Acceptable Daily Intake for humans which resulted to 5 mg sodium nitrate or 3.7 mg nitrate per kg body weight, which equals 222 mg for a 60-kg adult and has stood ever since (EFSA, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Reagents and chemicals

Sodium nitrate salt, Scott's water, Distilled water, Haematoxylin dye, Eosin stain, 1% acid-alcohol, Xylene, Ethanol, Distrene Plasticizer Xylene (DPX), 10% neutral buffered formalin and Normal saline.

3.1.2 Equipment and Apparatus

Analytical Weighing Balance, Stainless Steel Cages, Dissecting Board, Dissecting Set, Cotton Wool. Measuring Cylinder, Conical Flask, Cover slip, Slides, Universal Containers, 10ml syringes. Tissue Processor (Hestion - ATP7000 tissue processor-Germany), Embedding Machine (Hesition- E500 Germany), Leuckhart molds, hertz rotary microtome (Cambridge mode), Water Bath (Gallenkamp), Hot plate, household fridge (Chess, France), Staining rack, Forceps and Swift$^{(R)}$ binocular microscope (Olympus England), GFL shaker (No 3017 MBH, Germany).

3.1.3 Ethics Statement and Animal care

The animal studies were carried out in compliance with policies outlined in the 'Guide for the Care and Use of Laboratory Animals', published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). Ethical approval was obtained from the Ministry of Agriculture and Natural Resources (V.1034/37). Animals were obtained from the animal house of the Department of Anatomy and were housed in plastic cages with saw dust as beddings. They

acclimatize for 2 weeks, under standard condition of temperature (25±5ºC) and a light/dark periodicity of 12:12 hrs. The environment was cleaned regularly while sufficient food (Standard top feed®) and water were provided.

3.2 METHODOLOGY

3.2.1 Animal Grouping

Twenty (20) in-bred white rats of both sexes, aged 2-4 months and weighed between 160-200g were randomly selected into five (5) groups (n=4). Test groups were labelled A to D while group E was untreated and served as control.

3.2.2 Preparation of salt solution

The salt solution was prepared by measuring 5g using a weighing balance into a sterile conical flask. 1L of distilled water was added using a standard measuring cylinder. It was transferred to a GFL shaker and shaken for 5minutes.

3.2.3 Weight and Physical Measurements

The method described by Ajiboso *et al*., (2007) was used to determine body weight of experimental rats. All animals were weighed before and after administration. Individual rat was monitored for daily gain in body weight using digital electronic balance (Gilbertini, Italy). Gain in weight was obtained from the relationship given below: Daily gain in weight= Final day Weight – Initial day Weight, while the mean weight was recorded. Behavioral signs of acute toxicity in experimental rats were observed and recorded. These include: diarrhea, watery stool, hair loss, stretching, reduced activities, dullness, restlessness, paw licking and salivation.

3.2.4 Pre- Experimentation

Prior to the experiment, acute toxicity (LD_{50}) of sodium nitrate salt was conducted using modified Lorke's method (1983) which will serve as a guide extrapolating the required dosage to adopt in treatment plans.

3.2.5 Experimentation

Rats in groups A to D consumed sodium nitrate salt solution orally for 30days at an interval of 2days in the order of 5mg/kg, 10mg/kg, 15mg/kg and 20mg/kg body weight respectively while group E was untreated. Each rat was picked one at a time with a hand towel and appropriate volume of salt solution in (mL) was administered using a sterile 10ml syringe.

3.2.6 Post Experimentation

At the end of the experiment, all rats fasted till the next day and were sacrificed by cervical dislocation. Four (4) mL of blood was collected for liver and kidney function test. While the viscera organs (liver and kidney) were harvested for histology purposes.

3.2.6.1 Determination of Liver Enzymes

Actions of hepatic enzymes: Serum Aspartate and Alanine Transferase were assessed using the method described by Reitman and Frankel (1957) in which 0.2 ml of serum was measured with a micropipette and added to 1 ml of phosphate buffer, which contained a substrate. The mixture was incubated for 30 min peculiar to alanine aminotransferase (ALT) and 60 min for aspartate aminotransferase (AST). This was conducted at 37°C, before dispensing 1mL of dinitrophenylhydrazine into the mix and again incubated for another 20minute

at room temperature. Thereafter, 10 mL of 0.4% sodium hydroxide was dispensed and was mixed for 5minutes and read at 550nm against sample blank with a spectrophotometer. AST and ALT values were calculated using a sequence of standardized curves.

3.2.6.2 Determination of Electrolyte and Urea

Serum electrolyte (sodium, potassium, bicarbonate and chloride) levels were measured with the chemistry analyzer (Erba Chem 5X Analyzer). Serum creatinine was analysed using commercially available kits (HUMAN Diagnostics, Germany) and read with the spectrophotometer (Buck Scientific 210 VGP, China) at 490 nm wavelength whereas; urea was estimated using the urease-berthelot reaction, measured at 570 nm wavelength.

3.2.6.3 Histopathology

For each of the organs studied, the tissues were fixed for 36 hours and were cut at 3-5mm. They were processed in an automatic tissue processor for dehydration, clearing, and impregnation using molten paraffin wax, while embedding was done with the aid of the embedding machine. Sections of the tissues were obtained at 3-5microns using the hertz rotary microtome (Cambridge mode) to produce serial ribbons. Staining of the sections was according to haematoxylin and eosin staining technique.

3.2.6.3.1 Haematoxylin and Eosin Protocol

- Dewax and hydrate section
- Stain section in Cole's Haematoxylin for 10mins
- Wash section thoroughly in running tap water.

- Differentiate section in 1% acid alcohol briefly
- Blue in Scot's water for 5minutes
- Counter stain section in eosin for 3minutes
- Wash section in running tap water until excess eosin has been removed.
- Dehydrate sections, Clear in xylene and mount with DPX

3.2.7 Microscopy and Photomicrography

The sections were examined using Swift(R) binocular microscope with an in built light system and white films with an Olympus photomicroscope (Opticshot- 2; Nikon, Tokyo, Japan) at x 10 and 40magnification.

3.2.8 Statistical Analysis

Data were presented as Means ± SD and analyzed using one way ANOVA and Duncan post hoc test and significance was determined at $p < 0.05$ using Statistical package for social sciences (SPSS) version 16.0 (Inc Chicago, Illinois, US.

3.3 Location and Duration of Study

This study was conducted in the Department of Medical Laboratory Science, Histopathology sub-departmental Laboratory. Animal acclimatization, test administration, animal sacrifice, Grossing, fixation, processing, microtomy, staining, microscopic examination of histology slides and photomicrography were between November 2021 and February, 2022.

CHAPTER FOUR

RESULTS

Physically, all animals that took sodium nitrate salt solution showed signs of partial anorexia, dullness and developed reduced activities which were more pronounced in high dose treated animals in groups (C and D) in the 30 days duration. Animals that consumed more of the treatment solution lost more weight than those on lesser treatment regime (Table 4.1). Renal function test revealed that all parameters (K, Na, CL, HC03, urea and creatinine) for renal profile were within normal range. They were significantly expressed ($P \leq 0.003$) across all groups (Table 4.1) including liver function test (TB, CB, TP, ALB, AST, ALT and ALP) indices ($P \leq 0.05$) using one-way ANOVA (Table 4.2). Grossly, all organs both test and control showed no variation in colouration and histopathology findings were in keeping with normal histology of the liver and kidney with no evidence of necrosis or injurious effects (Plate 1 -6). In the analyses, photomicrographs obtained from control rats were compared with those administered with sodium nitrate solution.

Table 4.1**:** Physical and Weight Analysis of Experimental Rats for 30 Days

Cages	Dose in mg/kg b.w. in rats	Mean Average weight b4 Administration of decoction	Mean Average weight after Administration of decoction	Physical Weight loss / or gain	Activities / or dullness
A	10	197.20± 1.9	195.22± 1.3	↓	±
B	20	197.99±2.5	189.36 ± 3.5	↓	±
C	30	200.57± 3.3	195.77 ± 3.8	↓	±
D	40	198.40± 4.0	185.10 ± 4.2	↓	+
E	00	198.48± 1.4	205.11 ± 2.2	↑	-

Key

↑ → Slight increase in weight

↓ → Slight weight loss

⇟ → severe weight loss

\+ → Presence of features

± → Intermediate features

++ → Marked presence of features

\- → Absence of features

Table 4.2: Renal Test (Electrolyte and Urea/ Creatine) in Experimental Rats for 30days

Indices	Group A	Group B	Group C	Group D	Group E	P-value
K	4.00±0.07	4.45±0.01	4.45±0.01	7.91±1.68	4.07±0.01	0.006
Na	129.01±0.37	129.12±0.02	129.12±0.02	130.20±0.25	130.35±1.53	0.001
Cl	96.50±3.39	97.11±1.32	97.11±1.32	101.35±1.53	98.01±0.37	0.006
HCO3	17.80±2.09	17.53±0.54	17.53±0.54	19.00±0.91	17.00±0.91	0.002
Urea	23.09±0.01	22.07±5.09	23.07±5.09	23.50±2.12	23.90±0.05	0.001
Cr	0.39±0.07	0.39±1.042	0.39±1.042	1.30±0.00	0.40±0.02	0.001

All values presented as mean ± standard error of the mean for 4 rats per group with varying doses. Values significantly varied at $P \leq 0.05$ (One-way Anova). Bicarbonate (HCO3); Chloride (Cl); Sodium (Na); Potassium (K).

Table 4.3: Liver Function Test in Both Treated and Untreated Rats After 30days

Indices	Group A	Group B	Group C	Group D	Group E	P-value
TB	0.11±0.07	0.10±1.23	0.10±1.23	0.13±0.04	0.13±0.04	0.005
CB	0.07±0.03	0.06±0.11	0.06±0.18	0.06±0.12	0.06±0.12	0.004
TP	4.55±0.35	4.73±0.35	4.73±0.35	6.32±0.42	4.32±0.42	0.003
Alb	1.80±0.71	1.80±0.71	1.81±0.33	1.98±0.80	1.93±0.66	0.044
AST	40.50±0.7	40.50±0.7	41.52±1.27	41.02±4.04	40.52±1.27	0.001
ALT	16.50±2.12	16.01±1.51	16.01±0.72	16.69±0.16	16.12±0.01	0.041
ALP	19.03±1.41	19.22±0.91	20.08±0.22	20.22±0.91	19.22±0.91	0.002

Values presented as mean ± standard error of the mean for 4 rats per group with varying doses. Values significantly varied at $P \leq 0.05$ (One-way ANOVA). Total bilirubin (TB), albumin (ALB), conjugated bilirubin (B), aspartate aminotransferases (AST), alanine aminotransferases (ALT), Total protein (TP), alkaline phosphatase (ALP).

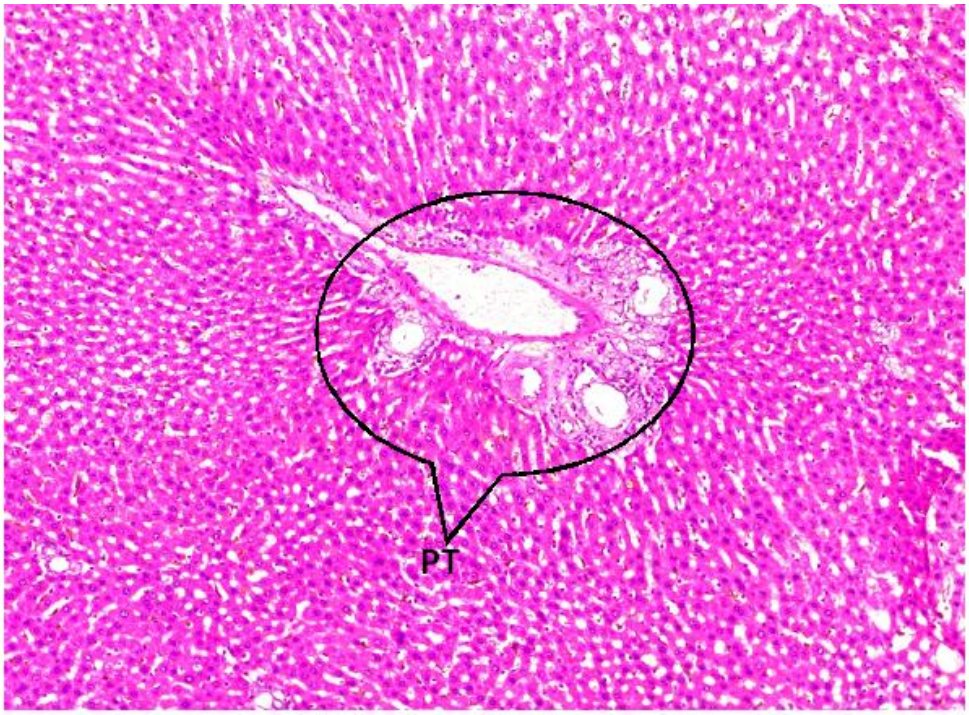

Plate 4.1: Untreated Liver Section (Group E)
Stained with H&E, x400magnification

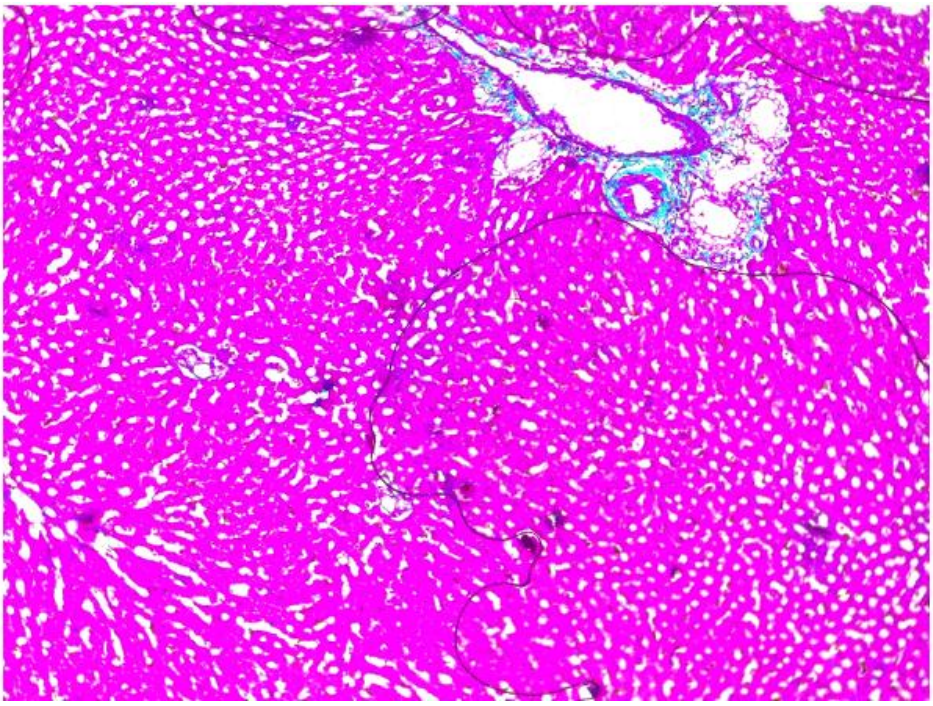

Plate 4.2: High Dose Treated Liver Section from Rats in (Group C) Stained with H&E, x400magnification

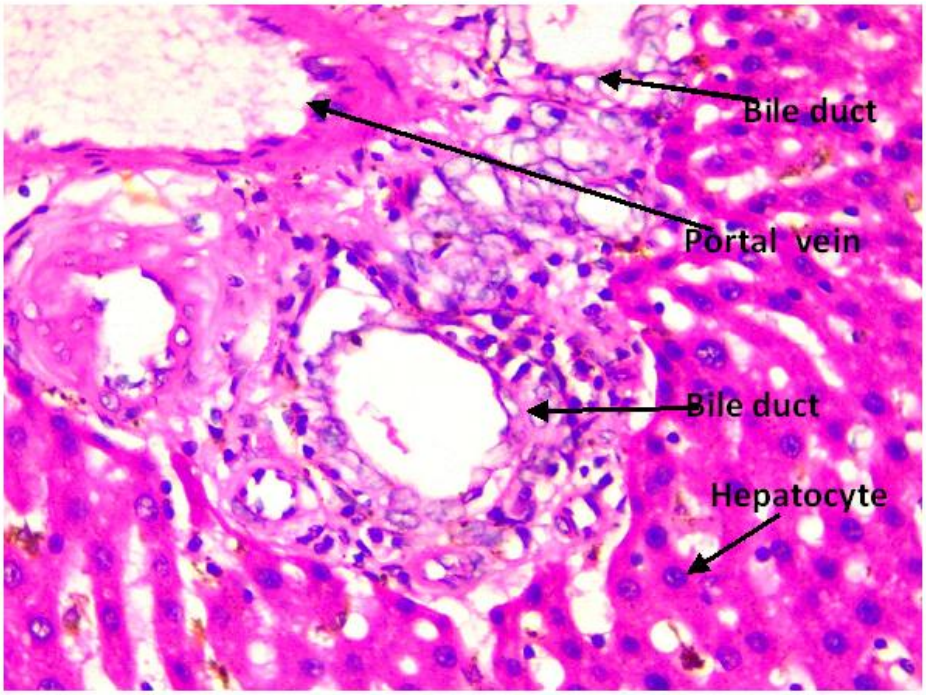

Plate 4.3: Highest Dose Treated Liver Section
Rats in (Group D) Stained with H&E, x400magnification

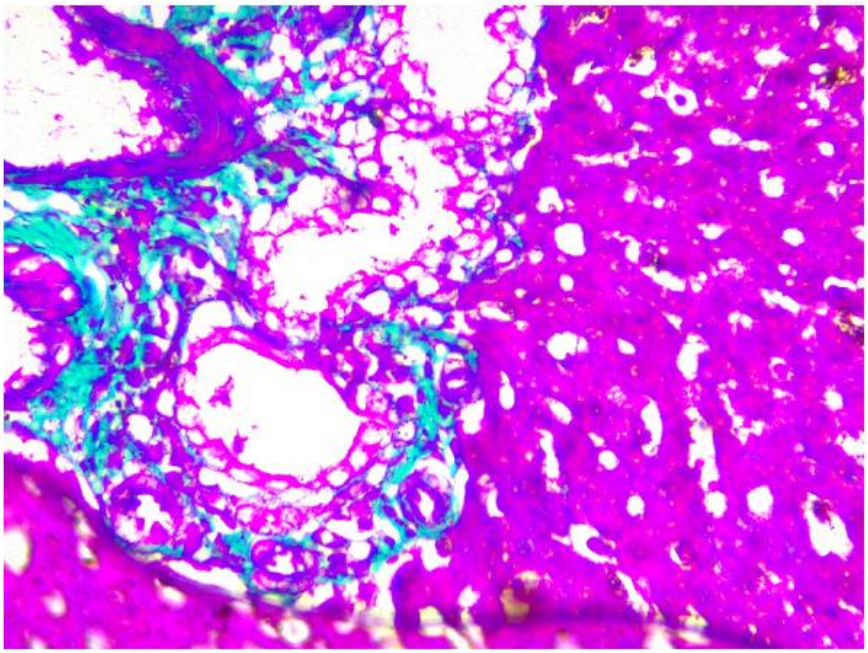

Plate 4.4: Untreated Kidney Section (Group E)
Stained with H&E, x400magnification

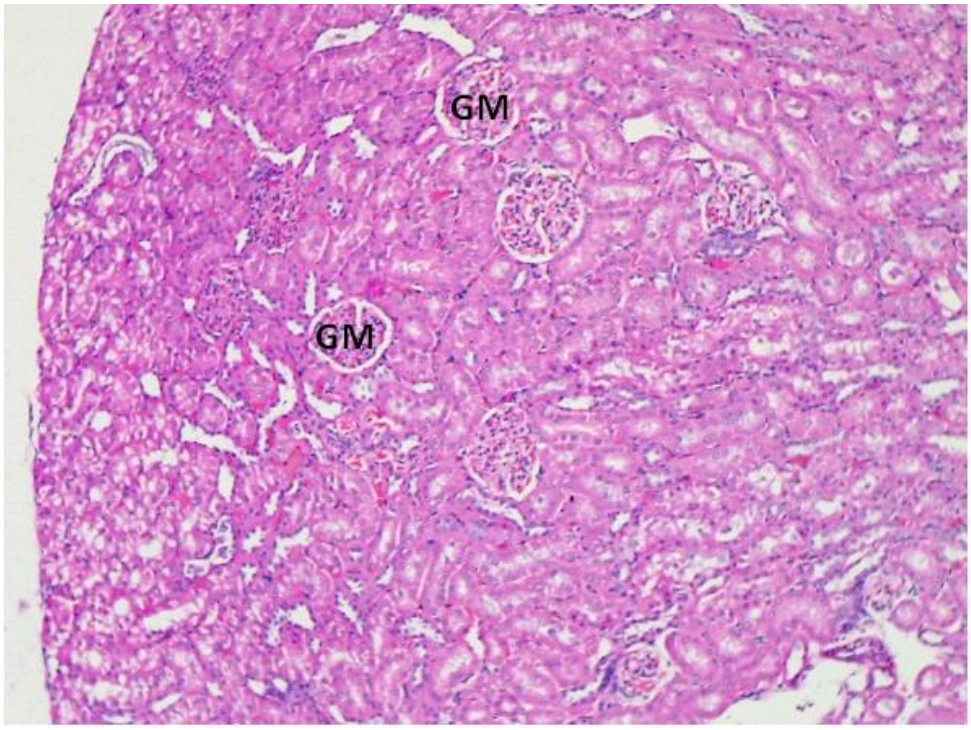

Plate 4.5: High Dose Treated Kidney Section from Rats in (Group C) Stained with H&E, x400magnification

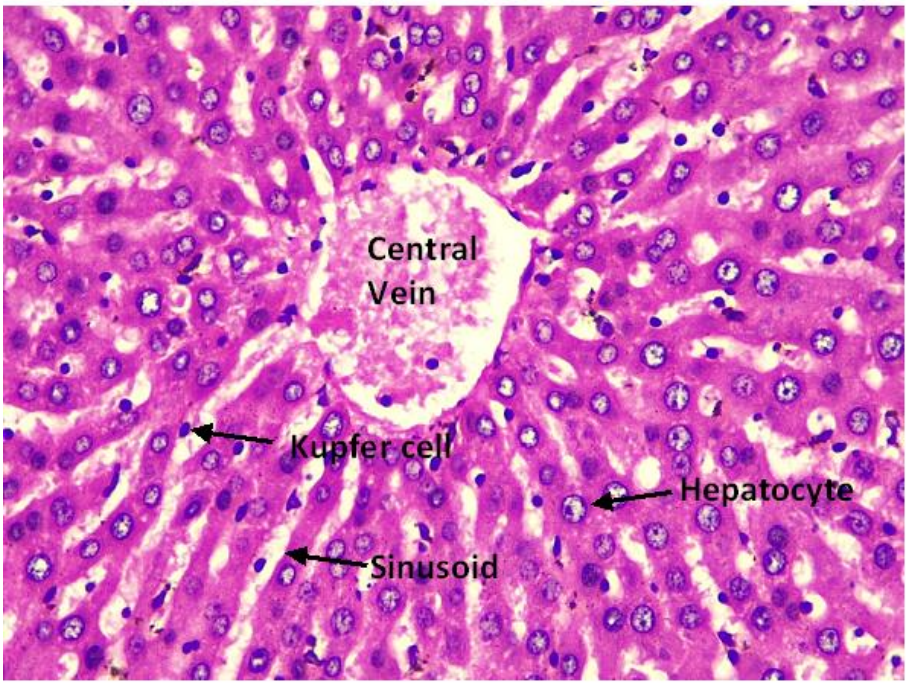

Plate 4.6: Highest Dose Treated Kidney Section
Rats in (Group D) Stained with H&E, x400magnification

Key:

GM: Glomeruli

PCT: Proximal Convoluted tubule

DCT: Distal Convoluted tubule

C: capillaries

BM: Bowman capsule

RC= Renal capsule

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The meat and poultry industry has greatly benefited from the use of sodium nitrate by allowing for the production of products with improved food safety and an extended shelf-life with excellent storage stability (Pegg and Shahidi, 2000). Many of today's processed meat products that are most enjoyed by consumers contain sodium nitrite, which allows for the existence of meat and poultry products with unique colours, textures, and flavours that may not be recreated using other ingredients (Sebranek, 1979).

Based on the foregoing, the present research is carried out to investigate possible histological alterations in organs of white rats exposed to sodium nitrate salt solution. From the result it was gathered that sodium nitrate salt solution had no pathological effects on organs of rats under investigation. This result is strongly supported by Bryan *et al*., (2007) where it was cited that sodium nitrate salt is safe for consumption but that the pathologic effects exhibited sometimes could be due to reducing effects of nitrate in the body leading to methaemoglobinemia. They further explained that it may occur via microbial actions in the surroundings or in the body (NTP, 2001). Further explanations suggest that bacteria in the mouth and gut convert nitrate into nitrite, while nitrite reacts with haemoglobin to produce methaemoglobin by converting the ferrous form to ferric form in the haemoglobin molecule, making it impossible to carry oxygen (Bryan and Hord, 2010).

Researchers have clearly shown that nitric oxide can be produced directly from nitrite and is involved in controlling blood flow in cardiac muscle and potentially from other tissues (Bryan *et al*., 2007; Bryan and Hord, 2010). In contrast, Powlson *et al.* (2008), revealed the role of intestinal infection in the cause of methaemoglobinaemia rather than nitrate. This was confirmed by an experiment, in which infants who were fed 100mg nitrate · kg^{-1} · d^{-1} did not develop methemoglobinemia. However, after feeding with bacteria harvested from contaminated wells, they developed methemoglobinemia. This suggests that the nitrate concentrations commonly encountered in foods and water are unlikely to cause methemoglobinemia. From another point of view, the present study did not agree with the report of the US Food and Drug Administration (2005), in which findings suggest that when sodium nitrate was fed to rats at levels up to 10% in the diet for their lifetime it resulted to a mild inflammation on the hepatocytes and renal corpuscle. But the former experimented on rats for a longer duration (for a life time) as against the present study in which exposure was merely for 30 days duration. Therefore, it can be inferred that duration of exposure may have significant adverse effects on organs of rats. In support of the present claims Hords *et al*., (2009) reported that nitrate and nitrite should be considered as nutrients explaining that an effect of exogenous nitrite on cancer also seems less likely because large amounts of nitrite are formed endogenously. Martijn *et al*., (2009) suggest that the evidence for adverse effects of dietary nitrate and nitrite provided by the US Food and Drug Administration Agency is rather weak, and that intakes above the legal limit might well be harmless.

According to results obtained on weight changes in experimental rats, this study aligned with Grant and Butler (1989), in which similar weight losses in

treated and gains in control animals were observed in their experiment but explained that it may be due to reduced food consumption or depletion in vitamin C according to Uchida *et al.,* (1990) resulting from increased consumption of sodium nitrite that in which the body becomes overloaded with the salt thereby increasing the body's catabolic processes. Porter *et al.,* (1993) also supported that changes in rat's weight may be due to decreased feeds consumption, hormonal imbalance or cytotoxic effects accumulated from sodium nitrite overload. Regarding liver and kidney functions, there is not enough literature either in support or against the effects exerted by sodium nitrate salt to adequately argue this segment of the experiment.

5.2 CONCLUSION

From the results obtained from this research, it can be concluded that sodium nitrate has no adverse deleterious effect on the histology of the visceral organs studied but daily intake abuse of the salt in a large concentration may be injurious to the liver and kidney of rats.

5.3 RECOMMENDATIONS

- Further research may also be needed to substantiate the information provided in this report.
- The use of sodium nitrate salt to preserve poultry foods in some cold rooms and farms should continue to be controlled at all strata of government.