ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2024; 9(1): 126 - 131

SJMLS - 9(1) - 014

Evaluating the Effects of *Moringa Oleifera* Leaf Extract on Testosterone Level of Male Wistar Rats Exposed to Petrol, Diesel and Kerosene

Bashir Saidu[1], Ashiru Dahiru[1], Aliyu Jibril[2], Ahmad Musa Dunfawa[1], Nafisat Abdulazeez[1] and Muawiyyah Muhammad Mahuta[3]

Department of Veterinary Physiology and Biochemistry, Usmanu Danfodiyo University, Sokoto[1], Department of Veterinary Theriogenology and Animal Production, Usmanu Danfodiyo University, Sokoto[2], Department of Veterinary Theriogenology, Federal University of Agriculture Zuru. Kebbi State[3]

Author for Correspondence: +234-808-597-5777/ ashiru.dahiru@udusok.edu.ng

ORCID: 0000-0002-5494-5312. DOI: 10.4314/sokjmls.v9i1.14

Abstract

Testes are primary organ of reproduction as they are responsible for the production and release of testosterone. Testosterone is the major androgenic steroid hormone produced primarily in the Leydig cells, it has both intratesticular and peripheral effects. Petroleum products are mixtures derived from crude oil and have similar chemical and physical properties. Exposure to petroleum product fumes has been reported to have both an effect on global warming and reproductive diseases. Infertility, low sperm count and decreased sperm viability that is affecting reproductive performance among individuals might be due to changes in testosterone level as a result of exposure to petroleum products. The aim of this study was to investigate the effect of *Moringa oleifera* leaf extract on testosterone levels of male Wistar rats exposed to Petrol, Diesel, and Kerosene. An experimental study was conducted with a total of Twenty-one (21) adults male Wistar rats randomly divided into seven (7) groups comprising three rats each. 40mg/kg/rat of moringa extract and 0.008 cm3/min/rat exposure dose were used in this study. The result showed an increase in serum testosterone level in the exposed groups and treatments with *moringa oleifera* leaf extract has shown a promising effect. In conclusion, the result of this study shows that exposure of Wistar rats to petroleum products through inhalation, causes marked increase in serum testosterone levels and treatment with *Moringa oleifera* leaf extract showed promising result.

Keywords: Wistar Rat, Moringa oleifera, Petrol, Diesel, Kerosene, Testosterone

Introduction

Testes are paired organs usually ovoid in shape but considerably compressed from side to side, they are the primary organs of reproduction in male. They are situated in the inguinal region, enclosed in a diverticulum of abdomen called Scrotum (Abiaezute *et al.,* 2020). Testosterone is the major androgenic steroid hormone produced primarily in the Leydig cells, it has both intratesticular effects (on spermatogenesis) and peripheral effects (on accessory sex organs as well as non-reproductive organs such as Muscle, Bone, and Skin to name a few (Shantanam *et al*., 2018). Serum biochemistry also known as clinical biochemistry refers to the analysis of the blood plasma (or serum) for a wide variety of substances; Hormones, Substrates, Enzymes, etc. and their use in disease diagnosis and monitoring (Whitbread, 2016). Several parameters influence the hematology and serum biochemistry of animals which are typically classified into two broad categories, for example genetic and non-genetic parameters. Genetic parameters include the breed and genotype of the animal while the non-genetic parameters include the age, gender, management system, medication, health status and environmental factors such as nutrition, hormone, and climate (Onasanya *et al.,* 2015). Infertility and associated reproductive problems could be due to changes in hormonal levels that could be associated with exposure to petroleum products. The low sperm count and decreased sperm viability that is affecting reproductive performance among individuals might be due to changes in testosterone levels that could result from exposure to petroleum products.

Petroleum is a naturally occurring mixture of hydrocarbons, generally in a liquid state, which may also include compounds of Sulfur, Nitrogen, Oxygen, Metals, and other elements (Rajamanickam, 2022). Petroleum products are mixtures derived from crude oil and have similar chemical and physical properties, example of petroleum products include Kerosene, Home heating oil, Diesel fuel and Gasoline (Speight, 2011). Non-hydrocarbon constituents of petroleum include organic derivatives of Nitrogen, Oxygen, Sulfur, and the metals Nickel and Vanadium, most of these impurities are removed during refining (Speight, 2011).

Moringa oleifera is a fast-growing, drought-resistant tree of the family Moringaceae, native to the Indian subcontinent (Cabi, 2019). It is widely cultivated for its young seed pods and leaves, use as vegetables and for traditional herbal medicine as well as for water purification (Sofowora *et al.,* 2013). A lot of publications have reported some of the specific effects of *Moringa oleifera* leaf extract on some body tissues or organs (Hassan *et al.,* 2020). It has an impressive range of medicinal uses in its leaves, roots, seed, bark, fruit, flowers (Patil *et al.,* 2022).

The Wistar rat also known as laboratory rat, are an inevitable part of today's biomedical research and are recognized as the preeminent model in numerous fields, including reproductive, neurobehavioral studies, cancer, and toxicology (Sengupta, 2013). Their use in scientific research started in the 16th century, although the development of the laboratory rat as an experimental model really began in 1906 when the Wistar Institute developed the Wistar rat model "Rattus norvegicus" (Sengupta, 2013). They are widely used in toxicological, nutritional, genetics, and environmental studies due to their short life span, short gestation length, smaller size and their genes are closely related to that of humans which make them preferable animals in conducting research due to the ease of housing and caring (Baker *et al*., 2013).

The aim of this study was to determine the effect of exposure to petroleum products on the testosterone level and the ameliorative effect of *Moringa oleifera* in male Wistar rats. This work will provide information on changes in testosterone level of male Wistar rats exposed to petroleum products. It will provide information on the ameliorative effects of *Moringa oleifera* on the fluctuation of testosterone level of male Wistar rats exposed to petroleum products.

Materials and Method

Materials: Wistar rats, Towel, Cages, Centrifuge, Test tubes, Pipette, Needle and Syringes, Hand gloves, Refrigerator, Beaker, measuring cylinder, Airtight container, Chloroform, Cotton wool, Plain bottles, Weighing balance, and Moringa oleifera leaf extract.

Study Location: The study was carried out in the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Sokoto State. The state lies between latitude 13° 3' 490N, longitude 5° 14' 890E and at an altitude of 272 m above the sea level (Nata, 2009). The state shares common borders with Niger Republic to the North, Kebbi State to the South and Zamfara State to the East and falls in the dry Sahel surrounded by sandy Sudan type Savannah (Jibrillah *et al*., 2018).

Study Design: Experimental study was conducted, and random sampling technique was employed. A total of 21 adults male Wistar rats were grouped into seven groups comprising of three rats in each and designated as group A to G. Group A: exposed to petrol only, Group B: exposed to petrol and later treated with *Moringa* extract. Group C: exposed to diesel only, Group D: exposed to diesel and later treated with *Moringa* extract. Group E: exposed to kerosene, Group F: exposed to kerosene and later treated with *Moringa* extract. Group G: exposed to *Moringa* oleifera leaf extract only for and served as positive control.

Preparation of Plant Material and Extraction: *Moringa oleifera* leaves were obtained from Nakasari area, Sokoto south, Sokoto, Nigeria. The plant was identified at the herbarium unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto (PCG/UDU/SOR1/0001). The leaves were thoroughly rinsed with tap water to remove any residual dirt, air dried at room temperature for

two to three weeks and then crushed into powdered form using mortar and pestle. 0.5kg (500g) of the grinded plant material was soaked into 1.8 liters of methanol and 0.4 liters of distilled water which was kept at room temperature free of dust for three days. It was sieved using soft cotton cloths and kept for seven days at room temperature for partial ethanol evaporation followed by 50^{0}C using rotary evaporator and subsequently freeze dried. The yield of the freeze-dried sample representing the aqueous extract was obtained.

Experimental Animals/Acclimatization: A total of twenty-one (21) adult male Wistar rats ranging between 4-10 weeks of age and weighing between 120-200g were obtained from Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. They were housed in cages and maintained in a room temperature under normal environmental conditions, with free access to water and feed. They were acclimatized for two weeks before the experiment commenced.

Exposure to Petroleum Products: Petroleum products used for this study were purchased from NNPC filling station beside Federal Government College Sokoto, Sokoto South LGA, Sokoto state in a sperate containers. A modified human nebulizer nose inhalation exposure method as described by Azeez *et al.* (2017) was used at 0.008cm3/min/rat for eight weeks.

Administration of Plant Material: A cannula attached to the 5 milliliters syringe was used in administration of the *Moringa oleifera* extract at the dose rate of 40mg/kg/rat for two weeks, while the rats were properly restraint using scrubbing method.

Determination of Testosterone Concentration: After exposure period of eight weeks, and treatment for two weeks, 5ml of blood sample was collected through cardiac puncture into plain sample bottles which was centrifuged at 3000 revolutions per minute for five minutes and the serum was collected for biochemical analysis to determine the Testosterone level with AccuBind® Testosterone kit using Enzyme Immunoassay, Colorimetric method (Monobind Inc., U.S.A.). The kit is based on the Competitive and Streptavidin-Coated plate principle.

Ethical approval: Ethical approval was sought from the Faculty Animal Research and Ethics Committee (FAREC) of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto (UDUS/FAREC/AUP-R16/2019). Procedures involving animals and their care were performed in accordance with the National Institute of Health (NHI) guidelines for the care and use of animals (NRC, 1996).

Statistical Analysis: Data are expressed as means ± standard error of means (SEM); statistical analysis was done using a one-way ANOVA and Behrens Fisher tests. The analysis was done using InVivoStat Software (version 4.2.0).

Result

The result of the study on the effect of petrol and *Moringa oleifera* leaf extract on testosterone level of male Wistar rats is presented in Table I. There was statistically significant increase ($P < 0.05$) between the group exposed to petrol only (group A) and the group treated with *Moringa* leaf extract after exposure to petrol (group B). There was statistically significant difference ($P < 0.05$) between the control group (group G) and the groups exposed to the petrol only (group A).

Table 1: **Means and standard error of means of testosterone of rats exposed to petrol, exposed to petrol & treated with *Moringa oleifera* leaf extract and administered *Moringa oleifera* leaf extract only (N = 9).**

Parameters	A	B	G
Testosterone (ng/ml)	5.03 ± 0.5^a	0.39 ± 0.3^b	2.00 ± 0.7^c

Key: A (Petrol only), B (Petrol + *Moringa oleifera* leaf extract) and G (*Moringa oleifera* leaf extract (positive control). Data is given as means ± standard deviation. ^{abc}Means with different superscript in a row with different superscripts differ significantly ($P < 0.05$)

The result of the study on the effect of diesel and *Moringa oleifera* leaf extract on testosterone level of male Wistar rats is presented in Table 2. There was statistically significant (P <0.05) between the group to diesel only (group C) and the group treated with *Moringa* leaf extract after exposure to diesel (group D). There was statistically significant difference (P <0.05) between the control group (group G) and the groups exposed to the diesel only (group C).

Table 2: **Means and standard error of means of testosterone of rats exposed to diesel, exposed to diesel & treated with *Moringa oleifera* leaf extract and administered *Moringa oleifera* leaf extract only (N = 9).**

Parameters	C	D	G
Testosterone (ng/ml)	0.19 ± 0.05^{a}	$1.50 \pm 0.^{b}$	2.00 ± 0.7^{c}

Key: C (Diesel only), D (Diesel + *Moringa oleifera* leaf extract) and G (*Moringa oleifera* leaf extract (positive control). Data is given as means ± standard deviation. ^{abc}Means with different superscript in a row with different superscripts differ significantly (P <0.05).

The result of the study on the effect of kerosene and *Moringa oleifera* leaf extract on testosterone level of male Wistar rats is presented in Table 3. There was also statistically significant difference (P <0.05) between the group exposed to kerosene only (group E) and the group treated with *Moringa oleifera* leaf extract after exposure to kerosene (group F). There was statistically significant difference (P <0.05) between the control group (group G) and the groups exposed to the kerosene only (group E).

Table 3: **Means and standard error of means of testosterone of rats exposed to kerosene, exposed to kerosene & treated with *Moringa oleifera* leaf extract and administered *Moringa oleifera* leaf extract only (N = 9).**

Parameters	E	F	G
Testosterone (ng/ml)	0.83 ± 0.5^{a}	1.79 ± 0.3^{b}	2.00 ± 0.7^{c}

E (Kerosene only), F (Kerosene + *Moringa oleifera* leaf extract) and G (*Moringa oleifera* leaf extract (positive control). Data is given as means ± standard deviation. ^{abc}Means with different superscript in a row with different superscripts differ significantly (P <0.05).

Discussion

This study was aimed at determining changes in testosterone levels of Wistar rats exposed to petrol, diesel and kerosene and the ameliorative effect of *Moringa oleifera* leaf extract on the rats. Upon exposure of groups A, C and E to Petrol, Diesel and Kerosine for eight (8) weeks via inhalation, a marked increase in serum testosterone level was observed. This is similar to the work of Njoroge *et al*. (2015). This can be due to the ability of benzene, toluene, and xylene in inducing oxidative stress as reported by (Zhou *et al*., 2011). Also, after exposure of group B, D, and F to Petrol, Diesel and Kerosine for eight (8) weeks and later treated with *Moringa oleifera* leaf extract for two (2) weeks, testosterone level was observed to be within the normal range. This may be due to the powerful antioxidant effect of *Moringa oleifera*, as reported by (Hassan *et al*., 2020). Moreover, (Oa *et al*., 2017) illustrated that the presence of flavonoids in *Moringa oleifera* leaf extract has a role in altering androgen levels.

Conclusion

In conclusion, the result of this study shows that exposure of Wistar rats to petroleum products through inhalation causes marked increase in serum testosterone levels. And treatment with *Moringa oleifera* leaf extract showed promising result.

Recommendation

We recommend exposure of humans and animals to petroleum products should be avoided or minimized. Humans with daily exposure to

petroleum products should include *Moringa oleifera* leaves in their food regimen.

Conflict of Interest Declaration

The authors did not receive support from any organization for the submitted work. This manuscript title "Evaluating the Effects of *Moringa Oleifera* Leaf Extract on Testosterone Level of Male Wistar Rats Exposed to Petrol, Diesel and Kerosene" has not been published elsewhere and it has not been submitted simultaneously for publication elsewhere.

Acknowledgment

We want to acknowledge the efforts of all staff of the Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

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Citation: Bashir Saidu, Ashiru Dahiru, Aliyu Jibril, Ahmad Musa Dunfawa, Nafisat Abdulazeez and Muawiyyah Muhammad Mahuta. Evaluating the Effects of *Moringa Oleifera* Leaf Extract on Testosterone Level of Male Wistar Rats Exposed to Petrol, Diesel and Kerosene**. *Sokoto Journal of Medical Laboratory Science*; 9(1): 126 - 131.** DOI: 10.4314/sokjmls.v9i1.14

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