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MITIGATIVE EFFECTS OF *Moringa Oleifera* ON PROGESTERONE LEVEL OF FEMALE WISTAR RAT EXPOSED TO PETROLEUM PRODUCTS

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ABSTRACT

Petroleum products are mixtures of complex compounds or materials derived from crude oil, the majority of petroleum is converted to petroleum products, which include several classes of fuels. Such as petrol, diesel, kerosene, heavy gas oil and many others and its by-products contribute to air pollution and global warming. Twenty (21) rats were randomly divided into seven groups of three rats each. Group A were administered with petrol only. Group B were administered with petrol and later treated with *Moringa oleifera*, Group C were administered with diesel only. Group D were administered with diesel and later treated with *Moringa oleifera*, Group E were administered with kerosene only. Group F were administered with kerosene and later treated with *Moringa oleifera*. Group G were administered with *Moringa oleifera* extract only and served as control group. A cannula attached to the 5 millilitres syringe was used in administration of the aqueous *Moringa oleifera* extract at the dose rate of 40mg/kg/rat while the rats were properly restraint using scrubbing method. Blood was collected through cardiac puncture and analyzed. The results showed exposure to petrol, diesel and kerosene decrease serum progesterone level in rats ($p < 0.05$). Treatment with *Moringa oleifera* effectively reversed this decline and normalized progesterone concentrations to control levels.

Keywords: Progesterone, *Moringa oleifera*, Petroleum Products, Wistar Rats

INTRODUCTION

Petroleum products are a group of hydrocarbon-based substances derived from crude oil through various refining processes. Crude oil, also known as petroleum, is a naturally occurring fossil fuel composed of hydrocarbons and other organic compounds (Esanmurodov, 2023). Unlike petrochemicals, which are a collection of well-defined usually pure organic compounds, petroleum products are complex mixtures (Walther *et al*., 2005). The majority of petroleum is converted to petroleum products, which includes several classes of fuels. Over 6,000 items are made from petroleum waste by-products, including: fertilizer, flooring (floor covering), perfume, insecticide, petroleum jelly, soap, vitamins and some essential amino acids (Dawe, 2000). Progesterone (P4) is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species (Jameson and De Groot 2015). It belongs to a group of steroid hormones called the progestogens and is the major Progestogen in the body (Jameson and De Groot 2015). Progesterone has a variety of important functions in the body. It is also a crucial metabolic intermediate in the production of other endogenous steroids, including the sex hormones and the corticosteroids, and plays an important role in brain function as a neuro-steroid (Adler *et al*., 2012). In addition to its role as a natural hormone, progesterone is also used as a medication, such as in combination with estrogen for contraception, to reduce the risk of uterine or cervical cancer, in hormone replacement therapy, and in feminizing hormone therapy (Stanczyk, 2002). *Moringa Oleifera* leaves meal fed tends to increase the concentration of progesterone during pregnancy and it decline sharply in post-natal. The higher the level of moringa leaves meal, the higher the production of prolactin hormone at natal period (Sinha *et al*., 2012). The *M. oleifera* have been reported to be a valuable source of both macro- and micronutrients, rich source of β-carotene, Protein, Vitamin C, Calcium, and Potassium and act as a good source of natural antioxidants; and thus, enhance the shelf-life of fat-containing foods (Sinha et al., 2012).

The aim of this research was to study the effects of *Moringa oleifera* on progesterone level of female Wistar rat exposed to petroleum products. Some studies have shown significant harmful changes in blood parameters of animals exposed to petroleum products, information on hormonal changes is however not adequate. Exposure to petroleum products toxicity has been said to have detrimental effect on both humans and animal, and the effect on reproductive tract or system have not been evaluated. This work will provide information on progesterone level of female Wistar rats exposed to petroleum products and the therapeutic effects of *Moringa oleifera* on progesterone level of female Wistar rats exposed to petroleum products.

MATERIALS AND METHODS

Experimental Animals

Twenty one female Wistar albino rats of approximately aged between 2-3month, weighing 128-233g were used for the study. The rats were obtained from the Animal House at the Department of Veterinary Pharmacology and Toxicology, Usmanu Danfodiyo University Sokoto. The animals were housed in sanitary cage and had access to wholesome water and a standard diet. All procedures involving the animals were conducted with strict adherence to the guidelines and procedure provided by the animal ethics committee. They were also acclimatized for two weeks before the experiment commenced.

Study Design

An experimental study was conducted between December 2022 – February 2023. The rats were maintained in standard conditions (28.5- 33°C, 12:12 hour dark/light cycle), They were fed on Pelleted feed (Vital feed® growers mash) and

water *ad libitum* throughout the experiment. The rats were randomly allocated into 7 groups comprising of 3 rats, the groups were leveled A to G. Group A, petrol was administered only; Group B, petrol was administered and later treated with 40mg/kg/rat *Moringa oleifera* extract; Group C, diesel was administered only; Group D: diesel was administered and later treated with *Moringa oleifera* extract only; Group E, kerosene was administered only; Group F, kerosene was administered and later treated with *Moringa oleifera* extract and Group G, *Moringa oleifera* leaf extract was administered and served as positive control.

Extraction of Plant Materials

Moringa oleifera leaves were obtained from Bodinga Local Government Area of Sokoto. The plant was identified at the Herbarium (PCG/UDU/SOR1/0001), Department of Biological Science, in Usmanu Danfodiyo University Sokoto. The leaves were washed thoroughly with clean water and allowed to dry at room temperature for two weeks and crushed into powder form using laboratory mortar and pestle. 0.5kg (500g) of the grinded plant material was soaked into 1.8 litres of methanol and 0.4 litres 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 ^{O}C using rotary evaporator and subsequently freeze dried. The yield of the freeze-dried sample represents the aqueous extract obtained.

Administration of Plant Extract

A cannula attached to a 5 millilitres syringe was used in administration of the aqueous *Moringa oleifera* extract, at the rate of 40mg/kg/rat, while the rats were properly restraint using scrubbing method.

Exposure to Petroleum Products

Petrol, diesel and kerosene were purchased from Nigerian National Petroleum Corporation (NNPC) Mega filling station, along Gusau road, Sokoto, in separate containers. A modified human nebulizer nose inhaler was used to dispense the agents at 0.008cm3/rat throughout the experimental period. After the exposure period the blood sample was collected using cardiac puncture into plain sample bottles which were centrifuged at 5000 revolutions per minute for five minutes and the serum was harvested and used for progesterone assay using Acqu Bind® kit.

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). $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 indicates the effect of petrol and ameliorative effects of *Moringa oleifera* leaf extract on progesterone level of female Wistar rats. There was statistically significant difference (P >0.05) among the control group (group G) and the groups exposed to Petrol (group A) and later treated with *Moringa oleifera* leaf extract (group B)

Table 1: Effect of petrol and ameliorative effects *Moringa oleifera* leaf extract on progesterone level of female Wistar rats. (N=9)

Parameters	A	B	G
Progesterone(ng/ml)	13.10±0.55^{bg}	9.00±0.50^{a}	16.96±1.38^{ade}

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 in a row with different superscripts differ significantly (P >0.05).

Table 2 indicates the effect of diesel and ameliorative effects of *Moringa oleifera* leaf extract on progesterone level of female Wistar rats. There was a statistically significant difference (P >0.05) among the control group (group G) and the groups exposed to Diesel (group C) and later treated with Moringa oleifera leaf extract (group D).

Table 2: Effect of diesel and ameliorative effects *Moringa oleifera* leaf extract on progesterone level of female Wistar rats. (N=9)

Parameters	C	D	G
Progesterone(ng/ml)	18.25±1.32^{dg}	19.66±1.87^{c}	16.96±1.38^{ade}

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 in a row with different superscripts differ significantly (P >0.05).

Table 3 below indicates the effect of Kerosene and ameliorative effects of *Moringa oleifera* leaf extract on progesterone level of female Wistar rats. There was a statistically significant difference (P >0.05) among the control group (group G) and the groups exposed to Kerosene (group E) and later treated with *Moringa oleifera* leaf extract (group F).

Table 3: Effect of kerosene and ameliorative effects *Moringa oleifera* leaf extract on progesterone level of female Wistar rats. (N=9)

Parameters	E	F	G
Progesterone(ng/ml)	12.25±0.80^{fg}	20.35±1.68^{e}	16.96±1.38^{ade}

Key: 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 in a row with different superscripts differ significantly (P >0.05).

Discussion

This study was aimed at determining changes in progesterone levels of Wistar rats exposed to petrol, diesel and kerosene and the possible ameliorative effects of *Moringa oleifera* leaf extract on petroleum product induce changes in progesterone levels in female Wistar rats. Upon exposure of groups, A, C and E, to petrol, diesel and kerosene, respectively for eight (8) weeks via inhalation, a marked decrease in serum progesterone level was observed and this could be as a result of the suppressing effects of polyaromatic hydrocarbons present in these petroleum products. This observation is similar to the findings of (Patrick-Iwuanyanwu *et al*., 2007). 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, progesterone level was observed to be within the normal range. This can be correlated to the ability of *Moringa oleifera* leaf extract to reverse the effects of the petrochemical compounds on progesterone levels of Wistar rats. This fact is likely due to amino acids contents of *Moringa oleifera* leaves particularly essential amino acids. The claim is supported by the fact that *Moringa oleifera* is a good dietary supplement for protein deficiencies and metabolic processes due to its distinct and well-balanced essential amino acid content such as tryptophan, valine, histidine, phenylalanine, leucine, lysine, methionine, and threonine, which directly contributes to its nutritional and therapeutic advantages (Flores et al 2015). Moreover, *Moringa oleifera' s* rich essential amino acid profile, especially methionine, arginine, and phenylalanine, which support steroidogenesis, boost luteinizing hormone signaling, and ameliorate oxidative stress in steroidogenic tissues, may be the reason progesterone levels returned to normal in rats exposed to petroleum hydrocarbons after treatment (Ojiako et al., 2018; Vergara-Jimenez et al., 2017).

CONCLUSION

In conclusion, exposure to petrol, diesel and kerosene decrease serum progesterone level in rats ($p < 0.05$). Treatment with *Moringa oleifera* methanolic leaf extract effectively reversed this decline and normalized progesterone concentrations to control levels. This restorative effect may be attributed to the leaf of the plant that is rich in amino acid profile and antioxidant properties, which likely mitigated the endocrine disruption induced by petroleum hydrocarbon.

RECOMMENDATIONS

Further studies should be conducted on the effects of petrochemical compounds on Estrogen and Prolactin, and the ameliorative effects of *Moringa oleifera*

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