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Evaluation of the Safety Profile of Ethanol Stem Bark Extract of *Mangifera indica* on Reproductive Functions of Male Albino Rats

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ABSTRACT

Medicinal-plants are widely used globally for prevention and treatment of wide range of diseases. It is sometimes used as alternatives and/or adjuvants to modern-medicine. However, detailed toxicological-evaluation of most herbal-plants especially on Reproductive-functions has not been fully explored. This research focused on evaluating the effect of ethanol stem-bark extract of *Mangifera indica* on the Hormonal-profile and Semen-quality of male albino rats. Twenty-four male rats weighing between 120-150g each were used for this study. They were divided into 4 groups (A, B, C and D) of six rats per group. Group D served as the control (without test substance) while groups A, B and C were fed with of 250mg/kg, 500mg/kg and 1000mg/kg body-weight (BW) of the extract for the period of 4 weeks and then tested for the hormonal-profile and semen-quality. The results obtained from hormonal analysis showed that the extract produced significant ($p<0.05$) increase in the levels of Luteinizing hormone-(LH) and Testosterone at 250mg/kg and also produced an increase in Follicle Stimulating hormone-(FSH) level at 1000mg/kg when compared with the control. However, the extract significantly ($p<0.05$) reduced the levels of Luteinizing hormone-(LH) and Testosterone at 500mg/kg and 1000mg/kg while the level of Follicle Stimulating hormone-(FSH) was reduced at 250mg/kg and 500mg/kg. For the Semen-analysis, the extract significantly ($p<0.05$) increased the sperm-motility, sperm-morphology and sperm-count of the rats in a dose dependent manner with more pronounced effect at 1000mg/kg when compared with the control, this may be as a result of the ability of the extract to increase the level of Follicle Stimulating hormone-(FSH) which is responsible for spermatogenesis. This suggests that the stem bark of *Mangifera indica* may be incorporated in the formulation of male fertility drugs.

Keywords: Male Reproductive hormones, Sperm quality, *Mangifera indica*, Hormonal profile

INTRODUCTION

Reliance on medicinal plants for primary health care need or treatment of diseases has greatly increased over the years (Ekor and Martins, 2013) and have been the preferred option for most people nowadays because of the serious side effects and high cost of conventional drugs (Abdelgader and Adil, 2018). Medicinal plants are noted for their various therapeutic potentials and these have contributed immensely to their recognition and acceptability worldwide. *Magniferia indica,* a flowering plant belonging to family Anacardiacea is a well-known important herb in the Ayurvedic and indigenous medical system. It is a large fruit-tree, it is capable of growing to a height and crown width of about 100 feet and trunk circumference of more than 12 feet. It is one of the most widely consumed fruit in the world. It has a range of health benefits which are attributed to its various parts (Shah *et al.,* 2010). Its reported therapeutic potentials has been attributed to presence of some chemical constituents/ secondary metabolites present in it such as polyphenolic, flavonoids, triterpenoids, mangiferin, tannins, gallic acid etc and this has greatly contributed to its acceptance in health care delivery system. Studies have shown that various part of M. indica such as leaves, stem bark, fruit peel and flesh, root and flowers possess anti-oxidant, antidiabetic, anti-tumor, anti-HIV, antispasmodic, antipyretic, antiviral, anti-helminthic, immunomodulatory, anti-diarrhea, anti-inflammatory, anti-bacterial, anti-fungal, anti-parasitic, hepatoprotective, gastroprotective activities (Martinez *et al.,* 2000; Ojewole, 2005; Peng *et al*., 2004; Prasad *et al*., 2007; Rodeiro *et al.,* 2007; Sujon *et al.,* 2008; Abbasi *et al.,* 2017). Utilization of *M. indica* for medicinal purposes and nutritional intake necessitate the need to thoroughly investigate its positive and negative effects especially on reproductive functions to support its nutritional intake as well as its therapeutic use. Thus, this research work focus on evaluating the safety profile of ethanoic stem bark extract of *M. indica* on the reproductive functions of male albino fats.

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MATERIALS AND METHODS

Animal collection and housing

Wistar rats ranging from 120-180g were purchased from the animal house in University of Ibadan, Oyo State. All animals were housed, fed and treated in accordance with the in house guidelines for animal care (Hedrich, 2006). Animals were kept for two weeks in other to acclimatize them prior to the investigation. During this time, they were given standard pellet diet and water ad libitum.

In this study, all the animal experimentation was carried out according to the guidelines of Institutional Animal Ethics Committees (IAEC).

Collection of plant samples

The stem bark of *Mangifera indica* were collected from Abeokuta, Ogun state, Nigeria. The plant was identified and its botanical identity confirmed and authenticated at the Herbarium section of the Department of Botany, University of Lagos, Akoka, Lagos, Nigeria, by Mr. Oyebanji.

Extract preparation

The fresh stem bark of *Mangifera indica* were air dried at room temperature and then oven dried at a temperature of 60OC. The dried stem were then reduced to coarse powdered using grinding machine and weighed. 500g of the powdered plant was extracted with 4litres of 90% Ethanol in a cold maceration process for 72 hours and then filtered using filter paper. After filtration, the filtrate was concentrated using rotatory evaporator. The extract was further concentrated to dryness in a water bath and stored in a refrigerator in air tight container (universal bottle) prior to use.

Extract Reconstitution

The extract was reconstituted by suspending 3g of crude extract in 10ml of distilled water to obtain a stock solution of 300mg/ml.

Experimental design

After acclimatization the animals were weighed and divided into four groups of five animals per group. The control group received a dose of 0.2ml of distilled water and the other groups were given graded doses of the extract orally for 4 weeks. Treatments were as follows;

Group 1: Normal control (distilled water).
Group 2: Extract MI at dose of 250mg/kg per Bwt.
Group 3: Extract MI at dose of 500mg/kg per Bwt.
Group 4: Extract MI at dose of 1000mg/kg per Bwt.

Hormonal assay

At the end of the experimental period, five rats from each group were sacrificed and blood samples collected. The blood samples collected were centrifuged at 2500rpm for 5min at 10-25^{0}C to obtain the serum for analyzing testosterone, follicle stimulating hormone(FSH) and luteinizing hormone (LH) level using enzyme linked immunosorbent assay (ELISA) technique according to Levine et al., 1985. Reagents used were of analytical grade.

Test procedure for Testosterone

Sufficient wells for calibrators, control and the test samples were properly placed in duplicate. 50µl of each calibrator, control and test samples was added to the corresponding labelled wells in duplicate followed by addition of 100µl of conjugate working solution. The solution was properly mixed and incubated on a plate shaker (approximately 200rpm) for 1hr at room temperature. Each well was washed properly with 300µl of diluted washed buffer and the plate firmly tapped against absorbent paper to ensure that it was dry. 150µl of tetramethylbenzidine (TMB) substrate was added to each well and incubated for 15 minutes at room temperature before the addition of 50µl stop solution. The absorbance was read at 450nm within 20 minutes after addition of the stop solution using spectrophotometer (Levine *et al.*, 1985).

Test procedure for Follicle stimulating hormone (FSH)

Sufficient coated wells in a holder were placed to run 0.0MIU/ml, 25MIU/ml, 50MIU/ml and 100MIU/ml for FSH calibrators, control and the test samples in duplicate. 50µl of each sample was pipetted into the corresponding coated well with addition of 200µl of the enzyme antibody conjugate solution. The solution was properly mixed and incubated at room temperature for 45 minutes. The wells were properly washed with wash buffer and de-ionized water and decanted. 100µl substrate-chromogen solution was added to each well, mixed thoroughly and incubated for 15 minutes at room temperature. Immediately after incubation, 100µl of 1NH2SO4 was added to each well and properly mixed. The absorbance of each well was read at 450nm spectrophotometrically. A graph of absorbance versus concentration of FSH was plotted to read off the actual amount of FSH in the test samples (Levine *et al*., 1985).

Test procedure for Luteinizing hormone (LH)

Sufficient coated wells in a holder were placed to run 0.0MIU/ml, 25MIU/ml, 50MIU/ml and 100MIU/ml for LH calibrators, control and the test samples in duplicate. 50µl of the calibrators, control and the test samples were added to the corresponding coated well. 200ml of the enzyme antibody conjugate solution was added to all the wells, properly mixed and incubated at room temperature for 45 minutes. The wells were properly washed with wash buffer and de-ionized water and decanted. 100µl of substrate-chromogen solution was added to each

well, mixed properly and incubated at room temperature for 15 minutes. This was followed by addition of 100µl of 1NH2SO4 stop solution. The solution was properly mixed and the absorbance was read at 450nm against water using spectrophotometer. A graph of absorbance versus LH concentration was plotted to obtain the actual amount of LH in the test samples (Levine *et al.,* 1985).

Semen analysis

Evaluation of sperm motility

Semen samples from the different treatment groups were dropped on a glass slide and viewed under the microscope. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 spermatozoa for each rat. The percentage of sperm motility was analyzed for progressive motile sperm (PMS), non-progressive motile sperm (NPMS) and non-motile sperm (NMS) distinguished by the movement of the sperm cells (WHO, 1992).

Estimation of mean sperm count

This was carried out according to the method of Ekaluo *et al.,* 2009. The epididymal content was obtained by macerating with fine scissors known weights of the caput and cauda epididymides in a glass petridish containing warmed buffered physiological saline in the ratio of 1:10w/v. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80µm stainless mesh. A tissue-free aliquot was loaded into the Neubauer haemocytometer. Five different counts were done for each sample, and the mean were taken as the mean count for each male rat.

Sperm count = (Total number of sperm cells in the cytometer)/(Mean value)

Where; mean value = Five.

Sperm morphology

A fraction of each of the sperm suspension was examined by placing the solution (10:1) for 30min on a glass slide. The slide was examined for percentage abnormalities in every 200 spermatozoa observed on each slide and five air dried smear was prepared on glass slide for each sample according to of Ekaluo *et al*., 2009.

Statistical Analysis

The results obtained were expressed as mean + standard error of mean (SEM), statistical analysis of the data was done using One Way Analysis Of Variance (ANOVA), followed by Dunnett Multiple Comparison Test. Differences were considered significance, if $p< 0.05$.

RESULTS

Effect of *Mangifera Indica* (MI) on Hormonal Profile

The results obtained showed that ethanolic stem bark extract of *Mangifera indica* (MI) significantly ($p<0.05$) decreased luteinizing hormone (LH) and testosterone levels of the rats treated with 500mg/kg & 1000mg/kg but increased the levels in the rats treated with 250mg/kg when compared with the control. It also significantly decreased the follicle stimulating hormone (FSH) of the rats treated with 250mg/kg and 500mg/kg but increased its level in the rats treated with 1000mg/kg (Table 1).

Table 1: Effect of *Mangifera Indica* (MI) on Hormonal Profile

	Luteinizing Hormone (LH) (mlU/ml)	Follicle Stimulating Hormone (FSH) (mlU/ml)	Testosterone (ng/ml)
Distilled water	6.489± 0.30	1.16± 0.76	0.90± 0.35
MI 250mg/kg	6.921± 0.54	0.94± 0.50*	1.45± 0.61*
MI 500mg/kg	6.161± 2.23	0.67± 0.59*	0.80± 0.43
MI 1000mg/kg	5.209± 1.49*	1.60± 1.00*	0.72± 0.39

Values expressed as mean± SEM (n=5) *p<0.05 Significant compared to control. Statistical analysis done using ANOVA followed by Dunnett multiple comparison tests.

Effect of *Mangifera Indica* (MI) on Semen Quality

For the semen quality, the results showed that the ethanolic stem bark extract of *Mangifera indica* (MI) significantly ($p<0.05$) increased the sperm motility and the sperm count of the treated rats in a dose dependent manner when compared with the control, and also significantly increased sperm morphology of the rats treated with 1000mg/kg but decreased those treated with 250mg/kg and 500mg/kg (Table 2).

Table 2: Effect of *Mangifera Indica* on Sperm quality

	Sperm Motility	Sperm Count (x106)	Sperm Viability (%)	Sperm Morphology
Distilled water	90.33± 1.45	14.70± 0.15	95.33± 1.33	8.00± 0.58
MI 250mg/kg	93.00± 0.00	15.20± 0.00	95.00± 0.00	7.00± 0.00*
MI 500mg/kg	93.50± 0.00	15.30± 0.00*	94.00± 0.00*	8.00± 0.00
MI 1000mg/kg	94.00± 0.00*	15.40± 0.00*	93.00± 0.00	9.00± 0.00*

Values expressed as mean± SEM (n=5) *p<0.05 Significant compared to control. Statistical analysis done using ANOVA followed by Dunnett multiple comparison tests.

DISCUSSION AND CONCLUSION

Medicinal plants play an important role over the years in improving human health and is still in widespread use today. Various plants have been exploited for their therapeutic potentials, *Mangifera indica* is one of such plants.

Administration of the ethanolic stem bark extract of *Mangifera indica* had a significant (P<0.05) effect on the levels of FSH, LH and Testosterone as well as on Semen quality of the treated rats. The extract significantly (P<0.05) reduced the levels of LH and Testosterone at highest dose used with corresponding increase in the level of FSH when compared with the control. It has been reported that some phytochemicals such as flavonoids and alkaloids were known to reduce plasma concentrations of fertility hormones (Bianco et al., 2006), the resultant low level of Testosterone and LH produced by the extract may be as a result of these phytochemicals present in the plant. Literature has shown that Cytochrome P450s enzymes are essential for various hydroxylation reactions that contribute to the synthesis of androgens and other steroid hormones, inhibition of these enzymes can result in reducing the synthesis as well as the circulating levels of testosterone and other steroid hormones and this can contribute to variety of anti-steroid hormone effects (Gerald, 2004). Reduction in the levels of LH and Testosterone by the extract at higher dose may be due to the ability of the extract to inhibit the enzymes necessary for synthesis of the hormones when used at such concentration with little or no effect on the enzymes responsible for FSH production. In this study, the extract also significantly (P<0.05) increased sperm count, sperm motility as well as sperm morphology of the treated rats. This suggest that the extract has no damaging effect on the testicular cells of the treated rats which could lead to decline in their sperm quality. The ability of the extract to produce significant increase in sperm quality may be due to the presence of bioactive compounds which are responsible for increase in sperm quality in the extract according to Ikpeme *et al.,* 2007. It can also be as a result of important phytochemical constituents which enhance hormonal production (Okwu and Ezenagu, 2008) and sperm quality in the extract. Enhanced sperm quality produced by the extract can also be as a result of the ability of the extract to produce a stimulatory effect on the hypothalamus which is responsible for the production of hormones required for spermatogenesis. According to Ikpeme *et al.,* 2014, it could be possible that Testosterone needed FSH to function effectively since FSH is indirectly linked with spermatogenesis. Increased level of FSH with the levels of LH and Testosterone produced by the extract may contribute to increase in the sperm quality since both FSH and LH work simultaneously with Testosterone during spermatogenesis.

CONCLUSION

In conclusion, administration of the extract produced an increase in sperm quality of the male albino rats, this may be due to the ability of the extract to increase the level of Follicle Stimulating hormone (FSH) with little increase in the levels of LH and Testosterone which are responsible for spermatogenesis. This suggests that the stem bark of Mangifera indica is safe without causing any damage to spermatogenic pathways and thus may be incorporated in the formulation of male fertility drugs.

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