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FERTILITY ENHANCEMENT PROSPECTS OF LEAVE EXTRACTS OF *MANGIFERA INDICA* AND *PEPEROMIA PELLUCIDA*

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ABSTRACT

Some non-communicable diseases like infertility and cancer are currently on the increase across the globe. This therefore necessitates the increase in the search for appropriate therapies which are more effective, less expensive and of minimal or no side effects. Herbal medicines are being regarded as suitable alternative to orthodox medicines considering their wide acceptability and availability. The plants extracts used in this study have shown hypoglycemic and hypolipidemic properties but there are dearth of information on the pro-fertility tendencies following exposure of normal rats to them. In this study, male albino rats of wistar strain were placed on the herbs-supplemented diets containing 30% and 70% respectively of each plant for three days after which they were sacrificed by cervical dislocation. Some tissues were collected and some gene expression assays were carried out. The results showed that the genes expressed in the testis, FSH-R and LH-R genes were significantly expressed (up-regulated) by *P. pellucida* (30%) and *M. indica* (70%). In the kidney, TNF-alpha gene was down-regulated by all the extracts while IL1-alpha was up-regulated. In the liver, PFK1, Pyr-Kinase and G6pD genes were expressed by all the extracts. It was observed that there is modulation of gene expression following the exposure of normal rats to these herbs, with *M. indica* and *P. pellucida* showing to be less toxic and more promising in expression of genes that are important in fertility management. This study offers a key to unlock new therapeutic targets for the treatment of infertility.

KEYWORDS: Non-communicable diseases, Pro-fertility, Up-regulated, Down-regulated.

INTRODUCTION

Herbal medicinal products are assuming greater roles in the lives of the people across the world in the face of global upsurge of drug resistance, toxicity, adverse effects and increasing costs of synthetic products. In Nigeria, several thousands of plant species have been claimed to possess medicinal properties and employed in the treatment of many ailments.[1] Many of these indigenous medicinal plants are used as spices and food plants and for medicinal purposes. Medicinal plants are believed to be an essential source of new chemical substances with potential therapeutic effects. Currently, medicinal plants continue to play an important role in the management of diabetes mellitus, especially in developing countries, where many people do not have access to conventional antidiabetic therapies.[2]

Metabolomics, pharmacogenomics, and toxicogenomics can be utilized to examine the chemical processes involving metabolites of medicinal herbs, to investigate the variations within the host genome and herbs, and to analyze the toxic effects of herbs.[3,4,5,6] Omics, such as functional genomics, transcriptomics and proteomics, can be applied to study the gene protein functions of medicinal herbs and to evaluate the herb/host interactions. Plants are known to be pharmacologically active, inactive or toxic by examining alterations caused by the plant extracts on the hematological and transcriptomic markers. One of the important methods of assessment of health in an animal is to assess the haematological parameters. Several studies have reported the effect of plant extracts on haematological parameters of rats following administration of the plant spices or extracts.[7] Also, transcriptomic analysis is important in understanding how animals exposed to plant extract can undergo altered expression of genetic variants, which contributes to complex diseases such as diabetes. Analysis of RNA expression provides insight into biological pathways and molecular mechanism that regulate cell fate, development and disease progression. Although different studies have independently reported the effect of plants on the alteration of haematological

parameters and transcriptomic library, this study aims to investigate some transcriptomic alteration occurring in experimental white albino rats when exposed to these selected plants.

Peperomia Pellucida (also known by common names pepper elder, shining bush plant, and man to man) is an annual, shallow-rooted herb, usually growing to a height of about 15 to 45 cm it is characterized by succulent stems. Flowering year-round, the plant is found in various shaded, damp habitats all over Asia and the Americas. It grows in clumps, thriving in loose, humid soils and a tropical to subtropical climate shiny, heart-shaped, fleshy leaves and tiny, dot-like seeds attached to several fruiting spikes. It has a mustard-like odor when crushed.

Peperomia pellucida has been used as a food item as well as a medicinal herb. Although mostly grown for its ornamental foliage, the entire plant is edible, both cooked and raw. It is also said that it can be a good refrigerant.[8]

The analgesic properties of the plant seem to be related to its effect on prostaglandin synthesis.[8] It may have potential as a broad-spectrum antibiotic, as demonstrated in tests against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli*.[9] Chloroform extracts from dried leaves *of P. pellucida* have been shown to exhibit antifungal activity against *Trichophyton mentagrophytes* in vitro.[10]

Anti-inflammatory activity (in paw edema) and analgesic activity has been demonstrated in rats and mice .Although the plant can cause asthma-like symptoms in patients with known hypersensitivity reactions to the species, no clinical data have yet been reported on human toxicity.[11]

Mangifera indica, commonly known as mango, is a species of flowering plant in the sumac and poison ivy family Anacardiaceae. It is found in the wild in Bangladesh, India and Pakistan where it is indigenous and cultivated varieties have been introduced to other warm regions of the world. It is a large fruit-tree, capable of a growing to a height and crown width of about 100 feet and trunk circumference of more than twelve feet. The species appears to have been domesticated in India at around 2000 B.C.[12] The species was brought to East Asia around 400-500 BCE from India; next, in the 15th century to the Philippines; and then, in the 16th century to Africa and Brazil by the Portuguese. The species was described for science by Linnaeus in 1753. Mangiferin (a pharmacologically active hydroxylated xanthone C-glycoside) is extracted from mango at high concentrations from the young leaves (172 g/kg), bark (107 g/kg), and from old leaves (94 g/kg).[13] Leaf of *Mangifera indica* commonly known as mango (family *Anacardiaceae*), a large evergreen tree of tropical and subtropical region has been used by traditional medicine by a number of people for centuries. The leaves of *Mangifera indica* plant are used as an antidiabetic agent in Nigerian folk medicine, although when aqueous extract given orally did not alter blood glucose level in either normoglycaemic or streptozocin induced diabetic rats. However, antidiabetic activity was seen when the extract and glucose were administered simultaneously and also when the extract was given to the rats 60 minutes before the glucose. The results indicate that aqueous extract of *Mangifera indica* possess hypoglycaemic activity. This may be due to an intestinal reduction of the absorption of glucose.[14] Several studies in animal models with diabetes have shown that both short and long term hypoglycemic effect of mango leaves and other source were proven. Its mechanism for lowering glucose level is unknown, and however, some studies suggest facilitation of glucose uptake peripherally. Definitely potential hypolipidemic effects in diabetic rat have also been shown with mango leaves extract and other natural sources.[15]

MATERIALSANDMETHODS

Collection and Identification of plant materials

Fresh leaves of mango plants (M*angifera indica*) were harvested from School of Health Technology Akure, while fresh leaves of Sliver bush (*Peperomia pellucida*) were harvested from the female hostel of Adekunle Ajasin University Akungba-Akoko. These plant specimens were identified at the Forestry Herbarium, Ibadan (FHI) and later deposited as voucher materials at Adekunle Ajasin University (AAU) Herbarium with voucher numbers, *Mangifera indica* no 131 and *Peperomia pellucida* no 165.

Sample Preparation

The leaves of *M. indica* were thoroughly washed, air dried for two months and later blended into fine powder using an electric blending machine (Marlex CM/L7371373). While that of *Peperomia pellucida* were freeze dried (Lyophilized) using a Lyophilizer at the Biochemistry Laboratory of Adekunle Ajasin University Akungba.

The ground plant materials were weighed into 30%weight/weight (30g leaf extract and 70g growers mash) and 70%weight/weight (70g leaf extract and 30g growers mash). The control group was 100% growers mash. 100ml of honey was measured using a graduated cylinder into the weighed powdered extract and into 100% (100g) growers mash and the honey was thoroughly mixed with the powdered leaf extracts. This is to facilitate the eating of the extracts by the rats.

Animal Treatment

A total number of twelve albino rats were used; three groups with four animals in a group. The third group is the control group. Prior to the experiment, the animals were weighed and stabilized for a period of seven days by giving them water and growers mash prepared by Guinea feed Nig. Ltd. This was done to ascertain the

animals were in good state of health. Different weights of the prepared extracts were used to feed the rats for three consecutive days after which the rats were fasted over night for 12 hours. Clean water and 100% growers mash were used to feed the control group. After the administration of extract the rats were weighed. On the fourth day the rats were sacrificed and vital organs like testis, liver and kidneys were removed and processed for genetic expression.

Gene expression
The maps of the organs were made and were arranged into the PCR machine (Multigene Optimax) made by Labnet international Inc. prior to polymerization reaction.

RNA isolation
Tissues excised from each animal were homogenized in Eppendorf tubes containing 50ul Trizol reagent. 100µl gradient separation medium (chloroform) was added to homogenates, vortex and centrifuged for 30 minutes at 1500rpm and the supernatant was aspirated, which contains the RNA, into new labeled tubes. 100µl of precipitating medium (isoamyl alcohol) was added to the supernatant and vortexes for 30mins at 1500rpm, RNA is recovered in pellet form and the supernatant decanted. The concentration of total RNA was determined by UV absorbance spectrophotometry (JENWAY 6305) for RNA (mRNA) quantification and all samples were diluted to the same concentration.

Reverse transcription
Reverse transcription was performed by adding 2µL reverse transcriptase from the cocktail which contain (1) the random oligo primer (2) the dNTPs (3) the reverse transcriptase (4) the reverse transcriptase buffer and (5) nuclease-free water.

2µl reverse transcriptase was aliquoted into 20µl of total RNA across the samples for the conversion into complimentary DNA (cDNA). The sample is then incubated in the thermocycler running for 4 hours at 65-degree Celsius in a total reaction volume of 22µl for the conversion.

Polymerase Chain Reaction (PCR)
Synthesized cDNA was diluted in nuclease-free water and 5µL was aliquoted into primer-specific PCR cocktail. In total, a 5.0µL PCR reaction mix containing 5µL cDNA template, 2µL forward and reverse primers (Inqaba Biotech, South Africa) and 2µL PCR Master Mix. As a rule, β-actin specific primer was used to track basal gene expression in each representative animal. The PCR was carried out using multigen optimax PCR machine. PCR amplification was done under 30 cycles with each cycle consisting of denaturation, annealing and extension.

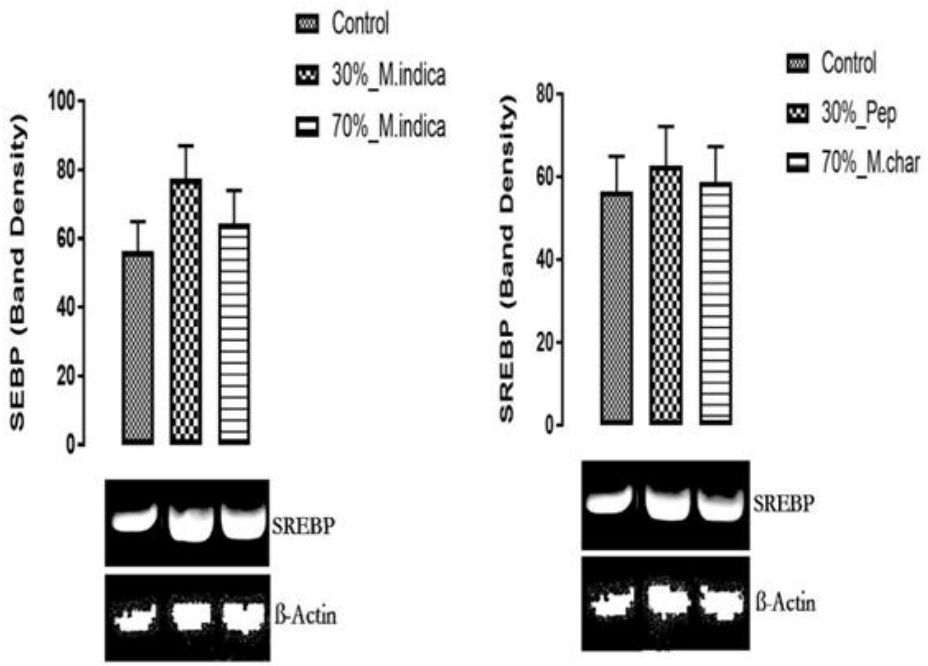
Agarose Gel Electrophoresis: PCR amplicons were submitted for densitometric run in agarose gel electrophoresis. Snapshots revealing the relative density of DNA bands were taken under UV-gel documentation.

Statistical Analysis of Data: Intensities of bands formed on agarose gel electrophoresis were quantified densitometrically using Image J software and the gene expression was normalized with β-actin gene, a basal gene. All data collected were subjected to one-way analysis of variance (ANOVA). The results were expressed as mean ± standard error of mean (mean ± SEM), and values of $p < 0.05$ (i.e., 95% Confidence Level) were considered statistically significant.

RESULTS

Sterol regulatory element binding protein (SREBP) was not significantly expressed by any of the extracts compared to the control. Luteinizing hormone receptor (LH-R) was significantly expressed in the testis by *M. indica* at 70% while with *P. pellucida;* it was significantly expressed at 30% concentration compared to the control, ($p<0.05$). Androgen –Receptor (And-R) was not expressed at all by any of the extracts compared to the control. Follicle stimulating hormone-Receptor (FSH-R) was significantly expressed by 70% *M. indica* and 30% *P. pellucida* compared to the control. In the testis of the normal rats, SREBP and androgen receptor were down regulated by the extracts, (Figures 1, 2, 3, and 4).

TNF-α was down regulated in the kidney by all the extracts. IL-1-alpha was significantly expressed by all the extract compared to the control, ($p<0.05$) Figure 5.



Key: M. indica= Mango (*Mangifera indica*), **Pep**= *Peperomia pellucida*, **M. char** = *Momordica foetida*. (**NB:** Applies to Figures.1-5)

Figure 1: The effects of plant extracts on the expression of sterol regulatory element binding protein (SREBP) on the testis of rats.

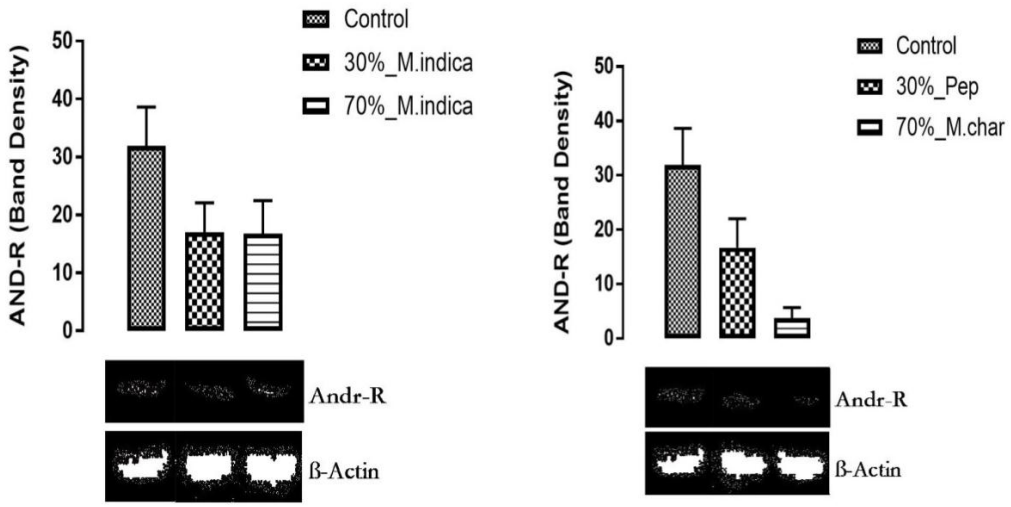


Figure 2: The effects of plant extracts on the expression of androgen receptor gene on the testis of rats.

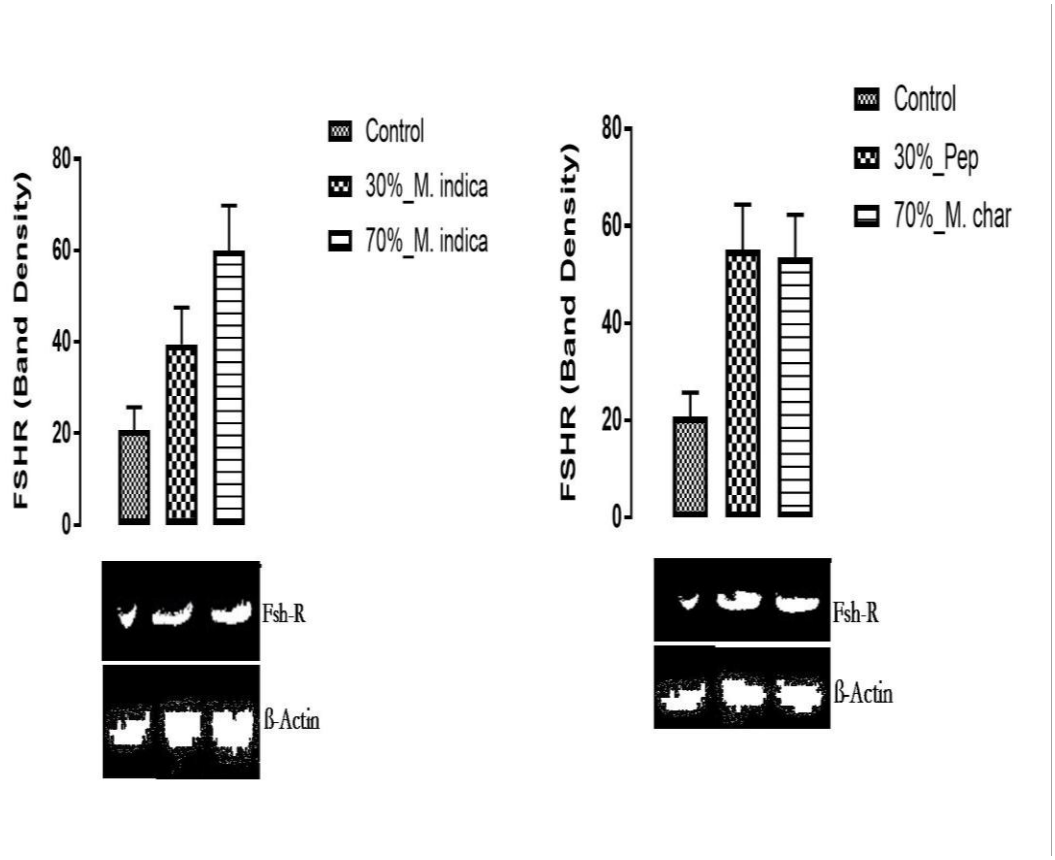


Figure 3: The effects of plant extracts on the expression of follicle stimulating hormone (FSH) receptor on the testis of albino rats.

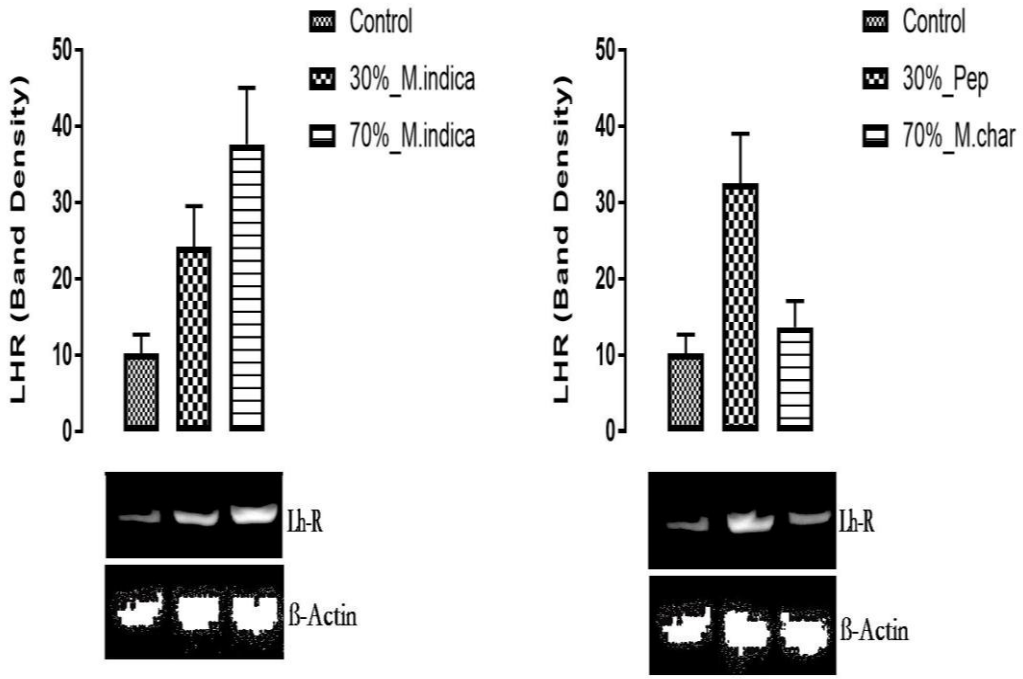


Figure 4: The effects of plant extracts on the expression of luteinizing hormone (LH) receptor on the testis of albino rats.

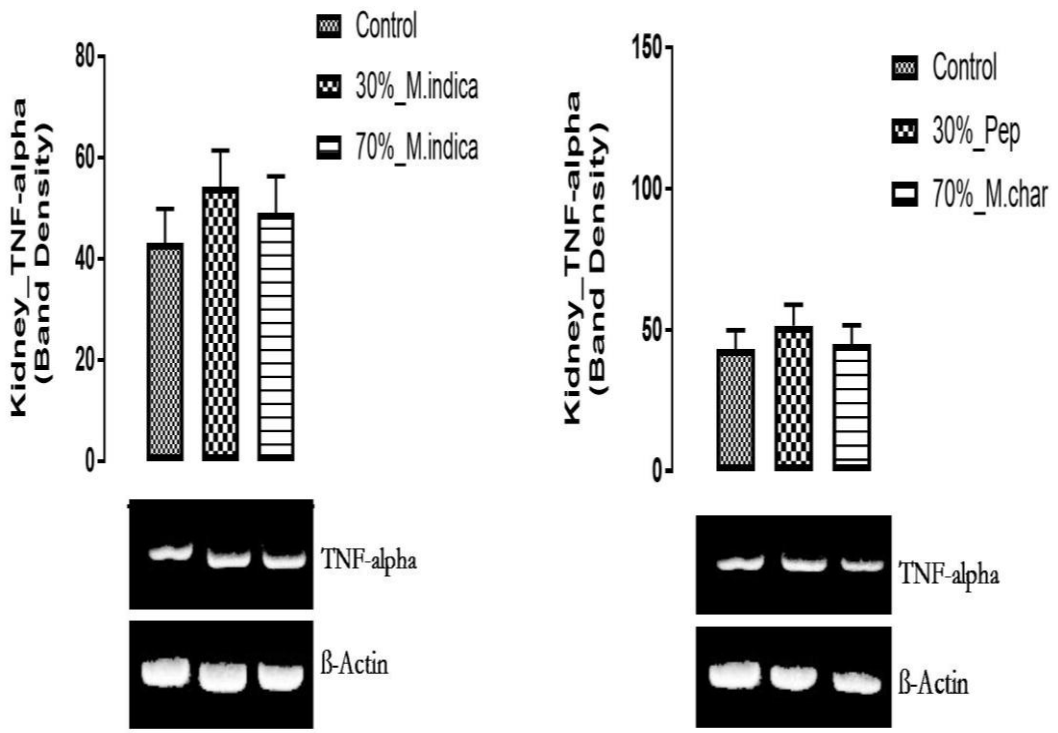


Figure 5: The effects of plant extracts on the expression of tumour necrosis factor-α (TNF-α) gene in the kidney of albino rats.

DISCUSSION AND CONCLUSION

Luteinizing hormone receptor (LH-R) has been reported to be found predominantly in the ovary and leydig cells of the testis where it plays pivotal role in testosterone production and support spermatogenesis. This trans-membrane receptor specifically acts to up-regulate the enzyme cholesterol side-chain cleaving enzyme, which leads to the greater conversion of cholesterol into androgen precursors required to make many steroid hormones, including testosterone and estrogens.[16] In this study the increase in LH receptor levels by *M. indica* and *P. pellucida* compared to the control may be due to low testosterone levels which then facilitated increased response of LH receptor as explained by the statement above. Many beneficial effects of medicinal plants on male reproductive function are associated with antioxidant effects.[16] This suggests that the potential of phytomedicine to improve male fertility is due to presence of antioxidants. Furthermore, antioxidants have been shown to improve various processes (spermatogenesis, steroidogenesis) of male reproductive function.[17,18] This result negates the work earlier done by some researchers; that the aqueous extract of mango leaves reduces male reproductive activity and can be used for birth control.

Androgen receptor is a type of nuclear receptor that is activated by binding either of the androgenic hormones (e.g. testosterone) in the cytoplasm and then translocating into the nucleus; it functions as a DNA-binding transcription factor that regulates gene expression. These extracts showed slight down regulation compared to the control, this then could safely be attributed to the fact that the active compounds in these herbs of interest may activate the steroidal, non-steroidal and N-terminal domain anti-androgens (which have been reported to antagonize the effects of androgen receptors) in the testis of their respective animals. The expression of And-R in the testis at this non-significant level as seen in the present study is not in concordant with works done by.[19, 20]

FSH receptor (FSHR) is a transmembrane receptor that interacts with the follicle-stimulating hormone (FSH), as its activation is necessary for the hormonal functioning of FSH. In the male, the FSH receptor has been identified on the sertoli cells that are critical for spermatogenesis. FSH binds specifically to receptors located on the membrane of sertoli cells.[21] In this study, FSH-R gene was expressed significantly in the testis of experimental rats fed with *M. indica* and *P. pellucida*.

FSH-R was significantly expressed in the experimental rats fed with extract of *M. indica*, this result indicates

that the leaf extract of *M. indica* and *P. pellucida* have the tendency to improve spermatogenesis thereby enhancing fertility in males. Even though some researchers have declared *M. indica* as anti-fertility plant and good as contraceptive agents[22,23], the Hindu scriptures call Mango the symbol of love, **fertility** and immortality. History has it that in India, mango leaves are used in wedding ceremonies in order to ensure that the couple bears many children.[24]

ETHICAL CLEARANCE: Ethical approval was obtained from Research and Development Center of Adekunle Ajasin University, Akungba-Akoko, Ondo State. Authors observed all ethical considerations in handling the animals used.

CONFLICT OF INTEREST: The authors declared that they have no conflicts of interest.

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