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Research Article
Biochemical and Hormonal Effects of *Nymphaea lotus* Aqueous Extract on Hyperprolactinemic Female Wistar Rats

Olubunmi Josephine Sharaibi and Anthony Jide Afolayan

Medicinal Plant and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, 5700 Alice, South Africa

Abstract

Background and Objective: *Nymphaea lotus* (*N. lotus*) is a medicinal plant used in traditional medicine for the management of hyperprolactinemia and infertility-related diseases. This aim of the study was to investigate the biochemical and hormonal effects of aqueous extract of *N. lotus* in metoclopramide-induced hyperprolactinemia in rats. **Methodology:** Hyperprolactinemia was induced by oral administration of 5 mg kg^{-1} b.wt., metoclopramide to female rats. This was accompanied by administration of graded doses (50, 100 and 200 mg kg^{-1} b.wt.,) of *N. lotus* to the rats daily for 21 days, while distilled water and bromocriptine were given to the hyperprolactinemia control and positive control, respectively. Biochemical and hormonal parameters were determined at the end of the experiment using the uterine homogenates and serum, respectively. **Results:** Significant increase ($p<0.05$) were observed in the serum prolactin and estradiol level, while follicle stimulating hormone (FSH), luteinizing hormone(LH) and progesterone witnessed reduction, following induction of hyperprolactinemia. Administration of aqueous extract of *N. lotus* significantly improved ($p<0.05$) the levels of these hormones especially prolactin and progesterone. It also tends to restore uterine glucose, cholesterol and protein concentration. **Conclusion:** Aqueous extract of *N. lotus* especially at dosage of 200 mg kg^{-1} b.wt., could ameliorate hyperprolactinemia in rats.

Key words: *Nymphaea lotus*, hyperprolactinemia, prolactin, follicle stimulating hormone, luteinizing hormone, progesterone, estradiol, glucose, cholesterol and protein concentrations

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Corresponding Author: Anthony Jide Afolayan, Medicinal Plant and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, 5700 Alice, South Africa Tel: +27635314474

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

World Health Organization (WHO) asserts that one out of every four couples in developing countries is affected by infertility[1]. Infertility can be due to myriad of factors such as genetic, anatomic, hormonal and immunological problems. It could also arise from secondary sources like sexually transmitted infections, surgery and obesity[2].

One of the hormonal causes of female infertility is hyperprolactinemia, which is an elevated level of serum prolactin. Prolactin is a peptide hormone secreted by the anterior pituitary gland which plays a pivotal role in a variety of reproductive functions. Its primary role is the secretion of milk and lactation, as well as in reproductive mammary development and parental behaviour[3]. On the contrary, excess prolactin can reduce the secretions of other pituitary gland hormones that are responsible for gametes development; these include follicle stimulating hormones (FSH) and luteinizing hormones (LH)[4]. Excess prolactin prevents the secretion of gonadotropic hormones; when the serum level of gonadotropin is reduced, the secretion of FSH and LH is reduced and this inhibits gamete production[5].

Several drugs especially dopamine agonist agents like bromocriptine, cabergoline, pergolide and quinagolide are usually the first choice for controlling hyperprolactinemia but their usage is associated with gastrointestinal, cardiovascular and neurological adverse effects[6]. This is why medicinal plants are being used as alternatives due to their efficacy, safety and easy accessibility[7]. Ethnobotanical survey of plants used in the treatment of hyperprolactinemia and related conditions in Lagos, Southwest of Nigeria implicated *Nymphaea lotus* as the best choice[8]. The phytochemical composition, antioxidant activities and safety of *Nymphaea lotus* leaf extracts have been reported by Afolayan *et al.*[9] and Sharaibi *et al.*[10]. The present study was designed to evaluate the effects of aqueous extract of *N. lotus* on hormonal concentrations and uterine biochemical parameters of metoclopramide-induced hyperprolactinemic female Wistar rats.

MATERIALS AND METHODS

Plant material: Fresh leaves of *Nymphaea lotus* were collected from Badagry Area of Lagos, Southwest Nigeria in June, 2012 and were identified by Mrs Sharaibi. Voucher specimen (LSH 2012/6) was prepared and deposited in the Lagos State University herbarium for proper documentation. The leaves were carefully rinsed under running water, air dried to constant weight in the laboratory and later pulverized before extraction.

Assay kits and chemicals: Assay kits for prolactin, progesterone, estradiol, follicle stimulating hormone and luteinizing hormone were supplied by Sigma-Aldrich Chemicals., Pomona/Kempton Park 1619, Johannesburg, South Africa. Assay kits for glucose, protein, cholesterol and alkaline phosphatase were procured from Randox Laboratories Ltd, Co-Atrim, United Kingdom. Metoclopramide and bromocriptine were bought commercially from Berea Pharmacy, East London, Eastern Cape, South Africa. All other reagents used were of analytical grade and were prepared using glass-distilled water.

Experimental animals: Female wistar rats weighing 220-250 g were obtained from the animal house of the School of Biological and Environmental Sciences, University of Fort Hare, 5700 Alice, South Africa. They were housed in clean plastic cages under standard environmental conditions of temperature (23±1°C) and relative humidity (45-50%) under a 12 h dark-light cycle. They were acclimatized to animal house conditions for 7 days before dosing and allowed free access to drinking water and standard pellets (Pioneer Food (Pty) Ltd, Huguenot, South Africa). All experimental protocols were approved by the Animal Ethics Committee of the University of Fort Hare, 5700 Alice, South Africa and according to the Guide for the Care and Use of Laboratory Animals[11].

Preparation of extract: A total of 100 g powdered plant material was extracted in 1.0 L distilled water for 48 h at 30°C on an orbital shaker (Stuart Scientific Orbital Shaker, UK) at room temperature. This was centrifuged at 1500 rpm for 5 min and the filtrate further filtered with Watman No 1 filter paper. It was then freeze dried using Virtis BenchTop (SP Sci-entific Series, USA) freeze dryer and the yield was 11.52 g. This was reconstituted in distilled water to give the required concentrations of 50, 100 and 200 mg kg^{-1} b.wt., used in this study.

Experimental design: Thirty female Wistar rats were broadly divided into two groups: (i) Normal control, which daily received 0.5 mL of distilled water (n = 5) and (ii) Experimental, which daily received 0.5 mL of 5 mg kg^{-1} b.wt., metoclopramide dissolved in distilled water (n = 25) to induce hyperprolactinemia. The experimental group was then further randomized into five groups of five animals each, which implies six groups in all. Group 1 received the distilled water alone; Group 2 were metoclopramide-induced hyperprolactinemic rats only; Groups 3-5 comprised metoclopramide-induced hyperprolactinemic rats administered 50, 100 and 200 mg kg^{-1} *N. lotus* extract

respectively. Group 6 consisted of metoclopramide-induced hyperprolactinemic rats administered bromocriptine (2.5 mg kg^{-1} b.wt.,). The extracts and drugs were suspended in distilled water and were orally administered for 21 days using orogastric tube and experiment was terminated on the 22nd day by humanely sacrificing the animals.

Preparation of serum and tissues: The animals were anaesthetized in a jar containing cotton wool soaked in diethyl ether. When the rats became unconscious, their neck region was quickly cleared of fur and skin to expose their internal jugular veins. The veins were slightly displaced (to prevent contamination of the blood with interstitial fluid) after which they were cut sharply with a sterile blade. The rats were then held head downwards, allowed to bleed into clean, dry centrifuge tubes. The blood samples were allowed to clot for 10 min at room temperature and subsequently centrifuged at 22.4 rpm for 10 min with Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, Essex, England). The sera were later aspirated with Pasteur pipette into sample bottles and used within 12 h for the hormonal assay.

The rats were thereafter quickly dissected in the cold; their uteri were excised and transferred into ice-cold 0.25 M sucrose solution. The uteri were freed of surrounding tissues, blotted with clean tissue paper and then weighed. This was then homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v)[12]. The homogenates were further centrifuged at 105.5 rpm for 15 min to obtain the supernatants which were kept frozen overnight at -20°C before being used for the various biochemical assays.

Determination of serum hormonal concentration: The serum hormonal concentration was carried out using a Roche E170 modular analytics immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany) as described by Ofem *et al.*[13] and de Almeida *et al.*[14].

Determination of uterine biochemical parameters: The method described by Aydin *et al.*[15] was used to determine the glucose, protein and cholesterol concentrations of the uterus. Alkaline phosphatase (ALP) concentration was determined using Piccolo x press automatic chemistry analyser (Abaxis Inc. Union City, CA 94587, USA).

Statistical analysis: Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, San Diego MA, USA). The data were subjected to one way analysis of variance (ANOVA) followed by Tukey test. All the results were expressed as mean±SEM (n = 5) and were considered statistically significant when $p<0.05$.

RESULTS

The effect of administration of aqueous extract of *N. lotus* on serum prolactin concentration of the animals was shown in Fig. 1. At the end of the administration of metoclopramide to the experimental group, the prolactin level in the experimental group was significantly higher ($p<0.05$) than the normal control. However, administration of *N. lotus* extract at all tested concentrations (50-200 mg kg^{-1} b.wt.,) as well as the standard drug (bromocriptine) significantly reduced ($p<0.05$) the concentration of the hormone to status similar to the normal control group.

The effects of administration of *N. lotus* extract on the concentration of other female reproductive hormones represented in Fig. 2 showed that the extract was not able to increase the concentration of the follicle stimulating hormone except at 200 mg kg^{-1} which was significantly higher ($p<0.05$) than the hyperprolactinemic control but lower than normal control (Fig. 2a). Luteinizing hormone also witnessed significant increase ($p<0.05$) following administration of 200 mg kg^{-1} extract and was similar to that of normal control

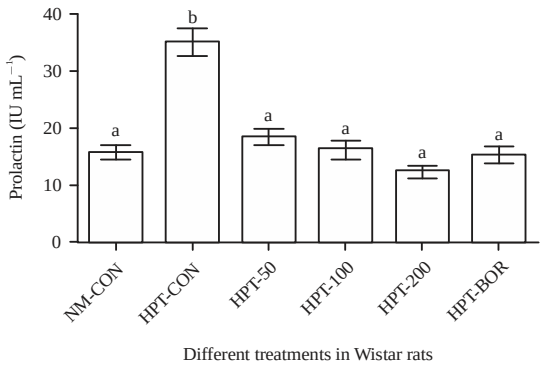

Fig. 1: Effect of administration of aqueous extract of *Nymphaea lotus* leaf on the serum prolactin concentration of metoclopramide-induced hyperprolactinemic rats.

NM-CON: Normal control, HPT-CON: Hyperprolactinemia control, HPT-50: Hyperprolactinemic rats treated with 50 mg kg^{-1} *Nymphaea lotus* extract, HPT-100: Hyperprolactinemic rats treated with 100 mg kg^{-1} *Nymphaea lotus* extract, HPT-200: Hyperprolactinemic rats treated with 200 mg kg^{-1} *Nymphaea lotus* extract, HPT-BCR: Hyperprolactinemic rats treated with 2.5 mg kg^{-1} bromocriptine. Values are mean±SEM of 5 rats per group. Bars carrying different superscripts are significantly different ($p<0.05$)

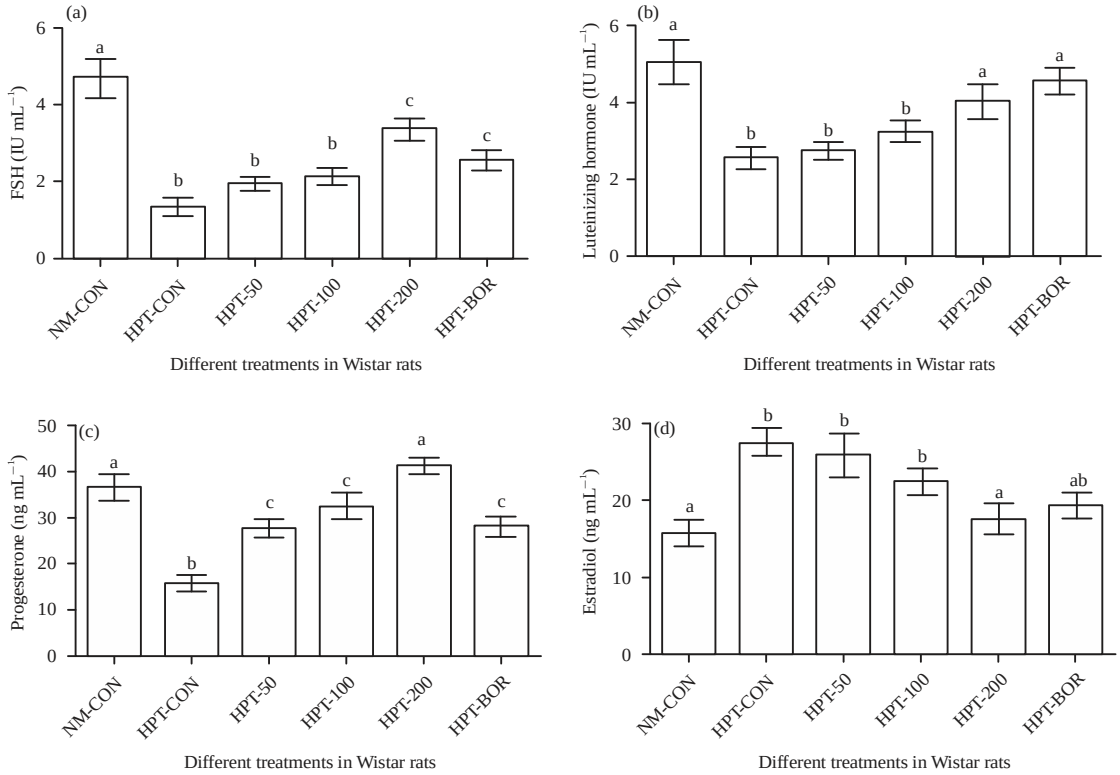

Fig. 2(a-d): Effect of administration of aqueous extract of *Nymphaea lotus* leaf on serum concentration of (a) Follicle stimulating hormone, (b) Luteinizing hormone, (c) Progesterone and (d) Estradiol in metoclopramide-induced hyperprolactinemic rats

NM-CON: Normal control, HPT-CON: Hyperprolactinemia control, HPT-50: Hyperprolactinemic rats treated with 50 mg kg^{-1} *Nymphaea lotus* extract, HPT-100: Hyperprolactinemic rats treated with 100 mg kg^{-1} *Nymphaea lotus* extract, HPT-200: Hyperprolactinemic rats treated with 200 mg kg^{-1} *Nymphaea lotus* extract, HPT-BCR: Hyperprolactinemic rats treated with 2.5 mg kg^{-1} bromocriptine. Values are mean±SEM of 5 rats per group. Bars carrying different superscripts are significantly different ($p<0.05$)

Table 1: Effect of administration of aqueous extract of *Nymphaea lotus* leaf on some uterine parameters of metoclopramide-induced hyperprolactinemia in rats

Groups	Glucose (mg/100 mg)	Protein (mg/100 mg)	Cholesterol (mg/100 mg)	Alkaline phosphatase (IU)
Normal control	2.95±0.52^{a}	25.67±3.27^{a}	0.75±0.03^{a}	1.25±0.06^{a}
Hyperprolactinemia control	1.56±0.67^{b}	12.50±1.56^{b}	0.23±0.01^{b}	3.65±0.25^{b}
Hyperprolactinemia+50 mg kg^{-1} NLE	4.15±0.32^{c}	15.15±2.67^{b}	0.18±0.05^{b}	4.12±0.22^{b}
Hyperprolactinemia+100 mg kg^{-1} NLE	3.56±0.71^{ac}	18.75±4.05^{c}	0.36±0.08^{c}	2.32±0.15^{c}
Hyperprolactinemia+200 mg kg^{-1} NLE	3.87±0.55^{ac}	17.29±3.03^{c}	0.45±0.03^{c}	3.27±0.06^{d}
Hyperprolactinemia+Bromocriptine	3.15±0.37^{ac}	21.27±5.87^{a}	0.56±0.05^{d}	2.56±0.12^{c}

Values are mean±SEM of 5 rats per group. Test values down the vertical columns carrying different superscripts are significantly different ($p<0.05$). NLE: *Nymphaea lotus* extract

as well as bromocriptine-treated animals (Fig. 2b). The extract caused dose-dependent increase in the serum progesterone concentration and produce effect (at all concentrations tested) that were significantly different ($p<0.05$) from the hyperprolactinemic control but comparable to the normal control (Fig. 2c). The concentration of estradiol that hitherto increased in the metoclopramide-induced hyperprolactinemic animals, faced decrease at all doses tested but was only significantly different ($p<0.05$) at 200 mg kg^{-1} compared to hyperprolactinemic control (Fig. 2d).

The effect of administration of aqueous extract of *N. lotus* on some uterine biochemical parameters of hyperprolactinemic rats was shown in Table 1. Administration of metoclopramide to the rats produced significant reduction ($p<0.05$) in the concentration of glucose, protein and cholesterol while elevating alkaline phosphatase. The extract (at all doses tested) significantly increased ($p<0.05$) concentration of glucose to a level similar to the normal control. Administration of 100 and 200 mg kg^{-1} extract to the hyperprolactinemic animals produced significant elevation

($p<0.05$) in protein and cholesterol concentration, though only bromocriptine elicited effect similar to the normal control. Though, the extract tends to lower the uterine alkaline phosphatase concentration in the hyperprolactinemic rats but the effect was not considerable and did not follow particular trend.

The effect of administration of *N. lotus* extract on some physical parameters of metoclopramide-induced hyperprolactinemic rats was represented in Fig. 2. Though, there were fluctuations but no difference exists in the amount of food and water intake across all groups of animals used in this study. There was significant reduction ($p<0.05$) in the body weight of the animals treated with 100 mg kg^{-1} b.wt., extract compared other groups while other groups were similar. Metoclopramide-induced hyperprolactinemia caused reduction in the uterine weights of the animals, while administration of the plant extract significantly increased ($p<0.05$), this index compared to the hyperprolactinemic control.

DISCUSSION

Hyperprolactinemia was the most common endocrine disorder productive of hypersecretion on the hypothalamic-pituitary axis, which occurs predominantly in young women (20–30%) and can lead to several abnormalities, including infertility[16]. Though prolactin was required for breast development and lactogenesis, its over-secretion constitutes reproductive disorder. Hyperprolactinemia tends to suppress the ovulatory cycle by inhibiting the secretion of follicle stimulating hormone (FSH) and gonadotropic-releasing hormones, which are necessary for ovulation[17]. Dopamine is a known prolactin inhibitory factor, which decreases secretion of prolactin from the anterior pituitary gland. The observed reduction in the serum prolactin levels in metoclopramide-induced hyperprolactinemic rats following administration of aqueous extract of *N. lotus*, suggests that the extract acts as dopamine agonist, which has high binding affinity for the dopamine receptors[18]. This result agreed with the reports of Chen *et al.*[19] and Ding *et al.*[20] in which the administration of plant extracts produced significant reduction ($p<0.05$) in prolactin serum levels in hyperprolactinemic women. However, Shoorideh *et al.*[21] and Siyahi *et al.*[22] reported that the administration of aqueous extracts of *Vitex agnus-castus* and *Foeniculum vulgare* produced a significant increase ($p<0.05$) in prolactin serum concentrations when compared with the control.

Follicle stimulating hormone (FSH) is essential for gonadal development and maturation at puberty, as well as gamete production. It stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells[14]. Reduction in the FSH level of the metoclopramide-induced hyperprolactinemic control may suggest gonadal dysfunction which delays maturation of ovarian follicles in the pre-ovulatory phase. Elevation of the FSH level following administration *N. lotus* (200 mg kg^{-1} b.wt.,) extract signifies the extract exerted its stimulatory effect on the anterior pituitary or hypothalamus because the secretion of the hormone is regulated by the gonadotropic releasing hormone of the hypothalamus[23]. This may increase production and maturation of ovarian follicles and thereby improves conception. Increase in FSH serum levels by the administration of aqueous extract of *Newbouldia laevis* was also reported by Egba *et al.*[24], but the report of Modupe[25], recorded a significant reduction in serum follicle stimulating hormone concentrations following the administration of a herbal decoction to female laboratory rats.

Luteinizing hormone stimulates secretion of sex steroids from gonads. Ovulation of mature follicles from the ovary is induced by a large surge of luteinizing hormone secretion during the pre-ovulatory periods[13]. Administration of metoclopramide may inhibit this release, thereby disrupting ovulation by reducing number of mature follicles or impaired oestrous cycle[26]. The rise in the luteinizing hormone level elicited by administration of the 200 mg kg^{-1} b.wt., extract may implies repair of the oestrous cycle and restoration of the normal ovulation in the animals. This will alleviates menstrual irregularities experienced in hyperprolactinemic women and promotes conception, thereby preventing infertility. Mboso *et al.*[27] reported no significant difference ($p<0.05$) serum luteinizing hormone concentrations of the experimental groups treated with the extract of *Ereromastax speciosa* when compared with the control.

Progesterone helps to regulate the monthly menstrual cycle, stimulate sexual desire, as well as prepare the body for conception and pregnancy[28]. Decrease in serum progesterone level by the metoclopramide administration in this study suggests impaired endometrial function, which disrupts normal secretion of special protein required to nourish an implanted fertilized egg and prenatal development[29]. The increase in serum levels of progesterone of *N. lotus* treated rats observed in this study was an indication that the extract ameliorated the damage caused to endometrium function, thus promoting reproduction. This may be due to the presence of steroids in the extract which were easily converted to progesterone[9,30]. This report was in agreement with the reports of Heidarifar *et al.*[31] and Moshfegh *et al.*[32] in which the administration of the extracts of *Anethum*

Table 2: Effect of administration of aqueous extract of *Nymphaea lotus* leaf on some physical parameters of metoclopramide-induced hyperprolactinemia in rats

Groups	Body weight (g)	Uterine weight (g)	Feed intake (g day^{-1})	Water intake (mL day^{-1})
Normal control	275.43±6.22^{a}	3.25±0.05^{a}	134.60±4.29^{a}	210.65±9.15^{a}
Hyperprolactinemia control	271.66±5.01^{a}	1.72±0.03^{b}	138.52±3.78^{a}	207.12±6.25^{a}
Hyperprolactinemia+50 mg kg^{-1} NLE	279.04±6.24^{a}	2.93±0.05^{c}	128.93±6.41^{a}	202.39±8.72^{a}
Hyperprolactinemia+100 mg kg^{-1} NLE	255.72±8.05^{b}	2.54±0.03^{d}	133.15±6.30^{a}	213.10±6.39^{a}
Hyperprolactinemia+200 mg kg^{-1} NLE	273.50±8.37^{a}	3.02±0.07^{c}	137.62±4.32^{a}	205.71±7.32^{a}
Hyperprolactinemia+Bromocriptine	269.70±7.82^{a}	2.95±0.04^{c}	129.13±7.15^{a}	217.61±8.65^{a}

Values are mean±SEM of 5 rats per group. Test values down the vertical columns carrying different superscripts are significantly different ($p<0.05$). NLE: *Nymphaea lotus* extract

graveolens and *Phoenix dactylifera* significantly increased progesterone serum concentrations in treated rats.

Estradiol stimulates the growth of the uterine lining, causing it to thicken during the pre-ovulatory phase of the oestrous cycle. In synergy with follicle stimulating hormone (FSH), estradiol stimulates granulose cell proliferation during follicular development, hence helps in ovulation and promotes fertility[29]. However, high serum estradiol concentration as observed in hyperprolactinemic control, was associated with hyperprolactinemia[18]. Administration of 200 mg kg^{-1} b.wt., of the *N. lotus* extract normalized the serum estradiol level in the animals to a level similar to the normal control. This may promotes ovulation, zygote implantation and subsequent maintenance of pregnancy[33]. Reduction in estradiol serum concentrations by the extracts of medicinal plants were also reported by Modupe[25] and Poorfarid *et al*.[34]. However, the reports of Heidarifar *et al*.[31] and Hosseini *et al*.[35] were on the contrary.

Uterine biochemical parameters such as glucose, protein, cholesterol and alkaline phosphatase concentration can be used as markers to determine functional capacity of the female reproductive system. Metoclopramide-induced uterine dysfunction by decreasing concentration of glucose, cholesterol and protein, while increasing alkaline phosphatase (Table 1). The elevation in uterine cholesterol level of the animals following *N. lotus* extract administration could imply stimulation of steroidogenesis, thereby leading to increased steroid hormones' concentration. Increase in the glucose concentration of the uterus may suggest availability of required energy needed for the normal functioning of the reproductive system. This could also account for the rise in uterine protein level, which in turn improves conception and reproduction. Alkaline phosphatase is a membrane-bound enzyme that could be used to assess the integrity of plasma membrane of the uterus. Its increase in all the treatment groups may suggest increased synthesis of plasma membrane proteins during repairs of the damage caused by metoclopramide. However this may improve adequate mobilization of carbohydrate and lipid metabolites needed by the gonad.

The significant increase in the weight of the uterus (Table 2) of the animals treated with *N. lotus* extract may be attributed to increased secretory activity of the organ. This was also corroborated by increase in uterine glucose, protein and cholesterol levels (Table 1). Food and water are essential for life and are required for the growth and development of all organisms. The fact that there was no difference in food and water intake of all groups of the animals, suggests metoclopramide, bromocriptine as well as the *N. lotus* extract did not affect the sense of taste or appetite of the animals. This may account for the similarity in the body weights of the animals with exception of the animals that received 100 mg kg^{-1} b.wt., of the extract.

CONCLUSION

The implication of this study is that the administration of aqueous extract of *N. lotus* can ameliorates hyperprolactinemia. It can also improve the activities of other reproductive hormones, as well as biochemical parameters, which could enhance conception and reproduction. This study finds applications in the development of novel drugs that can be used to treat hyperprolactinemia. However, further investigation of the effects of aqueous extract of *Nymphaea lotus* on female reproductive hormones using human subjects is recommended. The findings from this study support the folkloric usage of this plant in the treatment of hyperprolactinemia and related disorders.

SIGNIFICANCE STATEMENT

Excess prolactin negatively modulates other female reproductive hormones and aqueous extract of *Nymphaea lotus* produced a baseline reduction of serum prolactin in female Wistar rats.

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REFERENCES

1. Wiersema, N.J., A.J. Drukker, M.B.T. Dung, G.H. Nhu, N.T. Nhu and C.B. Lambalk, 2006. Consequences of infertility in developing countries: Results of a questionnaire and interview survey in the South of Vietnam. J. Translational Med., Vol. 4. 10.1186/1479-5876-4-54.
2. Larsen, S.H., G. Wagner and B.L. Heitmann, 2007. Sexual function and obesity. Int. J. Obesity, 31: 1189-1198.
3. Torre, D.L. and A. Falorni, 2007. Pharmacological causes of hyperprolactinemia. Ther. Clin. Risk Manage., 3: 929-951.
4. Melmed, S., F.F. Casanueva, A.R. Hoffman, D.L. Kleinberg, V.M. Montori, J.A. Schlechte and J.A.H. Wass, 2011. Diagnosis and treatment of hyperprolactinemia: An Endocrine Society clinical practice guideline. J. Clin. Endocrinol. Metab., 96: 273-288.
5. Grattan, D.R., C.L. Jasoni, X. Liu, G.M. Anderson and A.E. Herbison, 2007. Prolactin regulation of gonadotropin-releasing hormone neurons to suppress luteinizing hormone secretion in mice. Endocrinology, 148: 4344-4351.
6. Colao, A., A. Di Sarno, E. Guerra, M. de Leo, A. Mentone and G. Lombardi, 2006. Drug insight: Cabergoline and bromocriptine in the treatment of hyperprolactinemia in men and women. Nat. Clin. Pract. Endocrinol. Metab., 2: 200-210.
7. Hasani-Ranjbar, S., H. Vahidi, S. Taslimi, N. Karimi, B. Larijani and M. Abdollahi, 2010. A systematic review on the efficacy of herbal medicines in the management of human drug-induced hyperprolactinemia; Potential sources for the development of novel drugs. Int. J. Pharmacol., 6: 691-695.
8. Sharaibi, O.J., O.T. Ogundipe, A.J. Afolayan and D.O. Aworinde, 2014. Ethnobotanical survey and phytochemical analysis of medicinal plants used for the treatment of hyperprolactinemia in Lagos State, Nigeria. J. Med. Plants Res., 8: 1284-1288.
9. Afolayan, A.J., O.J. Sharaibi and M.I. Kazeem, 2013. Phytochemical analysis and *in vitro* antioxidant activity of *Nymphaea lotus* L. Int. J. Pharmacol., 9: 297-304.
10. Sharaibi, O.J., O.T. Ogundipe, O.A. Magbagbeola, M.I. Kazeem and A.J. Afolayan, 2015. Acute and sub-acute toxicity profile of aqueous leaf extract of *Nymphaea lotus* Linn (nymphaeaceae) in wistar rats. Trop. J. Pharm. Res., 14: 1231-1238.
11. NAS., 2011. Guide for the Care and Use of Laboratory Animals. 8th Edn., National Academic Press, Washington, Pages: 246.
12. Yakubu, M.T., O.S. Awotunde, O.T. Ajiboye, A.T. Oladiji and M.A. Akanji, 2011. Pro-sexual effects of aqueous extracts of *Massularia acuminata* root in male Wistar rats. Andrologia, 43: 334-340.
13. Ofem, O.E., A.B. Antai, N.M. Essien and V.O. Oka, 2014. Enhancement of some sex hormones concentrations by consumption of leaves extract of *Viscum album* (mistletoe) in rats. Asian J. Med. Sci., 5: 87-90.
14. Imeida, B.E., J.E. Oliveira, R. Damiani, S.L. Dalmora, P. Bartolini and M.T.C.P. Ribela, 2011. A pilot study on potency determination of human follicle-stimulating hormone: A comparison between reversed-phase high-performance liquid chromatography method and the *in vivo* bioassay. J. Pharm. Biomed. Anal., 54: 681-686.
15. Aydin, M., A. Cevik, F.M. Kandemir, M. Yuksel and A.M. Apaydin, 2009. Evaluation of hormonal change, biochemical parameters and histopathological status of uterus in rats exposed to 50-Hz electromagnetic field. Toxicol. Ind. Health, 25: 153-158.
16. Rossi, A.G.Z., R.C.T. Gomes, M. de Jesus Simoes, R. dos Santos Simoes, P.B. Oliveira, J.M. Soares and E.C. Baracat, 2010. Effects of metoclopramide-induced hyperprolactinemia on the prolactin receptor of murine endometrium. Fertil. Sterility, 93: 1643-1649.
17. Fitzgerald, P. and T.G. Dinan, 2008. Prolactin and dopamine: What is the connection? A review. J. Psychopharmacol., 22: 12-19.
18. Yuan, H.N., C.Y. Wang, C.W. Sze, Y. Tong and Q.R. Tan *et al*., 2008. A randomized, crossover comparison of herbal medicine and bromocriptine against risperidone-induced hyperprolactinemia in patients with schizophrenia. J. Clin. Psychopharmacol., 28: 264-270.
19. Chen, Z., G. Wang, X. Wang, L. Li and D. Xiao, 2008. Effects of Zhuangyang capsules on the serum prolactin of schizophrenic patients treated with risperidone. Med. J. Wuhan Univ., 29: 798-800.
20. Ding, Y., H.Z. Qian and Y.Q. Wang, 2008. Effect of tongdatang serial recipe on antipsychotic drug-induced galactorrhea-amenorrhea syndrome. Zhongguo Zhong Xi Yi Jie He Za Zhi, 28: 263-265, (In Chinese).
21. Shoorideh, Z.M., M. Azadbakht, A. Zarifkar, A. Jafari and S. Hossienie, 2007. The effect of *Vitex agnus castus* folio extract on serum prolactin concentration of female rats in Gastation. Iran. J. Biol., 20: 99-109.
22. Siyahi, M., A. Shiravi and N.M. Heydari, 2009. Effect of hydro extract of fennel on prolactine and lactation in female Rat (wistar). J. Anim. Sci., 1: 55-63.
23. El-Kashoury, A.A., A.F. Salama, A.I. Selim and R.A. Mohamed, 2010. Chronic exposure of dicofol promotes reproductive toxicity in male rats. Life Sci. J., 7: 5-19.
24. Egba, S.I., G.I. Sunday and E.G. Anaduaka, 2014. The effect of oral administration of aqueous extract of newbouldia laevis leaves on fertility hormones of male albino rats. J. Pharm. Biol. Sci., 9: 61-64.
25. Modupe, A.E., 2015. Effects of oral administration of a decoction on serum levels of leutinizing hormone, follicle stimulating hormone, progesterone and estradiol in female dutch-white rabbits. Res. J. Med. Plants, 9: 141-145.

26. Rahman, S.A., N.A. Ahmad, N.H.A. Samat, S. Zahri, A.R. Abdullah and K.L. Chan, 2017. The potential of standardized quassinoid-rich extract of *Eurycoma longifolia* in the regulation of the oestrous cycle of rats. Asian Pac. J. Trop. Biomed., 7: 27-31.
27. Mboso, O.E., E.U. Eyong, P.E. Ebong, A. Iwara and M. Odey, 2013. The effects of the ethanolic extract of *Ereromastax speciosa* leaf on the serum levels of leuteinizing hormone, follicle stimulating hormone, progesterone and estradiol in female pubertal rats. Ann. Biol. Res., 4: 136-140.
28. Montaserti, A., M. Pourheydar, M. Khazaei and R. Ghorbani, 2007. Anti-fertility effects of *Physalis alkekengi* alcoholic extract in female rat. Iranian J. Reprod. Med., 5: 13-16.
29. Hsia, S.M., C.L. Yeh, Y.H. Kuo, P.S. Wang and W. Chiang, 2007. Effects of adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf.) hull extracts on the secretion of progesterone and estradiol *in vivo* and *in vitro*. Exp. Biol. Med., 232: 1181-1194.
30. Noguchi, E., Y. Fujiwara, S. Matsushita, T. Ikeda, M. Ono and T. Nohara, 2006. Metabolism of tomato steroidal glycosides in humans. Chem. Pharm. Bull., 54: 1312-1314.
31. Heidarifar, R., H. Farahani, M. Mirizadeh, A. Yousefi and M. Dolatshahi *et al*., 2015. The effect of hydroalcoholic extract of *Anethum graveolens* (Dill) on serum estrogen and progesterone level in female rats. Qom Univ. Med. Sci. J., 9: 42-49.
32. Moshfegh, F., J. Baharara, F. Namvar, S. Zafar-Balanezhad, E. Amini and L. Jafarzadeh, 2016. Effects of date palm pollen on fertility and development of reproductive system in female Balb/C mice. J. HerbMed Pharmacol., 5: 23-28.
33. Hadley, M.E., 2000. Hormones and Female Reproduction Physiology. In: Endocrinology, Hadley, M.E. (Ed.). Vol. 141, 5th Edn., Prentice Hall Inc., Englewood Cliffs, New Jersey, pp: 445-472.
34. Poorfarid, M., J.H. Karimi and F. Houshmand, 2013. The effects of *Aloe vera* sap on progesterone, estrogen and gonadotropin in female rats. J. Jahrom Univ. Med. Sci., 10: 6-10.
35. Hosseini, E., M. Frozanfar and A. Payehdar, 2013. The effect of hydroalcoholic extract of purslane on serum concentration of esterogen, progesterone, prolactin and gonadotropins in mature female rats. J. Shahrekord Univ. Med. Sci., 15: 12-21.