



Effects of ethanol extract of *Ricinus Communis* seed on serum Prolactin, LH and FSH levels in Metoclopramide-induced Hyperprolactinemic female albino rats.

Agbai Emmanuel Onuka^{1*}, Nwafor Arthur²

1. Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Madonna University, PMB 48 Elele, Rivers State, Nigeria.

2. Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, PMB 5323, Choba, Port Harcourt, Rivers State, Nigeria.

ABSTRACT

This present study was undertaken to determine the effects of *ricinus communis* ethanol extract on serum prolactin and gonadotrophin levels after administering 10mg/kg (body weight) of metoclopramide in female albino rats. A total of thirty female albino rats divided into 5 groups (Groups I, II, III, IV and V) consisting of 6 rats in each group were used for the study. Group I served as Control, while II, III, IV and V served as experimental groups. The experimental groups received 10mg/kg of metoclopramide (i.v.) 4 times daily for 4 weeks. Groups III, IV and V were given respectively 200mg/kg/day, 500mg/kg/day and 900mg/kg/day of *ricinus communis* orally for 4 weeks. Group I received distilled water and normal rat chow. Results showed statistically significant decrease in prolactin level at $P < 0.05$ between Group I (13.38 ± 0.92 ng/ml), III (18.73 ± 4.78 ng/ml), IV (17.53 ± 3.33 ng/ml) and V (16.95 ± 1.20 ng/ml) compared to Group II (44.70 ± 2.77 ng/ml). There was statistically significant increase in serum LH level between Group I (CONT) (7.07 ± 0.71 mIU/ml), Group III (7.82 ± 0.88 mIU/ml), Group IV (8.44 ± 0.53 mIU/ml) and Group V (8.88 ± 0.72 mIU/ml) at $P < 0.05$ compared to Group II (6.42 ± 1.14 mIU/ml) at $P < 0.05$. There was also statistically significant increase in serum FSH level between Group III (6.71 ± 1.46 mIU/ml), Group IV (6.84 ± 0.59 mIU/ml), and Group V (7.87 ± 0.41 mIU/ml) at $P < 0.05$ compared Group I (CONT) (5.20 ± 0.9 mIU/ml) compared to Group II (4.85 ± 0.70 mIU/ml). Data suggested that *ricinus communis* extract reduced serum prolactin levels and increased serum LH and FSH levels respectively in metoclopramide-induced hyperprolactinemic female rats.

Keywords. *ricinus communis*. hyperprolactinemia. prolactin. luteinizing hormone. follicle stimulating hormone. metoclopramide.

*Corresponding Author Email: emmanuelagbai207@yahoo.com

Received 01 January 2014, Accepted 07 February 2014

Please cite this article in press as: Agbai EO *et al.*, Effects of ethanol extract of *Ricinus Communis* seed on serum Prolactin, LH and FSH levels in Metoclopramide-induced Hyperprolactinemic female albino rats. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Ricinus communis (Linn), commonly called castor bean, belongs to the family *Euphorbiaceae*. Different parts of the plant have been reported to have several medicinal values. The plant's leaf extract is hepatoprotective¹. It is a potent phytomedicine for diabetics². The seed extract has a strong antioxidant activity³, contraceptive effect in females⁴, and in males⁵ and decreases testosterone and sperm functions^{6,7}. The oil from the seed of *ricinus communis* plant was used in inducing labor at term^{8,9}. The methanol extract of *ricinus communis* seed was found to prevent implantation and when implantation occurred, it induced abortion in female guinea pigs¹⁰. It has also been shown to prolong the estrous cycle with a marked effect on the diestrous phase¹⁰.

Metoclopramide is an antiemetic and gastroprokinetic agent. It is commonly used to treat nausea and vomiting. The mechanisms of action are complex and involve 5-hydroxytryptamine (5HT₄-) receptor agonism, vagal and central 5-hydroxytryptamine (5HT₃-) antagonism, and possible sensitization of muscarinic receptors on smooth muscles, in addition to dopamine receptor antagonism both centrally and peripherally¹¹. Metoclopramide is used to induce hyperprolactinemia in mice^{12,13}. Hyperprolactinemia is a common endocrinological disorder that may be caused by several physiological and pathological conditions¹⁴. Most symptoms of hyperprolactinemia involve the reproductive system and are due to both a direct action of prolactin on target tissues and indirect effects mediated by the decrease in gonadotropin pulsatile secretion that leads to gonadal dysfunction. Therefore, this study was undertaken to determine the effect of *ricinus communis* on prolactin and gonadotropin levels in metoclopramide-induced hyperprolactinemia in female albino rats.

MATERIALS AND METHODS

Thirty female albino wistar rats (150 - 200 g) were purchased from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka. They were assigned into 5 groups (n=6) and housed in a wire mesh cage (under temperature of 25°C - 30°C, 14 hours light and 10 hours dark cycle) in the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Madonna University Elele, Rivers State. They were acclimatized for two weeks. The rats were fed with normal rat chow and had access to tap water *ad libitum*. The Animal Ethical Committee of the University approved all the protocols of the study.

Experimental grouping of animals

Hyperprolactinemia was induced by intravenous (i.v.) injection of metoclopramide hydrochloride 10mg/kg body weight 4 times daily for four weeks. During the experiment the

animals were weighed and randomly selected into four experimental groups. Control group: (Group 1) received distilled water and normal rat chow. Experimental Group II received 10mg/kg of metoclopramide only. Experimental Group III received 10mg/kg of metoclopramide plus oral administration 200 mg/kg of *ricinus communis* extract. Experimental Group IV received 10mg/kg of metoclopramide plus 500 mg/kg of *ricinus communis* extract. Experimental Group V received 10mg/kg of metoclopramide plus 900 mg/kg of *ricinus communis* extract^{15,16}

Extract preparation

The outer coating (husks) of the seeds were manually removed and the residual flesh. The residual flesh was sundried for one week and ground in a grinder into pulp. The wet ground pulp (736 g) was extracted by maceration with ethanol to afford pale yellow oils (274 g) and whitish scums. The pale yellow oily form was suspended for 48 hours in 90 % ethanol to remove excess oil and extracted using a mechanical stirrer after which it was filtered with Whatman filter paper (No 1). The filtrate was then concentrated to dryness at 35°C in an electric oven (gallenkamp) for 24 hours. It produced a semi-solid mass when dried and stored in an air tight container in the refrigerator below 10°C. 1.1 g of the extract is then measured using an electric weighing balance and then dissolved into 11 ml of distilled water to give a stock solution of 100mg/ml. The extract was administered orally using a 2 ml syringe without needle. This was done carefully to prevent damage of the alimentary canal of the rats.

Sample collection

At the end of four weeks experiment, the animals were sacrificed under anesthesia with the use of chloroform. Blood was obtained via cardiac puncture and was put in a labeled EDTA anticoagulant bottle for Enzyme-linked Immunosorbent Assay of prolactin, FSH and LH.

Test for prolactin.

The test tubes were labeled appropriately, 4 tubes for plasma from test animals and 4 tubes for control animals. 150µl of plasma and 200µl of enzyme conjugate reagent were dispensed into each of the four tubes and gently mixed for 10 minutes and incubated at room temperature for 45 minutes. The incubation mixture was removed and rinsed with distilled water five times. The tubes were struck onto absorbent paper to remove all residual water droplets. 100µl of TMB reagent was added into each test tube and gently mixed for 10 seconds, and incubated at room temperature in the dark for 20 minutes. The reaction was stopped by adding 100µl of stop solution to each and gently mixed for 30 minutes. The OD was read at 450nm with a microtitre plate reader within 15 minutes.

Test for FSH and LH

100µl of plasma from test animals were pipette into each of the 4 test tubes and the test tubes were labeled A, B, W and X. The same procedure was repeated for plasma from control animals and the test tubes labeled C, D, Y and Z. Tubes A-D used to test for FSH while tubes W-Z were used for LH. 100µl of FSH EIA magnetic antibody and 100µl of LH EIA magnetic antibody was added to tubes A-D and W-Z respectively. The tubes were then covered with aluminum film and briefly water mixed. After mixing, the tubes were transferred to the water bath. Tubes A-D was allowed to incubate for 15 minutes and tubes W-Z for 30 minutes. The temperature of the water bath was 37°C. To separate the hormone bound to magnetic particles from other components of plasma, the samples were washed. 500µl of diluted FSH/LH EIA wash buffer were briefly water mixed. The rack of tubes were placed on a magnetic separator and allowed for 5 minutes. After which the supernatant liquid was decanted by inverting the rack and separator.

Labeled antibody reaction

The rack was removed from magnetic separator and 250µl of diluted FSH EIA labeled antibody was added to tubes A-D and 300µl of diluted LH EIA labeled antibody was added to tubes W-Z. All test tubes were then covered and vortex mixed. After mixing, the tubes were transferred to the water bath. Tubes A-D were left to incubate for 60 minutes and tubes W-Z for 2 hours, after which all tubes were then washed twice as described above. The tubes were washed twice to ensure that all unbound labeled antibody were removed.

Colour development step

The tubes were removed from the magnetic separator. 500µl of substrate was pipette into all test tubes plus one empty tube that served as the substrate blank tube. The tubes were covered and vortex mixed. After mixing, the tubes placed into a water bath. Tubes A-D were left for 30 minutes and tubes W-Z for 1 hour, after which 1ml of diluted stop buffer was added to all nine tubes and the test tubes were briefly vortex mixed. The tubes were then placed in the magnetic separator for a minimum of 10 minutes.

Statistical Analysis

Results are expressed as mean \pm SEM. Statistical significance of the differences observed between control and experimental groups (ANOVA) was evaluated by Turkey's multiple comparison at $P < 0.05$.

RESULTS AND DISCUSSION

Group I (CONT) (13.38 ± 0.92 ng/ml) was statistically significant ($P < 0.05$) in prolactin level compared to Group II (44.70 ± 2.77 ng/ml). Group II (44.70 ± 2.77 ng/ml) was statistically

significant ($P < 0.05$) compared to other Groups [Group III (18.73 ± 4.78 ng/ml), Group IV (17.53 ± 3.33), and Group V (16.95 ± 1.20) ng/ml]. However, there was no statistically significant difference in serum prolactin between Group I compared with Groups III, IV, and V at $P > 0.05$.

There was statistically significant difference in LH between Group I (CONT) (7.07 ± 0.71 mIU/ml) compared to Group II (6.42 ± 1.14 mIU/ml) at $P < 0.05$. There was also statistically significant difference in LH between Group II (6.42 ± 1.14 mIU/ml) compared to Group III (7.82 ± 0.88 mIU/ml), Group IV (8.44 ± 0.53 mIU/ml) and Group V (8.88 ± 0.72 mIU/ml) at $P < 0.05$. There was no statistically significant difference in LH between Group I compared with Groups III, IV, and V at $P > 0.05$.

There was statistically significant difference in FSH between Group I (CONT) (5.20 ± 0.9 mIU/ml) compared to Group II (4.85 ± 0.70 mIU/ml) at $P < 0.05$. There was statistically significant increase in Group III (6.71 ± 1.46 mIU/ml), Group IV (6.84 ± 0.59 mIU/ml), and Group V (7.87 ± 0.41 mIU/ml) at $P < 0.05$ compared to Group I and II.

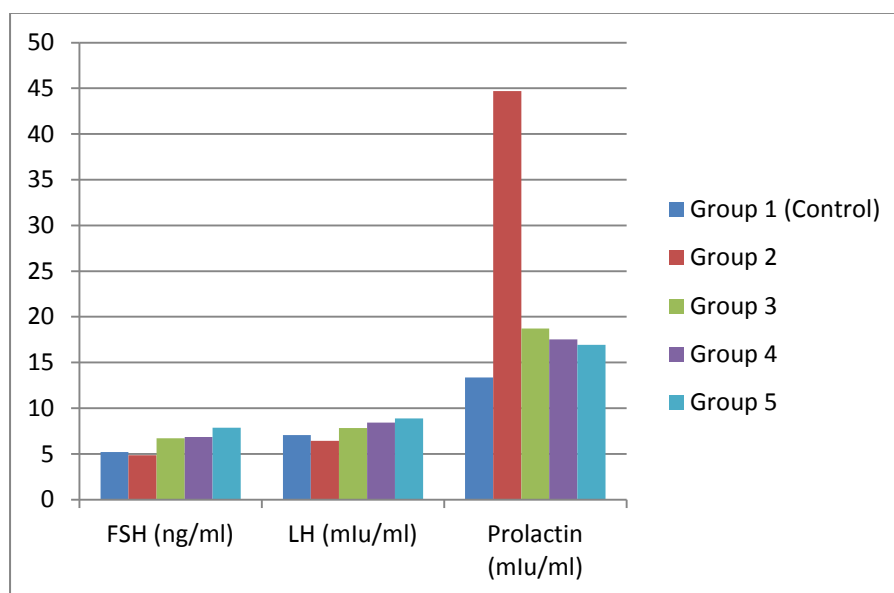


Figure 1. Effect of *ricinus communis* extract on serum prolactin, LH and FSH in metoclopramide-induced hyperprolactinemia

Studies have suggested that hyperprolactinemia directly affects a hypothalamic site which ultimately alters the luteinizing hormone releasing hormone pulse generator, thereby changing the secretion of luteinizing hormone releasing hormone¹⁷. Chronic hyperprolactinemia decreased luteinizing hormone secretion by primarily decreasing gonadotropin-releasing hormone secretion¹⁸. Report that hyperprolactinemia decreased gonadotropin pulsatile secretion

corroborated with result in figure 1 which showed that rats administered only metoclopramide decreased LH and FSH levels respectively in contrast to rats that received different doses of *ricinus communis* extract which increased LH and FSH in dose-dependent manner. Since studies have shown that hyperprolactinemia decreased gonadotropin level and directly decreased LH secretion^{14, 17, 18}, therefore, the increase in LH and FSH could be due to the lowering effect of *ricinus communis* extract on prolactin level after metoclopramide administration in this present study as evidenced by (Figure 1).

Seed extract of castor bean has been reported to possess high antifertility activity in female reproductive system, which is due to progestational activity and alternation in oestrogen/progesterone balance as well as direct effect on the uterus and fallopian tube⁴ and prevents ovulation in humans¹⁹ Thus, it is credible to suggest that *ricinus communis* extract reduced hyperprolactinemia caused by metoclopramide, and this reduction of prolactin by the extract may alter hyperprolactinemic- lowering effect on LH and FSH levels thereby resulted in an increase in gonadotropin levels. The mechanism of action of *ricinus communis* extract lowering-effect on gonadotropins may be dependent on the prolactin action.

CONCLUSION:

Hyperprolactinemia altered pituitary gonadotrophins which can impair fertility. Therefore, it can be concluded that *ricinus communis* extract reduced hyperprolactinemia with an increase in gonadotrophins and can be employed in the treatment of hyperprolactinemia in women.

REFERENCES

1. Visen PKS, Shukla B, Patnaik GK, Tripathi SC, Kulshreshtha DK, Srimal RC, Dhawan BN. Hepatoprotective activity of *ricinus communis* leaves. *Pharmaceutical Biology* 1992; 30(4):241-250
2. Shokeen P, Anand P, Murali YK, Tandon V. Antidiabetic activity of 50% ethanolic extract of *ricinus communis* and its purified fractions. *Food and Chemical Toxicology* 2008; 46:3458-3466
3. Williamson EM. In *Major herbs of ayurveda*, 1st edition, London, UK: Churchill Livingstone, 2001, p252-254
4. Okwuasaba FK, Das SC, Isichei CO, Ekwenchi MM, Onuruvwe O, Olayinka AO, Uguru VE, Dafur SJ, Ekwere EO, Parry O. Pharmacological studies on the antifertility effects of RICOM-1013-J from *ricinus communis var minor* and preliminary clinical studies on women volunteers. *Phytotherapy Research* 1997; 11(8):547-551

5. Nath S, Das B, Chetia P, Saikai R, Dutta Choudhury M, Sharma GD. Ricinoleic acid as male contraceptive. An in silico study. In Conference abstracts of the “International Conference on Recent Advances in Bioinformatics” held in KIIT University, Bhubaneswar, Orissa India. 2010; 53:74-75
6. Raji Y, Oloyo AK, Morakinyo AO. Effect of methanol extract of *ricinus communis* seed on reproduction of male rats. Asian J Androl 2006; 8(1):115-121
7. Roychoudhury S, Massanyi P, Slamecka J, Chilebec I, Trandzik J, Bulla J, Okab AB, Taha TA, Salem MH, Ayoub MA. In vitro gossypol induced spermatozoa motility alterations in rabbits. Journal of Environmental Science and Health 2009; 44(7):730-741
8. Garry D, Figueroa R, Guillaume, Cucco V. Use of castor oil in pregnancies at term. Altern Ther Health Med 2000; 6:77-79
9. Kelly AJ, Kavangh J, Thomas J. Castor oil, bath and/or enema for cervical priming and induction of labor. Cochrane Database Syst Rev 2001; 2:CD003099
10. Makonnen E, Zerihun L, Assefa G, Rostom A A. Antifertility of *ricinus communis* seed in female guinea pigs. East Afr Med J 1999; 76:335-337
11. Pasricha PJ. Treatment of disorders of bowel motility and water flux; antiemetic; agents used in biliary and pancreatic disease. Goodman and Gilman’s the pharmacological basis of therapeutics. 11th edition New York; Mc Graw-Hill 2006; p983-1008
12. Rossi AGZ, Soares Jr. JM, Motta ELA, Simoes MJ, Oliviero-Filho M, Haidar MA, Rodriguez de Lima G, Baracat EC. Metoclopramide-induced hyperprolactinemia affects mouse endometrial morphology. Gynecol Obstet Invest 2002; 54:185-190
13. Kauppila A, Isotalo H, Kirkinen P, Makila UM, Orava M, Vihko R. Metoclopramide-induced hyperprolactinemia: Effects on corpus luteum function, endometrial steroid receptor concentrations and 17 β -hydroxysteroid dehydrogenase activity. Clinical Endocrinology 2008; 26(2):145-154
14. La Torre D, Falomi A. Pharmacological causes of hyperprolactinemia. Ther Clin Risk Manag 2007; 3(5):929-951
15. Zylber-Haran EA, Gershman H, Spitz IM. Prolactin response to metoclopramide in the castrated male rat. Biology of Reproduction 1981; 25:1-5
16. Taur DJ, Patil RY. Antiasthmatic activity of *ricinus communis* L. roots. Asian Pacific Journal of Tropical Biomedicine 2011;13-16
17. Cohen-Becker IR, Selmanoff M, Wise PM. Hyperprolactinemia alters the frequency and amplitude of pulsatile luteinizing hormone secretion in the ovariectomized rat.

Neuroendocrinology 1986; 42(4):328-333

18. Fox SR, Hofer MT, Bartke A, Smith MS. Suppression of pulsatile secretion, pituitary GnRH receptor content and pituitary responsiveness to GnRH by hyperprolactinemia in the male rat. Neuroendocrinology 1987; 46(4):350-359
19. Goncim HY, Mador ES, Ogunranti JO. *Ricinus communis var minor* inhibits follicular development and possibly ovulation in human subjects as shown by ultra sound follicle tracking. Reproductive Health 2010; 4:35-38



AJPHR is
Peer-reviewed
monthly
Rapid publication
Submit your next manuscript at
editor@ajphr.com / editor.ajphr@gmail.com