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# Cottonseed Extract and Anti-fertility: Metabolic Versus Hormonal Changes in Rat Model

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## ABSTRACT [ENGLISH/ANGLAIS]

Cotton seed [*Gossypium- hirsutum*] has been reported to distort spermatogenesis and cause infertility in adult male rats. The present study was designed to investigate the effects of ethanolic extract of cotton seed on the metabolic activity of uterus and serum level of reproductive hormones in adult female wistar rats. A total of 18 adult female rats weighing between 180 g- 200 g were randomly selected into 3 groups n= 6. Group A received phosphate buffered saline, Group B and C were treated with doses of 20 mg/kg and 60 mg/kg body weight of the extract intraperitoneally twice daily at 0700 hours and 1900 hours for 21 days respectively. Animals were sacrificed by cervical dislocation 12 hours after the last administration and blood sample was collected from descending thoracic aorta for estimation of serum levels of oestrogen, progesterone, LH and FSH. Uterus excised following abdominal incision, homogenized in 5 % sucrose solution to estimate the enzyme activity of Glucose - 6- phosphate dehydrogenase and Lactate dehydrogenase. Significant {p<0.05} decrease in the serum level of oestrogen, FSH and LH were observed, while progesterone level was increased. Enzymes of Carbonhydrates metabolic activities, LDH and G-6-PDH were significantly {p<0.05} higher, The consequent changes observed in the result will interfere with ovulation and implantation thus promoting infertility.

**Keywords:** Cotton seed, anti-fertility, uterus, rats, hormone and enzyme

## RÉSUMÉ [FRANÇAIS/FRENCH]

Graines de coton [*Gossypium hirsutum*-] a été rapporté de fausser la spermatogenèse et provoquer la stérilité dans des rats mâles adultes. La présente étude a été conçue pour étudier les effets de l'extrait éthanolique de graines de coton sur l'activité métabolique de l'utérus et le taux sérique des hormones de reproduction chez les rats femelles Wistar adultes. Un total de 18 rats femelles adultes pèsent entre 180 g-200 g ont été choisis au hasard en 3 groupes n = 6. Groupe A une solution saline tamponnée de phosphate a reçu, le groupe B et C ont été traités avec des doses de 20 mg / kg de poids corporel et 60 mg / kg par voie intrapéritonéale de l'extrait deux fois par jour à 0700 heures et 1900 heures pendant 21 jours respectivement. Les animaux ont été sacrifiés par dislocation cervicale 12 heures après la dernière administration et de sang ont été recueillis à partir aorte thoracique descendante pour l'estimation des taux sériques d'oestrogènes, progestérone, LH et FSH. Utérus excisé après incision abdominale, homogénéisés dans une solution de saccharose à 5% pour estimer l'activité enzymatique de la glucose -6 - phosphate déshydrogénase et de lactate déshydrogénase. {Significative p <0,05} diminution de la concentration sérique de l'oestrogène, la FSH et de LH ont été observés, alors que le taux de progestérone a été augmenté. Les enzymes du métabolisme des activités Carbonhydrates, la LDH et la G-6-PDH étaient significativement {p <0,05} plus, les changements conséquents constatés dans le résultat va interférer avec l'ovulation et l'implantation favorisant ainsi l'infertilité.

**Mots-clés:** Graines de coton, anti-fertilité, de l'utérus, des rats, d'hormones et enzymes

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## INTRODUCTION

Cottonseed oil is naturally hydrogenated; its fatty acid profile is 50 % monosaccharide, 21 % polysaccharide and 29 % saturated [1]. The saturated acids contain natural

oleic, palmilic and stearic acid [1]. Gossypol is the active component extracted from cottonseed, it accounts for about 1.5 % of the seed weight and mostly used as medicine in the form of Gossypol acetate [1, 2]. Gossypol

was first identified as an anti-fertility agent as a result of epidemiologic studies conducted in China during the 1950s [2]. Investigators had been puzzled by the extremely low birth rate in a particular geographical region, the men had very low sperm counts and many women had amenorrhea [2]. Eventually, the phenomenon was attributed to the exclusive use of crude cottonseed oil for cooking [1, 2]. Investigations have shown that extract of cottonseed is a very potent arrestor of spermatogenesis [2]. Gossypol is a nonsteroidal compound that inhibits sperm production and motility in a variety of male animals and in humans [3]. It does not affect sex hormone levels or libido and its mechanism is distinct from that of steroidal oral contraceptives used by woman [3]. Gossypol exerts its contraceptive action by inhibiting an enzyme that plays a crucial role in energy metabolism in the sperm and spermatogenic cells. The target enzyme lactate dehydrogenase X is found only in sperm and male gonadal cells, it is involved in glycolysis and plays a role in inducing mitochondria in producing energy [3]. A variety of lactate dehydrogenases are found throughout the body and gossypol exerts a degree of inhibition on many of these enzymes. However, the drug exhibits its greatest inhibitory effects on lactate dehydrogenase X [3].

It is not known if administration of this extract has similar detrimental effect in the female rats [3]. Although there are scanty reports that Gossypol, the active ingredient in cottonseed extract prevents implantation [4], there are no data to determine enzymatic changes in the endometrial wall of Gossypol treated animal [4]. In female animal, the hypo-thalamic-pituitary-ovarian axes as well as the uterus play critical role in the biology of reproduction [5] and they are targets of most contraceptives [5]. With continued use of cotton seed extract and oil for food and anti-fertility agent, it is important that every alteration in cellular activities associated with ingestion of cotton seed extract be elucidated. This present work which uses female rat (as opposed to male rat which has been extensively investigated for cotton seed extract) becomes significant in balancing our understanding of sex differences in response to effect of ethanolic extract of cotton seed.

## MATERIALS AND METHODS

### Experimental Animals

A total of 18 adult female rats weighing between 180-200 g were obtained from animal holding facility, Anatomy Department, University of Ilorin, Ilorin. The animals

were given food and water *ad libitum* and allowed to acclimatize to the laboratory environment over a period of two weeks. Ethical approval on animal act right was obtained from the institutional animal care committee of University of Ilorin, Ilorin.

### Extract Preparation

Cottonseeds were obtained from Oja Oba Market, Ilorin, Kwara State. The seeds were grinded into a powder form; 35 g of it was soaked in 350 ml of 70 % ethanol for 24 hours and then filtered. The filtrate was concentrated using a water bath maintained at 70 °C, 7 g of extract was obtained from concentrate which was dissolved in phosphate buffered saline for dosage preparation and media for administration and preserved in refrigeration throughout the experimental period.

### Extract Administration

The extract was given intraperitoneally twice daily at 0700 hour and 1900 hour for 21 days of experiment. This was given according to the average body weight of animal in each group (180-200 g). Group A received phosphate buffered saline served as control, Group B and C received 20 mg/kg and 60 mg/kg body weight of the extract respectively.

### Animal Sacrifice

Animals were sacrificed by cervical dislocation 12 hours after the last administration. Blood sample was collected, following abdominal incision, from descending thoracic aorta for hormonal assay. The uterus was homogenized in 5 % sucrose solution for biochemical enzyme assay.

### Analytical procedure

Blood sample was obtained from the descending aorta in EDTA bottle for FSH, LH, Pg, oestrogen serum level using micro-well enzyme immuno-assay method produced by Syntron Bioresearch Inc. of United State of America (USA). The principle of the test was based on the sandwich principle for LH and FSH to form antibody-antigen antibody enzyme complex, while the micro-well competitive binding principle was utilized for oestrogen and progesterone assay. 0.5 g of uterus from each animal was homogenized with the aid of a clean laboratory mortar and pestle, cooled by ice jacket and centrifuged at 6000 rpm for 10 minutes, using Dietz and Labrano procedure for LDH and Peter and Liadsky 2006 procedure for G-6- PDH.

**Statistical Analysis**

The values are recorded as mean ± SEM. Student t-test was used for statistical inference/test and p< 0.05 was seen as significant difference.

**RESULTS**

There was significant decrease (P < 0.05) in serum oestrogen level; this decrease was more pronounced in the group that was treated with high dosage of 60 mg/kg body weight of the extract. Increase in the serum progesterone level was also observed in the rats treated with dose of 60 mg/kg of the extract when compared with the control rats but significant reduction was observed in the group treated with 20 mg/kg body weight of the extract as shown in Table I.

The mean concentration of LH and FSH was significantly lower (P < 0.05) in the rats treated with both 20 mg/kg and 60 mg/kg body weight of the extract when compared with the control rats as shown in the Table I. Biochemical Analysis revealed significant (P < 0.05) increase in Lactate dehydrogenase activity and increase in G-6-DPH activity of the treated rats when compared with the control rats as shown in Table II. The activity of carbohydrates metabolic enzymes was statistically higher in the group treated with the higher dosage.

**Table 1:** This table shows mean concentration of circulating reproductive hormone

	Group A Mean± SEM	Group B Mean± SEM	Group C Mean± SEM
Oestrogen (ng/mol)	59.99±0.27 *	50.25±0.42 *	5.77±0.62
Progesterone (ng/mol)	7.51±0.28 *	3.59±0.31 *	12.06±0.29
FSH (ng/mol)	11.24±0.12*	6.07±0.08 *	9.03±0.04
LH (ng/mol)	8.37±0.45 *	4.37±0.35 *	2.00±0.56

\* Sign of significant p< 0.05; FSH Follicles Stimulating Hormone; LH Luteinizing Hormone

**DISCUSSION**

Oestrogen concentration was lowered in the serum of the treated rats; this reduction in the serum level of oestrogen might be due to the degeneration or reduction in the number of follicular cell responsible for the production of oestrogen hormone in the ova [5]. The consequent effect of reduction in oestrogen level will be more pronounced in the uterus endometrium causing

atrophy or shrinkage of the endometrial lining and reduce glandular secretion which may interfere with implantation of fertilized ovum resulting in infertility in animal. The increase in progesterone concentration will suppress production of FSH and LH a mechanism called negative feedback effect on the pituitary gland from the anterior part. Reduction in the level of FSH and LH has an end product of preventing maturation and release of ovum or rupturing of follicle which may prevent fertility in animal. FSH is usually essential for the maturation of follicles and release of matured oocytes. This hormone was lowered in the treated rats as evident from the Table 1.

**Table 2:** This table shows serum level of enzymes activity

	Group A Mean± SEM	Group B Mean± SEM	Group C Mean± SEM
G-6-PDH (IU/L)	1100±2.89 *	1220±1.20 *	1465±1.62
LDH (IU/L)	197±1.03 *	315±2.05 *	217±3.56

\* Sign of significant p< 0.05; G-6-PDH Glucose-6-phosphate dehydrogenase; LDH Lactate dehydrogenase

There is a threshold level of FSH that is required for maturation and release of matured ovum, [5] if this threshold is not reached, no ovulation can occur. Also, it has a minimum level beyond which there is multiple release of ovum that leads to multiple fertilization [5]. Progesterone and oestrogen from the ovary suppress the FSH and LH, preventing follicular maturation and ovulation and are referred to as contraceptive hormones. The functional layer of endometrium depends on these hormones. LH is responsible for the rupturing of follicles and release of oocytes from corpus luteum which produces progesterone and oestrogen to ensure fertilization of the released ovum and implantation of fertilized ovum. Reduction in LH will lead to delay in the development of follicles and ovulation and consequent change in the level of progesterone which acts as a negative feedback mechanism on LH.

The hypothalamus responds to changes in the blood level of oestrogen and progesterone. It is switched off by high level and stimulated when low to secrete LHRH which stimulates the anterior pituitary gland to secrete FSH and LH [5] Hypothalamus only stimulates secretion of these hormones but cannot suppress them. This is

pointing to the fact that oestrogen and progesterone are two major hormones involved in the use of contraceptive and can be deduced that gossypol has a pronounced effect on these hormones.

The result observed in the present study also showed that the ethanolic extract of cotton seed alter the activities of enzymes of carbohydrate metabolism in the uterus of a treated rat. There is an increase in the activities of G-6-PDH which are more significant in the group that receives high dose when compared with the control. Increase in G-6-PDH activities indicates that: Carbohydrate metabolism is being facilitated more along glycolytic pathway and hexoses monophosphates shunt. Increase in G-6-PDH activities consequently facilitates production of ribose which leads to secretion of more nucleic acid. G-6-PDH is also a scavenger of residue of oxidative stress indicating that the uterus of the treated rat has undergone cellular alteration; this might consequently prevent implantation in the uterus in the treated rat. The LDH activity was increased in the treated rat when compared with the control; this enzyme catalysis inter - conversion of pyruvate and lactate with concomitant interconversion of NADH and NAD. However, breaking down of tissue will elevate the levels of LDH; the increase in the LDH activity of the uterus in the treated rat indicates an alteration in cyto-architecture of the uterus.

## CONCLUSION

It is concluded that ethanolic extract of cotton seed does not only prevent fertility but also has deleterious effect on the uterus of rat, as shown in the increased level of carbohydrate metabolism, indicating a stressed or damaged tissue.

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## CONFLICT OF INTEREST

No conflict of interest was declared by authors.

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