DOI: https://doi.org/10.53350/pjmhs2023171145

ORIGINAL ARTICLE

Effect of Piperine on Diameter of Graafian Follicles of Adult Female Albino Rats

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ABSTRACT

Background: Piperine is an alkaloid present in black pepper. It causes increase in diameter of Graafian follicles when given to adult female albino rats thus leading to decreased fertility.
Aim: To assess the harmful effects of piperine on ovaries of adult female albino rats on diameter of Graafian follicles.
Study design: Experimental Study.
Place and duration of study: Anatomy Department, Shaikh Zayed PGMI, Lahore from 1st August 2013 to 31st January 2014.
Methodology: Thirty female rats were randomly separated into three equal groups A, B and C, each having 10 animals. Group A was control and did not receive any medication except for normal saline at a dose of 10ml/kg body weight/day by orogastric tube for 30 days. Group B and C received piperine suspended in saline by orogastric tube, at a dose of5mg/kg/day and 10 mg/kg/day respectively for 30 days. After 30 days of experiment, the rats of all three groups were euthanized and ovaries were removed by dissection and then fixed in 10% formalin. With rotary microtome, 4-5µm sections were prepared and stained with hematoxylin and eosin solutions.
Results: The Graafian follicle in ovaries of rats in group B showed increased diameter as compared to control group A. The diameter of Graafian follicles of group C was greater than of group B. In both groups comparison was made, the p-value was less than 0.001.
Conclusion: Piperine administration in female adult albino rats showed significant increase in size of Graafian follicle which is likely to produce polycystic ovarian syndrome (PCOS) and in this way hamper fertility in females during child bearing age.
Keywords: Piperine, Albino rat, Ovaries, Fertility, Polycystic ovarian syndrome (PCOS)

INTRODUCTION

Piperine is present in black pepper and long pepper, scientifically known as Piper nigrum L. and Piper longum L., respectively[1]. These plants belong to the Family Piperacea[2]. Piperine is an alkaloid that imparts the black pepper its typical biting taste[3]. Besides its use as a condiment, black pepper is also shown to exhibit numerous therapeutic benefits as evidenced by studies on cells, humans and animals. The most valuable of the properties possessed by piperine are its action as a bioenhancer[4,5], used as an antiinflammatory[6], antioxidant and anticarcinogenic agent[7]. Studies on central nervous system demonstrate that in rats having "chronic unpredictable mild stress", piperine can relieve depression by influencing "hypothalamic-pituitary-adrenal axis"[8]. The study demonstrated improvement in cognition in rats with Alziemer's Disease (AD) and suggested that it is effective and safe for long-term use in the management of AD[9]. It possesses anti-seizure activity[10]. In a study the effect of piperine on improvement of inflammatory bowel syndrome were shown[11,12]. The role of piperine to manage obesity was also shown in a study[13]. It is also reported in a study to be helpful in managing arthritis[14]. Piperine is also used as a treatment for asthma[15,16].

Acute and subacute toxicity of piperine is proven in hamsters, rats and mice resulting in their death. The LD_{50} values for a single IV, IGIP, SC and IM dispensation of piperine to adult male mice were 15.1, 330, 43, 200 and 400 mg/kg body wt, respectively. In female adult rats, the i.p. LD_{50} value was 33.5 mg/kg body weight while the IG LD_{50} value was augmented to 514 mg/kg body weight[17]. In an experiment, piperine at 10mg/kg body weight dose resulted in marked damage to seminiferous tubules, decrease in diameters of seminiferous tubules and nuclei of Leydig cells and spermatocytes and spermatids desquamation were also observed[18].

Received on 14-08-2022
Accepted on 23-12-2022

In a study on adult male rats, piperine was administered orally at 1 mg/kg, 10 mg/kg and 100mg/kg b.w. daily for a period of 30 days. The movement and number of spermatozoa in epididymis also decreased at 10 mg/kg and 100 mg/kg body weight. The life span of spermatozoa was significantly reduced at 100 mg/kg[19]. In an experiment on Swiss albino mice, toxicity on reproductive system was assessed. Related short range investigations were carried out to judge the effect on sexual coupling, estrous cycle, damage to male gametes, fertilization, implantation and thriving of pups. It was demonstrated that piperine at higher doses resulted in augmentation of diestrous phase period which resulted in diminution of fertility and mating performance. Piperine was given orally for five days after copulation and a marked hampering of implantation was observed. There was total failure of implantation after injecting piperine into the uterus of concerned female mice. Thus it was shown that piperine damaged various essential happenings in the process of reproduction.[20] This study is designed to evaluate effect of piperine on diameter of Graafian follicles of adult female albino rats.

MATERIALS AND METHODS

Thirty female albino rats of Wistar strain aged 3-4 months (weighing about 200-250 gm) were obtained from Veterinary Research Institute, Lahore. They were kept in cages for 15 days in the animal house for the purpose of acclimatization. A 12 hours light/dark cycle was maintained. The animals were allowed free access to food and water. The rats were randomly divided into three equal groups labelled as A, Band C. Group A was labelled as control, the other two groups were experimental, groups B and C. Each group comprised of 10 animals. Piperine was given orally to rats according to following schedule:

Group A (Control): This was labeled as the control group and ten female rats of this group were not given any medication except for saline with a dose of 10ml/kg body weight/day by orogastric tube for 30 days.

Group B (Experimental): This was experimental group and animals of this group were fed by orogastric tube with piperine suspended in saline, at a dose of 5mg/kg/day for 30 days.

Group C (Experimental): This was also experimental group and ten animals of this group were given piperine suspended in saline, at a dose of 10mg/kg/day by gastric intubation, for 30 days.

Dissection and Fixation of Ovaries: At 30th day of experiment, after the cessation of administration of piperine, the animals of all the groups were weighed and recorded in proforma. To determine estrous cycle, vaginal smear process was used for each rat separately and it was noted accordingly.[21]It was made sure that all animals were in the same phase of monthly cycle.

These animals were then euthanized by injecting sodium pentobarbital as anaesthetic intraperitoneally in doses of 45mg/kg and morphine as analgesic in doses of 5mg/kg intraperitoneally.[22] This is the most acceptable agent for anesthesia, less expensive, acts quickly and humanely kill all types of rodents.

By making an incision at the base of abdomen, it was opened. The anterior abdominal viscera were reflected and the ovaries were removed after identifying the uterine tubes for the detailed morphological study. Ovaries were then weighed and fixed in 10% formalin for further study. Each fixed ovary was further processed by carrying out procedures of dehydration, clearing, infiltration, embedding and paraffin tissue blocks were made for proper tissue sectioning and staining[23]. With rotary microtome, 4-5µm sections were prepared and stained with hematoxylin and eosin solutions[24].

There were made 5 sections of each ovary and the diameter of Graafian follicles were measured by using ocular micrometer.[25] Diameters of group A were taken as reference. Experimental groups B and C were compared with the control group.

Data was analyzed by using SPSS-22. Diameter of Graafian follicles was depicted by Mean±SD and comparison between the groups were accomplished by ANOVA. The p-value <0.05 was regarded as statistically significant.

RESULTS

Histological examination of ovaries of rats in the control and experimental groups was conducted which showed ovarian follicles to be found in various stages of development in stroma of ovaries. The cortex and the medulla were well defined. Primordial, primary, secondary and Graafian follicles were found in the cortex of the ovary. Corpora lutea were observed occupying the cortex in different stages of development. In the medulla were present blood vessels and lymph vessels embedded in connective tissue and smooth muscles. In groups A, B and C the mean diameter of Graafian follicles was 50.7±3.8µm, 60.4±4.8µm and 69.5±2.5µm respectively (Table1). The difference among these groups was revealed to be highly significant with p-value <0.001 (Table 2).

Table 1: Mean diameter (µM) of Graafian follicles of rats between groups after piperine administration

Group	Diameter of Graffian follicle
A	50.7±3.8
B	60.4±4.8
C	69.5±2.5

A Control Group
B Experimental Group
C Experimental Group

Table 2: Comparison of mean diameter (µm) of Graafian follicles in rats after administration of piperine amongst three groups

	Sum of Squares	Df	Mean Square	F	Sig.
Between group	1767.8	2	883.9	60.5	0.000**
Within group	394.5	27	14.6		
Total	2162.3	29			

Based on ANOVA
Df: Degree of Freedom, **Highly Significant Difference, F:f-Test (Ratio of Variances)

Group wise comparison depicted that both experimental groups B and C had significantly greater diameter in comparison to control group A. Moreover, the group C had significantly higher diameter in comparison to group B. the p-values for all group wise differences were <0.001, which are highly statistically significant (Table 3, Fig, 1). The diameters of Graafian follicles in groups A, B and C is shown in Figs 2, 3 and 4 depicting hypertrophy in groups B and C.

Table 3: Group wise comparison of mean diameter (µm) of Graafian follicles of rats amongst various groups after administration of piperine

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.
Group A	Group B	-9.7*	1.7	0.000**
	Group B	-18.8*	1.7	0.000**
Group B	Group B	-9.1*	1.7	0.000**

Based on TUKEY'S test
A: Control Group, B: Experimental Group, C: Experimental Group,
**Highly significant difference

Fig 1: Bar diagram presenting mean diameter of Graafian follicles in rats after piperine administration with error bars showing standard deviation

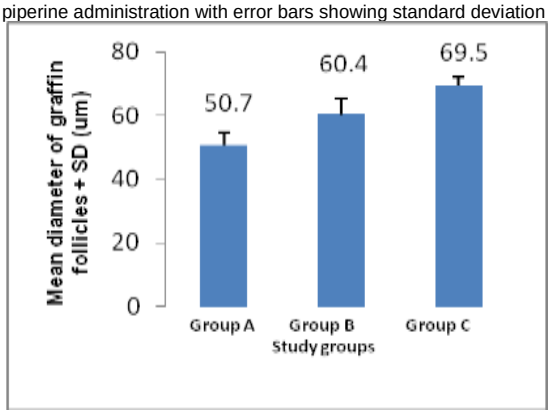


Fig 2: Photomicrograph – Panoramic view of ovary of adult female albino rat of control group a showing measurement of Graafian follicle (GF), zona granulosa (ZG), theca interna (TI), theca externa (TE), follicular antrum (FA) H E, 10X)

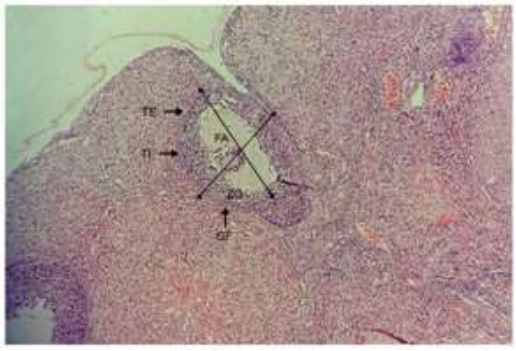


Fig 3: Photomicrograph – panoramic view of ovary of adult female albino rat of experimental group B showing Graafian follicle (GF), Zona Granulosa (ZG), Theca interna (TI), Theca externa (TE), Follicular antrum (FA), Cumulus oophorus (CO), Primary oocyte (O) (H&E, 10X)

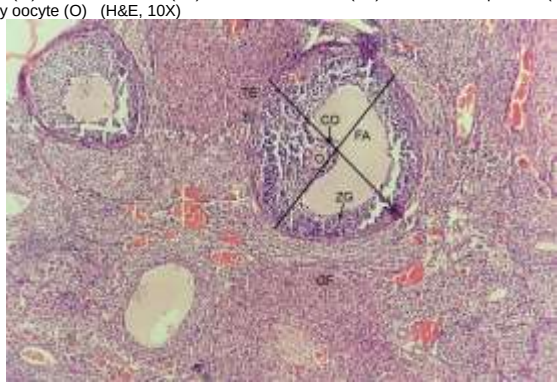
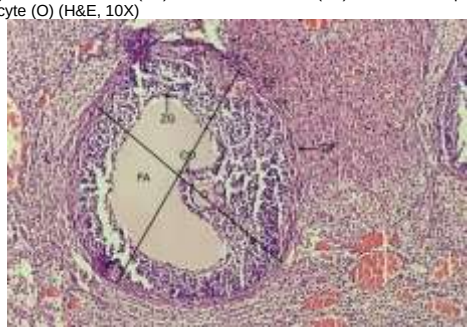


Fig 4: Photomicrograph – panoramic view of ovary of adult female albino rat of experimental group C showing Graafian follicle (GF), Zona Granulosa (ZG), Theca interna (TI), Theca externa (TE), Follicular antrum (FA), Cumulus oophorus (CO), Primary oocyte (O) (H&E, 10X)



DISCUSSION

Piperine is an alkaloid accounting for pungency of black pepper[26] and later is widely used as a spice all over the world.[27]Piperine increases the bioavailability of various drugs, and this is the most important characteristic possessed by it.[4,5] Due to enormous intake of piperine as a spice and multiplying use as bioenhancer, and its insufficiency of research studies on effects on the morphology of ovaries, this experiment was performed to look into the likely concurrent effects of piperine on the morphology and histology of the adult ovaries and hormonal profiles therewith.

In the present study, the comprehensive histological study of control group A animals' ovaries showed the ovulatory cycle in various stages of folliculogenesis, advancing from the primordial follicle to the Graafian follicle. Further, the differentiation of granulosa cells as well as theca interna cells, development of antrum, cumulus oophorus, and the corpus luteum were also seen and showed the normal histological findings which tally with the findings described by Westwood[27] in his experiment of rat histological guide to staging. It was seen that these follicles were embedded in the connective tissue stroma. The stroma of the cortex comprised the cellular portion which contained the above mentioned different stages of folliculogenesis, while the medulla captured the central portion of the ovary.

The comparison of the Graafian follicles among three groups revealed that these were normal in ovaries of all 10 animals of group A. There was hypertrophy in all 10 animals each of group B and C. The difference amongst groups was significant with p-value <0.001. Group wise comparison showed that both experimental groups B and C had significantly larger diameter as compared to group A. The difference of groups B and C from group A, and of group B from C was <0.001, which is highly significant (Table 3).This showed that diameters of Graafian follicles in group B and C was greater than control group rats. Similarly, Nirala et al[28] in their experiment showed antifertility effects of Piper nigrum alcoholic extract. The histological parameter they used was the number of mature follicles which decreased in a dose dependent manner.

CONCLUSION

This study showed that Piperine when given to adult female albino rats for 30 days in higher doses i.e. 5mg/kg/day and 10mg/kg/day body weight orally has negative effect on the morphology and histology of their ovaries. As rats are mammals like humans, this effect of Piperine on fertility may also apply to young adult human females. However, further research work is also required relating to the effects of Piperine on reproductive capability of female albino rats, and to explore these effects in more depth.

Conflict of interest: Nil

REFERENCES

1. Lin Y, Xu J, Liao H, Li L, Pan L. Piperine induces apoptosis of lung cancer A549 cells via p53-dependent mitochondrial signaling pathway. Tumor Biol 2014;35:3305-10.
2. Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, Khan MA. Biological role of Piper nigrum L (Black pepper): a review. APJTB.2012;2:1945-53.
3. Kolhe SR, Borole P, Patel U. Extraction and evaluation of piperine from piper nigrumlinn. Int J ApplBiol Pharm 2011;2:144-9.
4. Tatiraju VD, Bagade VB, Karambelkar JP, Jadhav VM, Kadam V. Natural bioenhancers: an overview. J Pharmacogn Phytochem 2013;2:55-60.
5. Meghwal M, Goswami TK. Piper nigrum and piperine: an update. Phytother Res 2013; 27: 1121-30.
6. Umar S, Sarwar AH, Umar K, Ahmad N, Sajad M, Ahmad S et al. Piperine ameliorates oxidative stress, inflammation and histological outcome in collagen induced arthritis. Cell Immunol 2013; 284:51-9.
7. Qu H, Lv M, Xu H. Piperine: Bioactivities and structural modifications. Mini-Rev Med Chem 2015; 15:145-56.
8. Park H, Hongbo L, Screen L, Dai-hong G, Yuyu W. Piperine regulating hypothalamic-pituitary-adrenal axis and acting as an antidepressant in rats. Chin J Integr Med 2009;7:667-70.
9. Elnaggar YS, Etman SM, Abdelmonsif DA, Abdallah OY. Novel piperine-loaded Tween-integrated monooleincubosomes as brain-targeted oral nanomedicine in Alzheimer's disease: pharmaceutical, biological, and toxicological studies.Int J Nanomedicine 2015; 10:5459-73.
10. Chen CY, Li W, Qu KP, Chen CR. Piperine exerts anti-seizure effects via the TRPV1 receptor in mice. Eur J Pharmacol 2013; 714: 288-94.
11. Gupta RA, Motiwala MN, Dumore NG, Danao KR, Ganjare AB. Effect of piperine on inhibition of FFA induced TLR4 mediated inflammation and amelioration of acetic acid induced ulcerative colitis in mice. Toxicol Lett 2015; 164: 239-46.
12. Hu D, Wang Y, Chen Z, Ma Z, You Q, Zhang X et al. The protective effect of piperine on dextran sulfate sodium induced inflammatory bowel disease and its relation with pregnane X receptor activation. J Ethnopharmacol 2015; 169: 109-23.
13. BrahmaNaidu P, Nemani H, Meriga B, Mehar SK, Potana S, Ramgopalrao S. Mitigating efficacy of piperine in the physiological derangements of high fat diet induced obesity in Sprague Dawley rats. Chem Biol Interact 2014; 221: 42-51.
14. Sabina EP, Nagar S, Rasool M. A role of piperine on monosodium urate crystal-inducedinflammation - an experimental model of gouty arthritis. Inflammation 2011;34:184-92.
15. Hussain A, Naz S, Nazir H, Shinwari ZK. Tissue culture of black pepper (Piper nigrum L.) in Pakistan. Pak J Bot 2011;43:1069-78.
16. Singotam M, Kumar BA. Anthelmintic activity of Piperine from black pepper. JGTPS 2013; 4: 1013-7.
17. Piyachaturawat P, Glinsukon T, Toskulkao C. Acute and subacute toxicity of piperine in mice, rats and hamsters. Toxicol Lett 1983;16:351-9.
18. Malini T, Manimaran RR, Arunakaran J, Aruldhas MM, Govindarajulu P. Effects of piperine on testis of albino rats. J Ethnopharmacol 1999;64:219-25.
19. D'Cruz SC, Mathur PP. Effect of piperine on the epididymis of adult male rats. Asian J Androl 2005;7:363-8.
20. Daware MB, Mujumdar AM, Ghaskadbi S. Reproductive toxicity of piperine in Swiss albino mice. Planta Med 2000;66:231-6.
21. Sahar MM, Abed el Samad O, Abed el Samad AA. Modified vaginal smear cytology for the determination of the rat estrous cycle phases, versus ordinary papanicolaou technique, verified by light and scanning electron microscopic examination of the endometrium. EJH 2007; 30: 397-408.
22. A Compendium of Drugs used for Laboratory Animal anesthesia, analgesia, tranquilization and restraint2013.
23. Spencer LT, Bancroft JD. Tissue processing. In:Theory and practice of Histological techniques. 6thedition. Philadelphia: Churchill Livingstone Elsevier; 2008.
24. Luna LG. American registry of pathology. Manual of histologic staining methods of the Armed Forces Institute of Pathology.3rd edition. New York: McGraw-Hill Publishers; 1960.
25. Todd JC. Clinical diagnosis by laboratory methods.2nd edition. Philadelphia; Saunders Company, 1998, 112-7.
26. Al Tae'e MF, Zaid K, Hadeel W. Immunological evaluation and acute toxicity study with fertility examination for the effect of aqueous extract from dried fruits of Piper nigrum L. in mice. IJS 2010; 51:465-70.
27. Westwood FR. The female rat reproductive cycle: a practical histological guide to staging. Toxicol Pathol 2008;36:375-84.
28. Nirala PK, Dwivedi SC. Antifertility effect of alcoholic p. nigram fruit extract on adult female Wistar rat. Models IJNTRISSN 2015;1:73-5.