

## EFFECT OF PIPERINE ON HYPOTHALAMIC PITUITARY GONADAL AXIS OF FEMALE ALBINO RATS: A RANDOMIZED CONTROLLED STUDY

Saqib Mansoor,<sup>1</sup> Muhammad Suhail,<sup>2</sup> Ahmad Bilal Suhail,<sup>3</sup> Amina Zia,<sup>4</sup>  
Ayesha Afzal,<sup>5</sup> Faiza Shafqat<sup>6</sup>

### Abstract

**Background and Objective:** Piperine is a constituent of black pepper. When administered to female adult albino rats, it might results in increased levels of LH and FSH, and thus causing diminished ability to procreate. The aim of this study is to determine the deleterious piperine influences on LH and FSH levels of adult female albino rats.

**Method:** It is a randomized controlled trial conducted in the department of Anatomy, FPGMI, Lahore. By random selection from 30 rats, three equal groups were made, each with 10 animals. Control status was assigned to group A which was not given any medicine, but only N/S administered with the dosage 10ml/kg body wt./day by using NG tube for a span of 30 days. Group B was given piperine at dosage of 5mg/kg/day for a period of thirty days. The C group was administered drug at the dosage of 10mg per kg per day for the same period of time as A and B via the same route. At completion of thirty days of the study, the animals of the 3 groups were given euthanasia. Dissection was performed, their ovaries were removed, and then fixed in 10% formalin. 4-5 micrometer sections preparation was done by using the microtome and then H&E staining was carried out.

**Results:** FSH and LH levels in serum of group B rats were shown to have increased in comparison to control group A. The FSH and LH levels in rats of C group were greater than that in rats of B group. When the two groups were compared, this difference was determined to be extremely of significance having a p-value less than 0.001.

**Conclusion:** Giving Piperine to female adult albino rats was shown to cause significant increase in levels of FSH and LH which is likely to produce negative feedback on hypothalamic-pituitary-gonadal axis and in this way hamper female fertility during their productive years probably by causing PCOS.

**Key words:** Piperine, Albino rat, ovaries, LH, FSH, fertility, hypothalamic-pituitary-gonadal axis, PCOS

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The piperine is a constituent of the black pepper, and long pepper, scientifically termed as Piper

nigrum L. and Piper longum L., respectively.<sup>1</sup> These plants are part of Family Piperaceae.<sup>2</sup> The piperine is regarded as alkaloid and black pepper owes its characteristic stinging flavor to the former<sup>3</sup>. We know its use as a condiment, but black pepper also possesses a lot of therapeutic benefits as depicted by studies on cells, animals and humans. The most important of the properties that piperine does have are its effect as a bioenhancer,<sup>4,5</sup> use as an anti-inflammatory,<sup>6</sup> anticarcinogenic and antioxidant agent.<sup>7</sup> A study showed cognitive improvement by giving piperine to rats with Alzheimer Disease and the experiment suggested that piperine is beneficial, and its usage over a long period is safe

1. Department of Anatomy, Niaz Medical and Dental College, Sargodha
2. Department of Anatomy, Amna Inayat Medical College, Lahore
3. Department of Radiology, Shaikh Zayed Federal Post Graduate Medical Institute, Lahore
4. Department of Anatomy, Azra Naheed Dental College, Lahore
5. Life Care Hospital, Lahore
6. Department of Anatomy, CMH Lahore Medical College and Institute of Dentistry, Lahore

### Correspondence:

Dr. Saqib Mansoor ; Assistant Professor of Anatomy, Niaz Medical and Dental College, Sargodha.  
E-mail: [drsajib.mansoor@gmail.com](mailto:drsajib.mansoor@gmail.com)

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to manage AD.<sup>8</sup> The value of piperine in managing obesity was also depicted in a study.<sup>9</sup> In a study it is demonstrated to be helpful in management of arthritis.<sup>1</sup> Piperine is also found to be useful in asthma cases.<sup>11,12</sup>

Piperine has acute and subacute toxic effects in rats, hamsters, and mice even causing their death. LD50 value of one i.e. dose of piperine to male adult mice was 330. Milligram per kilogram body wt. In the adult female rats, the i.g. LD50 value was greater i.e., 514 mg/kg body weight.<sup>13</sup>

A study showed that piperine at the dosage of 10mg/kg body weight clearly damaged the seminiferous tubules, caused the Leydig cells and their nuclei seminiferous tubules diameters to decrease; spermatocytes and spermatids desquamation were also shown.<sup>14</sup> In another study carried out on Swiss albino mice, toxic effects on the reproductive system were measured. Relevant short-term tests were employed to evaluate the impact on estrous cycle, sexual coupling, damage to male germ cells, fertilization, implantation and the growth of the pups. The study showed that piperine at greater doses enhanced diestrus phase period which in turn caused diminution of mating performance and fertility. Piperine was given by the oral route for a span of five days after mating and a pronounced hinderance in implantation was shown. Complete failure of the implantation was observed when piperine was injected in womb of corresponding feminine rats. In this way, they depicted that the piperine possesses harmful effects to many crucial steps in the reproduction process.<sup>15</sup> Our experiment intends to evaluate the influence of the piperine on the biochemical markers i.e., FSH and LH of feminine adult albino rats.

## METHODS

30 feminine albino rats of the Wistar ancestry having age three to four months, weight ranging from 200 to 250 grams were arranged from the Research Institute of Veterinary, Lahore. We acclimatized the rats by keeping them in the cages in animal house for a span of 15 days. A light / dark cycle of 12 hours was provided. Free access to water and food was given to these rats. The animals were divided in 3 equal groups

A, B, and C randomly.

Rats of A group were taken as control group, while B and C were the experimental groups. These three groups consisted of ten rats each.

Oral administration of the piperine to animals as per dosages given below:

### GROUP A

Here, 10 animals received no drug but the N.S. with dosage of 10 milliliter per kilogram body weight per 24 hours with N.G. tube over a span of thirty days

### GROUP B

Here the animals were administered piperine dissolved in saline, with a dosage of five milligram per kilogram every 24 hours for a span of 30 days via NG tube.

### GROUP C

Here 10 animals received the piperine dissolved in the saline solution, with a dosage of ten milligram per kilogram per 24 hours with N.G. intubation, over a span of 30 days.

### Dissection and fixation of ovaries

As the study finished on 30th day, the animals were given euthanasia by giving Inj. sodium pentobarbital as anesthetic by intraperitoneal route at a dose of forty-five milligram per kilogram and morphine as the analgesia at a dose of five milligram per kilogram via same route.<sup>16</sup> This is the most suitable medicine for anesthesia, being low-priced; acts briskly and in a humane manner kills all sorts of rodents. Blood was drawn after twenty-four hours of giving last dosage of the Piperine for FSH and LH levels of all 3 groups. Lidocaine, was used topically in cream form on rats' tails before drawing samples of blood for making the process pain-free<sup>17</sup>. Vaginal smear process was made use of to determine the Estrous cycle<sup>18</sup> for all the rats separately and it was recorded accordingly. It was a must to determine Estrous cycle to correlate it with the levels of LH and FSH.

The comparison of levels of FSH and LH was performed as serum levels expressed in ng/ml. The serum levels in our A group were regarded as the reference. Comparison of B and C groups was done with

the reference.

Analysis of data with the help of SPSS Version 22 was carried out. Levels of LH and FSH were expressed as Mean, + S.D and comparison between three groups was done by applying ANOVA. A less than 0.05 p-value was considered significant statistically.

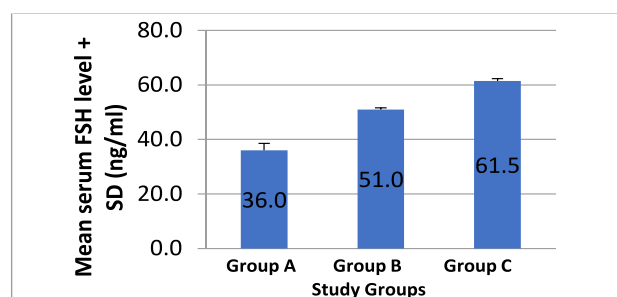
## RESULTS

### 1. FSH

The comparison of the marker FSH showed that average value for rats in first group was  $36.0 \pm 2.5$  nanogram per milliliter and the levels of the second and third group were  $51.0 \pm 0.6$  nanogram per milliliter and  $61.5 \pm 0.8$  nanogram per milliliter correspondingly. The difference among the 3 groups was determined to be of significant with a p-value less than 0.001. When the comparison was done within the groups, the 3 differences in sequence were all found to be of significance with the p-values less than 0.001, showing significantly elevated FSH level for the third group. (table I, II, III Fig I)

**Table 1:** Group wise comparison of average serum FSH values (nanogram per milliliter) of the animals among three Groups after giving the piperine

(I) Control Group	(J) Experimental Group	Mean Difference (I-J)	Std. Dev	Sig.
Group A	Group B	-15.0*	0.7	0.000**
	Group C	-25.5*	0.7	0.000**
Group B	Group C	-10.5*	0.7	0.000**



**Figure 1:** Bar diagram showing average serum FSH values for the animals following piperine dosage and error bars to show SD

Tukey test based

\*\* Of highly significance, p-value less than 0.001

### 2. LH

Comparison of values of serum LH was also carried out and the average values of all the 3 groups were calculated to be  $14.6 \pm 0.7$  ng/ml,  $20.4 \pm 0.6$  ng/ml and  $25.3 \pm 0.7$  nanogram per milliliter correspondingly. The group wise and overall differences were all significant with p-values less than 0.001. Serum LH value was determined to be conspicuously elevated and significant for the third group (table IV, V, VI, Fig. II). Based on TUKEY'S Test

**Table 2:** Group wise comparison of mean serum levels (ng/ml) of animals among three groups after giving piperine

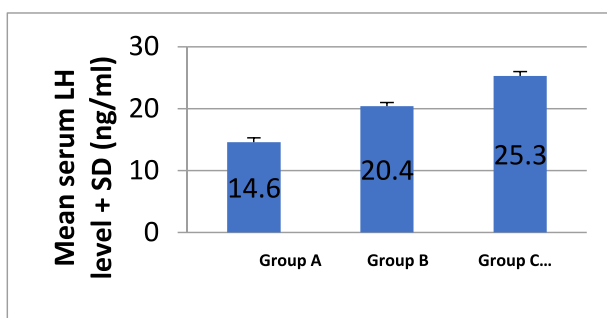
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.
Group A	Group B	-5.83*	0.29	0.000**
	Group C	-10.78*	0.29	0.000**
Group B	Group C	-4.95*	0.29	0.000**

A Control Group

B Experimental Group

C Experimental Group

\*\* Highly significant difference (P-value < 0.001)



**Figure 2:** Bar Diagram that shows average serum LH values of the animals after giving piperine, and the error bars that show SD

## DISCUSSION

Owing to increased usage of the piperine as a condition and its continuous increase as a bioenhancer,<sup>4,5</sup> and its less research studies carried so far on effects on the biochemical markers LH and FSH. This experiment was performed to probe the possible concomitant

influences of the piperine on these two hormones. FSH is responsible for stimulating follicles in the ovaries to cause ripening of numerous follicles. LH stimulates ovulation that is it causes release of the ovum from the follicles.

In the present study, a comparison was made between levels of LH and FSH in serum. For the FSH, variance among the 3 groups A, B and C was demonstrated to be of high significance having a p-value less than 0.001. When group-wise comparison was carried out, differences for the three groups were all shown significance with the p-values less than 0.001, that suggest markedly highest FSH level for the C group (table II, III).

For the LH, the differences group-wise as well as overall, were all of high significance statistically, having p-values less than 0.001. It was demonstrated that LH level in serum was most high and of statistical significance for C group (table V, VI). Similar results were also reported by other studies.

In one experiment, it was demonstrated that the piperine damaged epididymal conditions and the properties of sperm by decreasing concentration of the sialic acid and antioxidant enzymes.<sup>19</sup> In another experiment, the piperine was demonstrated to induce compromised ability to procreate in the rats (male) and to decrease event of spermatogenesis by modulating pituitary-testicular feedback axes. It was demonstrated that sperm motility, and the weight of the testes decreased, and the bioavailability of the testosterone decreased.<sup>14,15</sup> It was demonstrated in an experiment that piperine when administered to male rats decreased the number of spermatozoa and the concentration of the enzyme of antioxidant groups in epididymis.<sup>20</sup>

In yet another experiment, it was shown that piperine enhances the testosterone level in plasma of mice<sup>21</sup>. In another study, the probable use of Piperine in developing a reversible oral male contraceptive was demonstrated.<sup>22</sup> In a study, it was shown that serum levels of FSH and LH are raised by use of Piperine.<sup>23</sup>

In their study on mice, Al Tae'e MF et al reported significant elevation in the values of FSH and LH after

black pepper extract was given orally in the study groups as compared to the control group. FSH induces stimulation of the ovarian follicles to bring about release of various follicles. LH triggers the process so that ovum is released from the follicles.<sup>4</sup>

## CONCLUSION

Our study depicts that the piperine when administered to female adult albino rats for a span of thirty days at greater doses i.e., five milligram per kilogram every 24 hours and ten milligram per kilogram every 24 hours body wt. by oral route hampers feedback on hypothalamic-pituitary-gonadal axis badly and compromises fertility. As the rats are also mammals like the humans, this piperine influence on ability to procreate may also be applied to any adult females.

Nonetheless, more research is needed pertaining piperine influences on the ability to procreate of albino female rats, and to study these influences in additional detail.

**Conflict of Interest** *None*

**Source of Funding** *None*

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