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Effect of *Piper Nigrum* (Linn.) on Infertility Induced by Ethionamide and Para Amino Salicylic Acid in Female Sprague –Dawley Rats

G.V. Zodape and V. S. Gaikwad

Department of Zoology, S.S. and L.S. Patkar College of Arts and Science and V.P. Varde College of Commerce and Economics, S.V. Road, Goregaon (West), Mumbai- 400 062, India.
*Corresponding Author E-mail : drgautamvz5@gmail.com

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Chemiluminescence immunoassay of estrogen was performed. Ovaries were removed and immersed in Bouins solution for fixation and processed until the embedded in paraffin for histological analysis. Data was statistically analyzed by one way analysis of variance (ANOVA) by F –Test. The value p< 0.05 considered as significant. In our study it is observed that, Piper nigrum found much effective to compensate the level of estrogen to normal. This study suggests that the antituberculosis drugs in combination with Piper nigrum are effective to cure the disease and to maintain the fertility in rats. In the present investigation, the increased level of estrogen may be because of zinc and antioxidant property of Piper nigrum, which may also be responsible for the improvement of histological architecture of ovary of Sprague-dawley rats.

Keywords: Drugs; Estrogen; Fertility; Immunoassay; Rats.

Female reproductive system is very vulnerable to this infection and clinical presentation of this disease in a large majority of patients could be completely silent. This disease is an important cause of infertility, menstrual irregularity, pregnancy loss, morbidity to both the mother and child increases in pregnancy[1]. The clinical diagnosis of genital TB requires a high index of suspicion. Approximately 50% of patients might have had tuberculous pleurisy, peritonitis, or renal, osseous, or pulmonary TB. A history of primary infertility in a woman in whom examination reveals no apparent cause and who gives a family history of TB should arouse suspicion. A history of poor general health associated with weight loss, undue fatigue, low-grade fever, or vague lower abdominal discomfort is often elicited in patients. Medicines used to treat TB infection can also have adverse effects on contraception and other areas of female reproductive health. Though new, finer diagnostic tools of detection of TB are increasingly available in the form of bacterial cultures and polymerase chain reaction (PCR) based diagnostics, suspicion by clinicians remains the main tool for diagnosis of the condition. Hence, doctors need to be properly trained to become "Tuberculosis Minded" [2].

Ethionamide (ETH) is key drug used in MDR-TB drug resistance tuberculosis and have to shown physiological action[3,4,5]. It is listed as one of the essential medication in drug resistance tuberculosis in children and adults[6]. Para-amino salicylic acid (PAS) was the first antibiotic found

to be efficient in the treatment of tuberculosis in the 1940s.[7] PAS treatment is uncommon and a highly drug resistant strain seems to have limited resistance to this drug. Thus, PAS became the principle second line agent for the treatment of MDR-TB[8].

In developing countries, the traditional plant remedies are widely used to treat various ailments. Many varieties of plants have been used for treating different kinds of diseases including hepatoprotective potentials[9]. Now days the dietary supplements and herbal remedies have increase the interest of researchers to treat different kind of diseases. In India, over 40 poly-herbal commercial formulations have hepatoprotective, nephroprotective and many others action is being used[10,11].

Piper nigrum (Linn.) (family Piperaceae) is one of the most commonly used spices and considered as ''The King of spices'' among various spices. Piper nigrum is effective anti-M. Tuberculosis and is active against both drug sensitive and resistant strains of TB[12]. Piper nigrum along with other phyto-constituents contains major pungent alkaloid piperine which is known to possess many interesting pharmacological actions. Piperine has been found to enhance the therapeutic efficacy of many drugs, vaccines and nutrients by increasing oral bioavailability by inhibiting various metabolizing enzymes[13]. In view of the above property of *Piper nigrum* the present study has been undertaken to find the effects of *Piper nigrum* on infertility induced by Ethionamide and Para amino salicylic acid in female *Sprague-dawley* rats.

MATERIALS AND METHODS

Collection and identification of *Piper nigrum (Linn.)* Plant

Fresh seeds of *Piper nigrum* were procured from the botanical garden of Kokan Krushi Vidyapeeth, Dapoli, Ratnagiri. The initial identification was done by referring related literature and final identification and confirmation was done at the "Government of India, Ministry of Environment, Forest and Climate change, Botanical Survey of India, Western Regional Centre, 7, Koregaon, Road, Pune-411001.No.BSI/WRC/100-1/TECH./2019/53.Prior to process the sample at the department of Zoology S.S & L.S. Patkar College Goregaon (west), Mumbai India. After the identification of the plant ethanolic extract of the seeds was carried out by soxhlate extraction method. The sample was evaporated to dryness and powder was weighed and the yield so obtained was collected in a sterile container and kept at -200C till further use. The weight of the powder was calculated based on weight of the seeds.

Purchase of Ethionamide and Para aminosalicylic acid

The drugs ETH (Macleods Pharmaceuticals Ltd) and PAS (Lupin Ltd) were purchased following the Prescription of Physician from B.J. Medical College and Sassoon General Hospital, Pune, Maharashtra.

Procurement of animals

Sixty six (64) Sprague- dawley rats (average weight 150 - 240 g) of each sex were used for the experiment. They were purchased and procured from the National Toxicological Centre, APT Testing & Research Pvt. Ltd. (ATR) Pune. The experimental study was approved by Ethical committee at APT Research Foundation, Pune prior to the experimentation (CPCSEA NO. 40/PO /Re Bi Rc /S/99/. 11. 03. 2014).The animals were acclimatized, maintained and housed in APT laboratory for a week. The controlled humidity and temperature at 240 C; humidity, 12-hlight /12 hrs dark cycle was also maintained by feeding the rats with commercial rat pallets and water available ad libitum. Blood samples of the above groups were taken after 28th day by heart puncture for hormonal assay.

Hormonal assay of estrogen

Blood serum was analyzed for estrogen at the Unique Bio Diagnostics Enterprises (UBE) Veterinary Pathology Laboratory, B-20, Bhuvaneshwar, Dr. V. K. Valimbe Road, Near Gururani Nagkanya Chowk, Parel Village, Mumbai, for Chemiluminescence immunoassay of estrogen was performed as proposed by Tsang, 1980 and Gore-Langton,1988 by using Immulite 2000 immunoassay system,(Siemens).

Histological Analysis

Ovaries were removed and immersed in Bouins solution for fixation and processed until the embedded in paraffin for histological analysis. Five micron thick sections were prepared using microtome and staining using Hematoxylin

and Eosin (H&E) method as proposed by[14] and histological specimens were examined under the Biological digital microscope Motic B1 Series.

RESULTS AND DISCUSSION

Statistical analysis

Obtained data were statistically analyzed by one way analysis of variance (ANOVA) by F –Test. The value $p< 0.05$ considered as significant.

Table 1, showing administration of dosage of Piper *nigrum*, Ethionamide and Para amino salicylic acid in female rats for 28 days of study. Table No 2 Hormonal essay showing the mean body weight, mean weight of ovary and mean weight of estrogen on treatment of *Piper nigrum*, ETH and PAS in female *Sprague-dawley* rats.

The mean body weight was found in normal control rats was (216.21/gm). With respect to experimental groups the minimum mean body weight was found in rats treated with PAS, was (200.25/ gm), whereas maximum mean body weight was found (252/ gm) in rats treated with PnS. The mean weight of ovary was found in normal control rats was (479 mg). With respect to experimental groups the minimum mean weight of ovary was found in rats treated with ETH, was (350 mg), whereas maximum mean weight of ovary was found (766) in rats treated with PAS + PnS. The mean estrogen level was found in normal control rats was (33.82 Pg/ml). With respect to experimental groups the minimum mean estrogen level was found in rats treated with ETH, was (27.28 Pg/ml), whereas maximum mean estrogen level was found (55.97 Pg/ml) in rats treated with ETH+ PAS .

Fig No.1 Group A normal control showing normal histomorphology of ovarian

Table 1. Showing dosages administration of *Piper nigrum*, Ethionamide and Para amino salicylic acid in female rats for 28 days of study

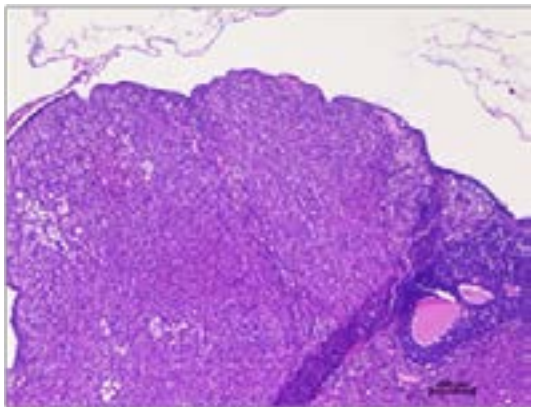
Groups	Number Of Male Animals	Treatment	Doses mg/kg.p.o./day	No Of Days
A	4	Rat Pellets and Ordinary water	Ad libitum	28
B	4	PnS	500	
C	4	ETH	132	
D	4	PAS	400	
E	4	ETH+PAS	132+400	
F	4	ETH+PnS	132+500	
G	4	PAS+PnS	400+500	
H	4	ETH+PAS+PnS	132+400+500	

ETH=Ethionamide. PAS=Para amino salicylic acid. PnS= Piper nigrum mg/kg/per. oral /per day.

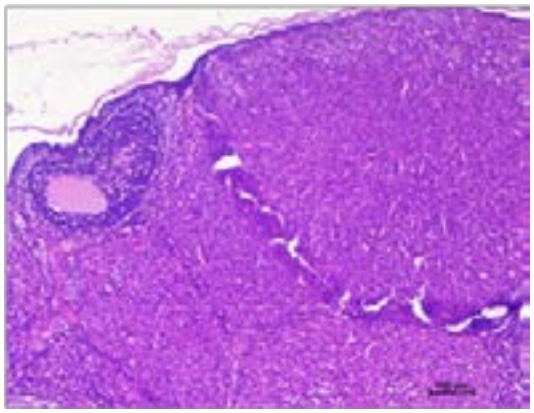
Table 2. Showing means body weight, mean Ovaries weight and estrogen level in female rats after 28 days of study

Group	Treatment	Body weight /gm	Ovary/gm	Estrogen Pg/ml
A	NC	216.25	0.479	33.82
B	PnS	252	0.384	29.82
C	ETH	221.75	0.35	27.28
D	PAS	200.25	0.392	50.65
E	ETH+PAS	206	0.406	55.97
F	ETH+PnS	211.5	0.324	51.77
G	PAS+PnS	204.25	0.766	32.57
H	ETH+PAS+PnS	202	0.459	43.82

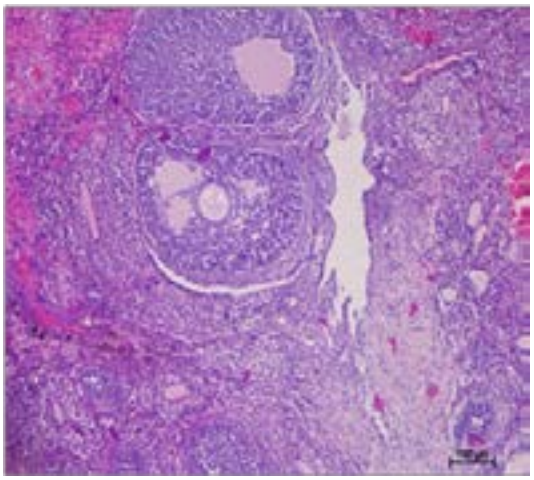
tissue with primary, secondary and tertiary follicles with an absence of degenerative or necrotic or inflammatory changes in the ovarian section. Fig No.2 Group-B-PnS- showing focal congestion in ovarian parenchyma with normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles. Fig No.3, Group C- ETH- showing normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles with an absence of degenerative or necrotic or inflammatory changes in the ovarian section. FigNo.4 Group-D-PAS- showing normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles with an absence of degenerative or necrotic or inflammatory changes in the ovarian section. Fig No.5, Group-E-ETH+PAS- Showing normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles with an absence of degenerative or necrotic or inflammatory changes in the ovarian section. Fig No.6, Group F-ETH+PnS- showing normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles with an absence of degenerative or necrotic or inflammatory changes in the ovarian. Fig No.7, Group-G-PAS+PnS- showing normal histomorphology of ovarian



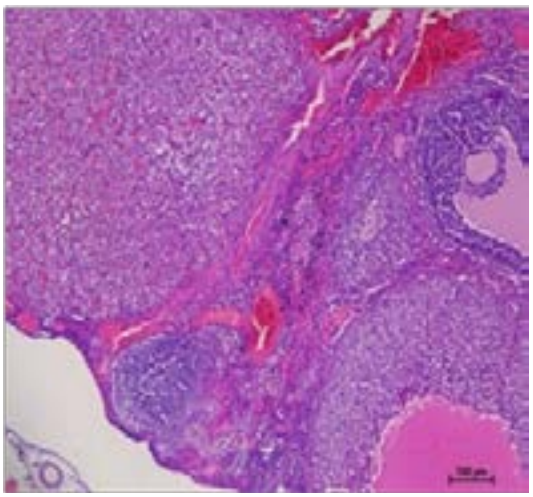
Group-A-NORMAL CONTROL-Normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles. Absence of degenerative or necrotic or inflammatory changes in the ovarian section.



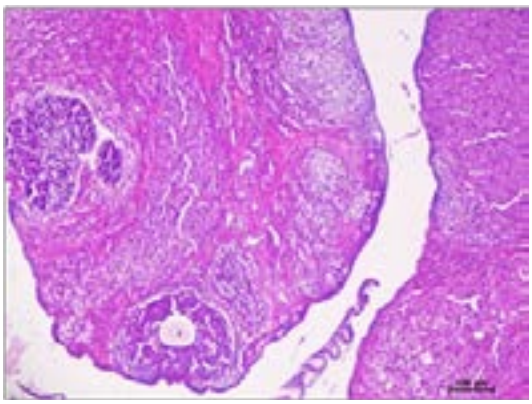
Group-B-PnS-Focal congestion in ovarian parenchyma. Normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles.



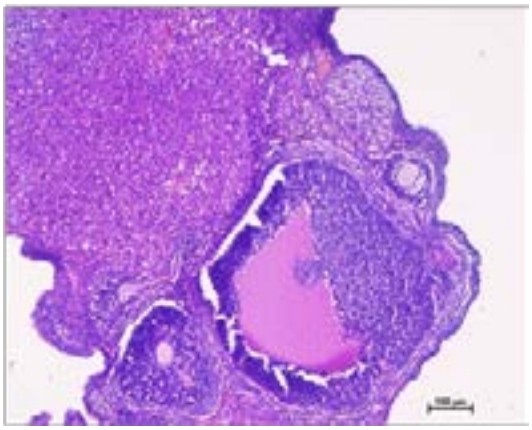
Group C-ETH-Normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles. Absence of degenerative or necrotic or inflammatory changes in the ovarian section



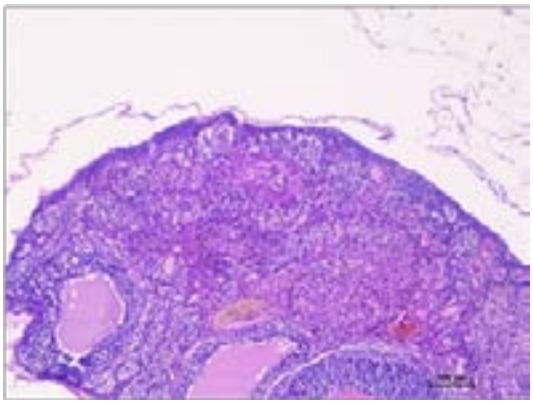
Group-D-PAS-Normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles. Absence of degenerative or necrotic or inflammatory changes in the ovarian section



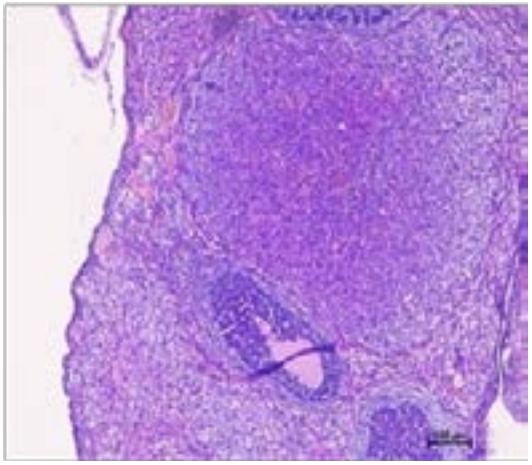
Group-E-ETH+PAS-Normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles. Absence of degenerative or necrotic or inflammatory changes in the ovarian section



Group F-ETH+PnS-Normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles. Absence of degenerative or necrotic or inflammatory changes in the ovarian section



Group-G-PAS+PnS-Normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles. Absence of degenerative or necrotic or inflammatory changes in the ovarian section



Group-H-ETH+PAS+PnS-Normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles. Absence of degenerative or necrotic or inflammatory changes in the ovarian section

Fig: A- H The effect of *Piper nigrum* administration on infertility induced by Ethionamide and Para amino salicylic acid in female rats showing histological architecture of ovary

tissue with primary, secondary and tertiary follicles with an absence of degenerative or necrotic or inflammatory changes in the ovarian section. Fig No.8, Group-H-ETH+PAS+PnS- showing normal histomorphology of ovarian tissue with primary, secondary and tertiary follicles with an absence of degenerative or necrotic or inflammatory changes in the ovarian section.

The study carried out by Pawinee *et al*., 1997 [15] evaluated effect of piperine on fertilization of egg in female hamsters from day first through day forth of the oestrus cycle at dose of 50 and 100 mg/ kg (b.wt. p.o.) they observed that there was enhancement of fertilization, 85.4± 4.1 and 82.8± 4.8 at dose of 50 and 100 mg/kg respectively at 9 hrs after artificial inseminated. The relaxation of this muscle allows more sperm to pass through and would facilitate fertilization at the ampulla since the isthmus serves as a ûlter for the transport of spermatozoa in the female reproductive tract.

However, examination of the embryo retrieved 48 hr after artificial insemination revealed no difference in the stage of embryonic development. There are two lines of evidence in support of this hypothesis. Jagdale 2009 [16] have studied the herb Piper *betle Linn* belonging to family piperacea to examine the antifertility activity where they have concluded that the *piper betle* have reversible potential for antifertile activity in female rats. The elevation in serum estrogen and prolactin was also reported in the same study in female rats due to *piper betle*, but they have not given clear cut idea about the fertility enhancement by supplementation of *piper betle* . Piperine, which has been reported to be rapidly absorbed and biotransformed after administration[17], as well as its metabolites might act on the oviduct and modify its luminal environment to enhance fertilization. One of its major metabolic products, vanillic acid[18], was evaluated in the present study. Thus the enhancing eûect of piperine on fertilization is unlikely to be due to the secretion of the metabolite, vanillic acid, into the oviduct resulting in a reduction in pH. In addition, the possible changes in the oviductal factors for embryonic development are less likely since the embryos obtained from the piperine-treated animals were not in a more advanced stage than those of the controls. Smith *et al*., 1987 [19] In their present study, it is suggested that the enhancement of in vivo fertilization by piperine occurred as a result of the relaxation of the isthmus circular smooth muscle allowing a higher number of spermatozoa at the fertilization site in the female oviduct. Piyachaturawat *et al*., 1982 [20] and Takaki *et al*., 1990 [21] have previously shown that piperine causes relaxation of the uterine smooth muscle in mice and guinea-pig ileum, respectively. Secondly, piperine also induces a release of epinephrine[22], which has been demonstrated to enhance fertilization [23], [24].

In our study we found that *Piper nigrum* was found that much effective to compensate the level of estrogen to normal. There is a paucity of the data on the reproductive toxicity of *Piper nigrum* in female rats. For reproductive toxicity critical examination of a test substance is required. There is a lack of safety data and unclear mechanism about antifertility profile of *Piper nigrum* in female rats. Where as in our study it is found that the estrogen level was highest 55.97 Pg/ml in the animals of group E treated with ETH+PAS and was lowest 27.28 in the animals of group C treated with ETH compared to normal control group. It is observed that the estrogen level is affected due to the administration of ETH and PAS. *Piper nigrum* showed the potential of fertility activity against ETH and PAS by controlling the body weights, ovary weight and estrogen secretion. We hereby in the present study have tried to pave new avenues for the researchers to study and resolve the issue of mechanism of action of *Piper nigrum* against infertility induced by anti-TB drugs in female rat. Molecular studies are still a maze to study mechanism of action of fertility potential of *Piper nigrum* against ETH and PAS induced reproductive toxicity by anti –TB drugs. We need more attention towards clinical data; accurate mechanism of action, bioenhancer capacity, safety profile of *Piper nigrum* to treat the antifertility caused by secondline antituberculous drugs ETH and PAS. The above data adds to a new evidence for understanding the fertility potential of *Piper nigrum* against infertility induced by Ethionamide and Para amino salicylic acid in female rat *Sprague-dawley* rats.

CONCLUSION

In female gonad study it is observed that the estrogen level is affected due to the administration of ETH and PAS. *Piper nigrum* showed the potential of fertility activity against ETH and PAS by controlling the body weights, ovary weight and estrogen secretion. This study suggests that the antituberculosis drugs in combination with *Piper nigrum* are effective to cure the disease and to maintain the fertility in rats. In the present investigation, the increased level of estrogen may be because of zinc and antioxidant property of *Piper nigrum,* which may also be responsible for the improvement of histological architecture of ovary of *Sprague-dawley* rats.

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