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ORIGINAL ARTICLE

Reversible Testicular Toxicity of Piperine on Male Albino Rats

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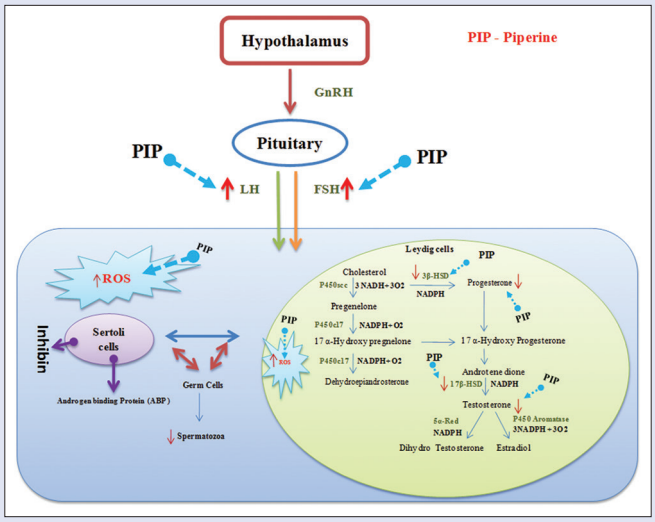
ABSTRACT

Background: Piperine was widely used in traditional medicine for inducing sterility and abortion. **Objective:** To evaluate the effect of the piperine on testis of male albino rats. **Materials and Methods:** Adult male rats were divided into four groups (n = 12). Group I (control): Rats were given vehicle p.o. i.e. 0.5% carboxymethyl cellulose in normal saline daily for 60 days, Group II (ED): Rats received piperine at a dose of 10 mg/kg body weight (b.w.) daily, Group III (E4D): Rats received piperine at a dose of 10 mg/kg b.w. on every 4th day, Group IV (E7D): Rats received piperine at a dose of 10 mg/kg b.w. on every 7th day. Half of the animals from each group were sacrificed after the treatment period (60 days), and the remaining were kept for drug-free withdrawal period (60 days) and then sacrificed. **Results:** Piperine significantly decreased the reproductive organ weights in groups ED and E4D. Piperine induced hormonal imbalance by altering the serum levels of follicle-stimulating hormone, luteinizing hormone, sex hormone binding globulin, serum, and testicular testosterone in groups ED and E4D. Furthermore, piperine decreased the activity of germ cell markers and Leydig cellular steroidogenic enzymes in the groups ED and E4D after 60 days. All the above-altered values returned to normal levels after withdrawal period. Histopathological findings also supported the above findings. **Conclusion:** From the above data, it can be concluded that piperine could be a good lead molecule for the development of reversible oral male contraceptive.

Key words: Androgen binding protein, germ cell markers, male oral contraceptives, serum hormones, sex hormone binding globulin

SUMMARY

- Piperine was employed for the contraceptive purposes in traditional medicine
- Piperine significantly impaired the spermatogenesis by decreasing the testicular hormone synthesis in groups ED and E4D
- Piperine disrupted the testicular antioxidant system by promoting the ROS production and hydroxyl radical generation in rat testis in groups ED and E4D
- Histopathological evidence supported the disruption of spermatogenesis by piperine
- All the effects of piperine after the treatment period (i.e. 60 days) were back to normal after the withdrawal period (i.e., after 120 days).



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INTRODUCTION

It is estimated that world's population will reach a staggering mark of 9.1 billion by 2050.[1] Even though sterilization is an effective and permanent means to control population explosion, social structure, health care infrastructure, and other factors prevailing in a particular region make it unsuitable in several circumstances. Alternatively, reversible or temporary contraception such as female oral contraceptives has gained popularity. Majority of men around the world are willing to take a contraceptive pill if such a method is available.[2] Despite this, very few contraceptive methods are available for men as compared to women. None of the hormonal contraceptives such as testosterone, testosterone ethanate, estrogen, anti-androgens, GnRH analogs, and dihydrotestosterone and chemical contraceptives such as depot medroxy progesterone acetate, cyproterone acetate, levonorgestrel, melatonin, α-chlorohydrin, metopirone, gossypol, and serotonin have the features of ideal male contraceptive.[2,3] Developing a reversible oral male contraceptive, which is rapidly effective, devoid of adverse events, does not influence the progeny, is acceptable to both the partners, and is still a dream for the researchers around the world. During the past century, natural products have served as a good source for modern drug discovery. Piperine is one such natural compound isolated from *Piper nigrum* Linn; *Piper longum Linn*. belongs to the family of *Piperaceae*. Piperine is responsible for the pungency of both the herbs.[4] A formulation containing these two herbs as active ingredients was employed for inducing menstruation and as abortifacient in Indian traditional medicine.[5] Preclinical studies revealed the effectiveness of piperine in treating ailments such as neuronal difficulties and depression.[6-8] Piperine is known to have antidiabetic,[9,10] anti-inflammatory,[11] antiplatelet,[12] antithyroid,[13] anti-leishmanial,[14] anti-asthmatic,[15] antidiarrheal,[16] antitumor,[17]

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anti-mutagenic,[18] and hepatoprotective activities.[19] Piperine is also known for its bioenhancing properties when simultaneously administered with gallic acid,[20] curcumin,[6] β-carotene,[21] tifferon,[22] and (−) epigallatocatechin-3-gallate.[23] It also enhances the bioavailability of clinically valuable drugs such as vasicine, sulfadiazine, isoniazid, ethambutol, phenobarbitone, phenytoin, dapsone, tetracyclines, rifampicin, pyrazinamide, carbamazepine, nimesulide, indomethacin, and ciprofloxacin.[24-26] Based on bioenhancing properties of piperine on antitubercular drugs such as isoniazid and rifampicin, synergistically acting formulations are available for clinical use in India since 2009.[4] A recent survey suggests that piperine is one among the 108 plant active constituents reported to have anti-fertility activity.[27] Furthermore, recent studies have proven the anti-fertility activity of piperine on male albino rats after 30 days of treatment.[28-30] However, the effect of piperine on complete spermatogenic cycle and its withdrawal effects were not investigated. The present study was undertaken to find out the effect of piperine on testes and prostate of male albino rats after the treatment (60 days) and drug-free withdrawal period (120 days) at a dose of 10 mg/kg body weight (b.w.).

MATERIALS AND METHODS

Chemicals

Piperine of 97% purity was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used for various assays were of analytical grade and were obtained from local commercial sources.

Animals

Healthy adult male albino rats of Wistar strain (age: 90 days) weighing 190–210 g procured from the Committee for the Purpose of Care and Supervision of Experimental Animals (CPCSEA) approved vendor (Biogen Laboratory Animal Facility, Bengaluru, Karnataka, India) were used for the present investigation. Animals were housed in polypropylene cages bedded with paddy husk and maintained under well-regulated light and dark (12 h: 12 h) schedule. Rats were fed with standard rat pellet diet (Om Sai Enterprises, Chennai, Tamil Nadu, India) and drinking water *ad libitum*.

Maintenance

All the protocols described in this research work were approved by Institutional Animal Ethical Committee (IAEC No: PU/IAEC/2014/25), Department of Biochemistry and Molecular Biology, Pondicherry University, Puducherry. All the animals were maintained according to the guidelines of CPCSEA, India.

Treatment

The dose of the piperine was selected according to the investigations carried out by D'cruz and Mathur, 2005.[29] Adult male rats were divided into four groups with 12 animals (n = 12) in each group. Group I (control): Rats were given vehicle p.o., i.e., 0.5% carboxy methyl cellulose (CMC) in normal saline daily for 60 days. Group II: Rats were treated with piperine suspended in 0.5% CMC at a dose of 10 mg/kg b.w. p.o. daily for 60 days (group ED). Group III: Rats were treated with piperine suspended in 0.5% CMC at a dose of 10 mg/kg b.w. p.o. on every 4th day for 60 days (group E4D). Group IV: Rats were treated with piperine suspended in 0.5% CMC at a dose of 10 mg/kg b.w. p.o. on every 7th day for 60 days (group E7D). Half of the animals from each group were sacrificed after 60 days of treatment. Remaining animals were kept for the drug-free withdrawal period of another 60 days and then sacrificed (total of 120 days). In this study, treatment period was considered as Phase I and withdrawal period as Phase II.

Collection of serum

Blood samples were collected from control and treated groups after treatment and withdrawal periods from the retro-orbital plexus. Blood was centrifuged at 1500 ×g for 15 min. Serum was separated and stored at −20°C in microfuge tubes until use.

Organ weights

At the end of the treatment (60 days) and withdrawal periods (120 days), the testes and ventral prostate were dissected out and weighed.

Testis index, testicular coefficient, and gonadosomatic index

Testis index was calculated by dividing the left testis weight with the total b.w. and multiplying with 100.[31] Testicular coefficient was calculated by dividing total organ weight by b.w. and then multiplying it with 100.[32] Gonadosomatic index (GSI) was calculated by dividing gonad weight with total b.w. and then multiplying with 100, where gonad weight = (weight of the right testis + weight of the left testis)/2.[33]

Determination of serum and testicular hormones

Hormones such as follicular-stimulating hormone (FSH),[34] luteinizing hormone (LH),[34] testosterone (T),[35] progesterone (P),[36] and sex hormone-binding globulin (SHBG)[37] were assayed using standard methods.

Preparation of homogenates

Homogenates of testes were prepared separately in ice-cold normal saline by using glass-Teflon homogenizer. Supernatants obtained after centrifugation were used for the biochemical assays.

Determination of testicular parameters

Testicular testosterone, total cholesterol, total acid phosphatase (ACP), alkaline phosphatase (ALP), and prostatic ACP were assayed using standard methods.[35,38-40]

Isolation of Leydig cells

Isolation of Leydig cells was carried out by using methods reported by Anand *et al*., with little modifications.[41,42] Animals were sacrificed by cervical dislocation and testes were removed. Decapsulated testes were incubated in modified's Eagles medium containing 0.25 mg/ml collagenase and 10% penicillin and streptomycin solution for 15 min. The seminiferous tubules were allowed to settle under unit gravity for 5 min, and the supernatant containing the interstitial cells were removed. The tubules were resuspended again in the medium and allowed to resettle and supernatants were collected. Supernatants were pooled together and transferred the Leydig cells enriched media into falcon tubes. The tube was inverted for 10 min and then filtered using nylon mesh. RBCs were removed by washing with 0.84% NH_4Cl for 15 min. Macrophages present in the pellet were removed by incubating the pellet in Falcon Petri dish containing 8 ml of complete media (Dulbecco's Modified Eagle's Medium, Hams F12 nutrient mixture in 1:1 ratio and 10% of fetal bovine serum [FBS]) for 45 min. Leydig cells were collected using the micropipette without disturbing the macrophages that settled at the bottom of Petri dish. Finally, Leydig cells were collected and counted using Neubauer chamber, cryopreserved using 95% of FBS and 5% of DMSO. The cells were counted using a hemocytometer and the purity was assessed by histochemical staining for the 3β-hydroxy steroid dehydrogenase (3β-HSD) using the procedure described by Sharpe and Cooper[43] It was observed that more than 95% of the cells were positively stained for Leydig cells [Figure 1].

Determination of steroidogenic enzymes, enzymatic and nonenzymatic antioxidants in testis and isolated Leydig cells

3β-HSD,[44] 17β-HSD,[44] glucose-6-phosphate dehydrogenase (G-6PD),[45] lactate dehydrogenase,[46] superoxide dismutase (SOD),[47] catalase (CAT),[48] lipid peroxidation (LPO),[49] glutathione-S-transferase,[50] reduced glutathione (GSH),[51] hydroxyl radical,[52] and γ-glutamyl transpeptidase[53] were assayed according to the standard procedures.

Histopathological studies

At the end of the experimental schedule, one testis from each rat was taken and fixed in Bouin's fluid, and processed, and the paraffin sections of 4 mm were stained with hematoxylin and eosin stain and then examined under microscope.

Statistical analysis

The data were computed using Prism GraphPad software (GraphPad Company, USA) program version 6.0 and presented as mean ± standard deviation if applicable. Statistical analysis was performed using one-way analysis of variance method, followed by Student's *t*-test. The significance of differences was set at $P < 0.05$.

RESULTS AND DISCUSSION

Recent studies have reported the toxicity of piperine in testis and epididymis of male rats after treating them with different doses.[28-30] However, there is no information available about the effect of piperine for one complete spermatogenic cycle, i.e., 48 days. In addition, the effect of piperine after the withdrawal period, i.e., reversibility of piperine's activity, is not reported. Here, we report the effect of piperine on male albino rats after a treatment period of 60 days and drug-free withdrawal period of 60 days. Various parameters related to spermatogenesis and male fertility were investigated and is discussed below.

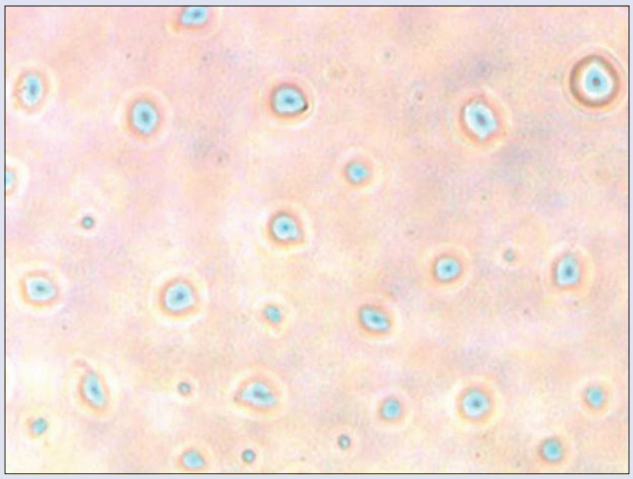

Figure 1: Immunohistochemical staining of Leydig cells (blue and dense blue color spots indicates the presence of Leydig cells)

Effect of piperine on body and organ weights of rats

Rats treated with piperine at a dose of 10 mg/kg (group ED, E4D) for 60 days have significantly reduced testicular weights. It is already reported that the weight of the reproductive organs always give a good measure of the degree of spermatogenesis in rats;[54] moreover, it is suggested that significant decrease or increase in the absolute or relative weight of an organ after administration of a drug indicates the toxicity of the particular drug.[55,56] Hence, weights of the testis and prostate were also measured. The observed decrease in testicular weights in groups treated with piperine (ED, E4D) returned to normal levels after the drug-free withdrawal period. The decrease in the testicular weight after the daily treatment of piperine indicates the toxic effect of piperine on rat testis. No significant differences in the testis index were observed in all the treatment groups (ED, E4D, and E7D) compared to the control group [Table 1]. However, piperine caused a significant increase in the GSI in the group ED treated with piperine at a dose of 10 mg/kg b.w. for 60 days. GSI is inversely proportional to the reproductive efficiency of the rats[57] and testicular coefficient (TCT) value, which is directly proportional to reproductive toxicity in testes.[58] A marginal increase in the GSI in group E4D and E7D was observed after the treatment with piperine for 60 days. GSI and TCT in all the treated groups returned to normal levels after the drug-free withdrawal period of 60 days [Table 1].

Decrease in the prostatic weight in the piperine-treated groups can be correlated with the deregulation of the steroid hormone status within the prostate gland[59] in comparison with the control group. Weights of the prostates were back to normal in all the groups after the withdrawal period; it may be due to the return of normal function in all the accessory organs [Table 1].

Effect of piperine on serum and testicular hormonal status of rats

No significant differences in the serum testosterone (T) levels were observed in all the groups (ED, E4D, and E7D) treated with piperine at a dose of 10 mg/kg, compared to the control group even after 60 days. However, significant reduction in the intra-testicular testosterone was observed in rats treated with piperine in group ED and E4D. This could be due to the nonspecific inhibition of NADPH-dependent cytochrome P450 enzyme, which plays an important role in the steroid hormone synthesis pathway through catalyzing the cholesterol side chain cleavage and other important hydroxylation reactions.[60-62] This decrease in

Table 1: Effect of piperine on rat body and organ weights

S.No	Parameter	Phase 1				Phase II			
		Control	ED	E4D	E7D	Control	ED	E4D	E7D
1	Body weight Phase (g)	307±13.8	275±6.89	286±8.72	291±11.1	418±13.1	390±23.9	393±18.1	381±18.5
2	Testicular weight (g)	3.16±0.14	2.46±0.09*	2.55±0.38*	2.87±0.10	3.02±0.08	2.63±0.15	2.71±0.16	3.02±0.13
3	Testis Index	0.52±0.05	0.66±0.01	0.52±0.02	0.53±0.02	0.64±0.03	0.62±0.02	0.59±0.03	0.63±0.02
4	Gonadosomatic index	0.26±0.02	0.30±0.01*	0. 26±0.03	0.26±0.01	0.36±0.01	0.37±0.02	0.43±0.03	0.44±0.02
5	Testicular Co-efficient	1.02±0.05	1.2±0.04*	1.09±0.06	1.03±0.04	0.72±0.03	0.74±0.05	0.87±0.07	0.89±0.04
6	Prostate weight (g)	0.57±0.06	0.26±0.04*	0.35±0.04	0.39±0.06	0.66±0.03	0.51±0.02	0.50±0.06	0.61±0.02

Values expressed as mean±SD, *n*=6, Control: Group treated with vehicle alone; ED: Treated with piperine 10 mg/kg body wt daily; E4D: Treated with piperine 10 mg/kg body wt on every 4th day; E7D: Treated with piperine 10 mg/kg body wt on every 7th day, Phase I-Treatment period, Phase II-Withdrawal period; *P<0.05, When compared with control group.

testicular testosterone levels correlates with the earlier reports published by Malini *et al.* and D'cruz and Mathur.[28,29] The testicular testosterone levels returned to normal levels in all the groups treated with piperine after the drug-free withdrawal period of 60 days [Table 2].

Piperine significantly increased the levels of the FSH and LH in group ED after 60 days of treatment. This could be due to the damage caused by the piperine to intra-testicular cells, i.e. Leydig cells and Sertoli cells, which in turn leads to a decrease in the testosterone biosynthesis or inadequate production of inhibin resulting in a decreased feedback signaling on pituitary gonadotropins.[28] This effect of piperine can be evidenced by a decreased serum testosterone concentration in the same group. However, increased levels of serum gonadotropins (FSH and LH) reinstated to normal values after the drug-free withdrawal period [Table 2].

Piperine caused only a minor hormonal imbalance in the group ED by increasing progesterone concentration. Progesterone plays a vital role in the estrogen balance and production of gonadotropins and also acts as a key regulator of male reproductive functions such as male sexual drive and ejaculatory potential.[63] However, progesterone levels were restored to normal level in all the piperine-treated groups after the withdrawal period [Table 2].

Serum SHBG was significantly increased in the group ED after the treatment period of 60 days, which further result in a decreased testosterone levels and reproductive functions.[64] Interactions of piperine with the active site amino acid residues of SHBG has already been reported in our previous *in silico* studies.[4]

Effect of piperine on functional status of reproductive organs of rats

In our previous study, we have demonstrated the reversible spermatotoxic effect of piperine on male albino rats.[65] Piperine significantly impaired the testicular function of rats in the group ED and E4D through elevating the testicular cholesterol levels, resulting in the inhibition of the steroidogenesis after the treatment period.[66] A slight increase in the testicular cholesterol level was observed in the group E7D. Elevated cholesterol levels were returned to normal levels after the drug-free withdrawal period. Inhibition of the steroidogenesis was also evidenced by a decrease in the activity of two crucial enzymes, i.e. 3β-HSD and 17β-HSD, which are responsible for testosterone synthesis in group ED and E4D[67] [Table 3].

Administration of piperine caused a significant disruption in the membrane lipid bilayers of the plasma membrane, resulting in the leakage of ALP into the extracellular matrix, which results in the functional impairment of the testicular cells. A marked decrease in ALP activity in groups ED and E4D after the treatment period was observed as compared to the control group. This effect of the piperine may also be attributed due to its fungicidal properties.[68,69] Along with the decreased level of ALP, a decrease in the total ACPs was also observed in groups treated with piperine (ED and E4D), which confirms the piperine-mediated inhibition of steroidogenesis in rats. The degree of steroidogenesis is always dependent on the concentration of ACP.[70] Hence, a decrease in the level of ACP by piperine decreases the steroidogenesis. Piperine negatively affected the development of spermatocytes by decreasing the detachable ACP of lysosomal origin.[71] However, after the drug-free withdrawal period, both the ALP and ACP levels were restored to normal in all the piperine-treated groups.

A decrease in testicular γ-gluamyl tranpeptidase (γ-GT) activity was observed in piperine treated groups indicating the dysfunction of the Sertoli cell. This could be due to the inhibition of androgen receptor by piperine as the function of γ-GT depends upon the regulation of androgen receptor. Our *in vitro* study also supports the same.[4,72,73] Lactate dehydrogenase (LDH) plays a crucial role in the production of energy during spermatogenesis, and it is also necessary for the proper functioning of Sertoli cells.[74,75] LDH activity was found to be inhibited in groups ED and E4D, indicating suppression of testicular function. Further, piperine caused dysfunction of Leydig cells by inhibiting the activity of G-6PD in groups ED and E4D after the treatment period. Determination of G-6PD activity gives a good measure of the functional status of Leydig cells.[76] The activity of γ-GT, LDH, and G-6PD was restored to normal level after the drug-free withdrawal period [Table 4].

Effect of piperine on enzymatic and nonenzymatic antioxidant status of rat testis

Oxidative damage caused to the rat testis can be shown by a decrease in the activities of two major antioxidant enzymes, namely, SOD and

Table 2: Effect of piperine of serum hormones (Testosterone, Progesterone, FSH, LH and SHBG)

S.No	Parameter	Phase 1				Phase II			
		Control	ED	E4D	E7D	Control	ED	E4D	E7D
1	Serum Testosterone (ng/ml)	5.19±0.31	3.77±0.35	4.3±0.42	4.7±0.38	3.77±0.18	3.68±0.17	4.43±0.48	4.61±0.30
2	Serum Progesterone (ng/ml)	28.72±1.81	21.91±1.58*	24.85±2.53	26.62±2.1	17.90±1.45	20.33±2.05	22.02±2.43	21.93±3.1
3	Serum FSH (mIU/ml)	13.9±2.32	19.2±1.39*	14.6±0.9	13.8±1.30	11.8±0.47	13.2±1.45	12.3±0.7	13.5±1.26
4	Serum LH (mIU/ml)	11.6±1.12	18.6±2.38 *	12.4±1.4	11.8±1.18	10.1±0.54	9.4±0.16	10.1±0.74	13.1±1.45
5	Serum SHBG (nmol/L)	15.2±1.36	26.6±3.13*	18.2±1.19	19.0±3.03	15.0±0.61	15.2±0.9	16.7±0.6	18.9±1.24

Values expressed as mean±SD, *n*=6, Control: Group treated with vehicle alone; ED: treated with piperine 10 mg/kg body wt daily; E4D: Treated with piperine 10 mg/kg body wt on every 4th day; E7D: Treated with piperine 10 mg/kg body wt on every 7th day, Phase I-Treatment period, Phase II-Withdrawal period; **P*<0.05; When compared with control group.

Table 3: Effect of piperine on biochemical status of rat testis

S.No	Parameter	Phase 1				Phase II			
		Control	ED	E4D	E7D	Control	ED	E4D	E7D
1	Testicular testosterone (ng/ml)	5.58±0.99	3.45±0.16*	3.95±0.11*	4.01±0.06	3.49±0.18	3.29±0.15	3.38±0.12	3.95±0.28
2	Total cholesterol (mg/dl)	312±23.8	593±18.9*	479±24.2*	362±16.3	226±10.4	242±24.0	248±21.3	242±24.0
3	Total acid phosphatase (U/L)	9.64±0.73	4.73±0.41*	5.76±0.45*	7.03±0.58	4.49±0.9	4.15±0.76	6.86±0.31	7.23±1.18
4	Alkaline phosphatase (U/L)	295±12.0	123±4.60 *	227±9.98	247±11.3	341±32.8	373±21.7	424±19.5	425±28.3
5	Prostate specific acid phosphatase (U/L)	2.32±0.64	0.62±0.26*	1.33±0.83	1.33±0.48	1.44±0.45	1.25±0.54	1.37±0.56	2.88±0.36

Values expressed as mean±SD, *n*=6, Control: Group treated with vehicle alone; ED: Treated with piperine 10 mg/kg body wt daily; E4D: Treated with piperine 10 mg/kg body wt on every 4th day; E7D: Treated with piperine 10 mg/kg body wt on every 7th day, Phase I-Treatment period, Phase II-Withdrawal period;**P*<0.05, When compared with control group.

CAT, to a significant extent in the groups (ED, E4D and E7D) after the piperine treatment period of 60 days. SOD converts the superoxide radicals to hydrogen peroxide (H_2O_2) and CAT is crucial for the conversion of H_2O_2 into water. These two enzyme systems act together to prevent oxidative damage.[77] Piperine is known to accelerate the membrane LPO of lipids in rat testis.[28] In this study, significant increases in the malondialdehyde (MDA) levels were observed in the groups ED and E4D treated with piperine for 60 days. Increase in the MDA content of the tissue results in excessive generation of free radicals.[78] Piperine-mediated oxidative damage through the excessive generation of reactive oxygen species (ROS) was supported with an increase in the hydroxyl radical content in the testes of the rats treated with piperine for 60 days (groups ED and E4D).[79] Testicular toxicity of piperine can be correlated with a significant depletion in the reduced GSH levels in groups ED and E4D after the treatment period. Perturbation of intracellular GSH levels leads to a negative alteration in the cellular metabolism. This results in decreased detoxification potential and redox imbalance in the testicular tissue.[80] Moreover, declined levels of GSH may be associated with an enhanced LPO[81] [Table 4].

The extent of oxidative damage caused to the testis can be shown by a decrease in the activity of glutathione-S-transferase (GST) in the groups ED and E4D treated with piperine for 60 days. GST plays a vital role in the adaptive defensive mechanisms and works against the free radical induced oxidative damage and eliminates the toxic products by catalyzing the conjugation reactions.[80] On the contrary to its catalytic ability, GST is important for the functioning of the sperm.[82] All the above-mentioned altered activities of enzymes were restored to normal levels after the drug-free withdrawal period of 60 days [Table 4].

Effect of piperine on antioxidant status of the Leydig cells

Piperine significantly disrupted the antioxidant defensive mechanism of Leydig cells in groups ED and E4D by decreasing the activity of SOD and CAT after the 60 days treatment. SOD protects the Leydig cells from the free radical induced damage by converting superoxide anions into hydrogen peroxide (H_2O_2) and impairs the LPO. Whereas, CAT eliminates the lipid, protein, and DNA destructive H_2O_2 by its catalytic activity.[83] Increase in the ROS production can be shown with concomitant increase in MDA levels in groups ED and E4D.[84] The decreased activity of SOD and CAT and increased MDA content were reinstated to normal levels after the drug-free withdrawal period of 60 days [Table 5].

Histopathological studies

Histology section of the group ED shows many desquamated spermatozoa with a decrease in the thickness of germ layer. The tubules showed both Type 1 and Type 2 spermatogonia and the rest composed of mostly mature spermatozoa. Primary spermatocytes are reduced in number compared to the control group. Section of the group E4D shows a decrease of 30% spermatozoa in the tubules, resulting in hypospermatogenesis. In contrary to this, section of group E7D shows a decrease of 10% spermatozoa in tubules, which represents mild hypospermatogenesis compared to the control group [Figure 2]. All the pathological changes appeared after treatment period returned to normal status after the drug-free withdrawal period of 60 days [Figure 3].

CONCLUSION

Compared to the synthetic molecules, natural and herbal products have been more successful in delivering safer drugs for the human kind.

Table 4: Effect of piperine on biochemical status of rat testis

S.No	Parameter	Phase 1				Phase II			
		Control	ED	E4D	E7D	Control	ED	E4D	E7D
1	Super oxide dismutase	1.18±0.03	0.67±0.03 *	0.85±0.04	0.91±0.04	0.88±0.05	0.76±0.04	0.74±0.03	0.80±0.04
2	Catalase	0.46±0.01	0.19±0.03*	0.29±0.03	0.41±0.05	0.14±0.02	0.13±0.05	0.16±0.02	0.11±0.02
3	Lipid peroxidation	1.21±0.06	2.85±0.02 *	2.21±0.04	1.62±0.01	1.09±0.36	0.93±0.17	1.06±0.22	1.14±0.13
4	Glutathione -S-transferase	1.10±0.24	0.33±0.16 *	0.83±0.13	0.60±0.17	0.59±0.14	0.60±0.02	0.51±0.10	0.69±0.09
5	Reduced Glutathione	5.47±0.53	3.07±0.49*	4.52±0.24	5.1±0.44	3.98±0.31	4.4±0.47	3.98±0.06	4.8±0.04
6	Hydroxyl Radical	1.14±0.37	2.98±0.05*	1.13±0.17	1.27±0.24	1.14±0.04	1.16±0.05	1.19±0.07	1.13±0.04
7	γ-Glutamyl transpeptidase	1.53±0.08	0.74±0.08*	0.80±0.17	1.56±0.15	1.37±0.13	1.41±0.22	1.53±0.16	1.36±0.21
8	Lactate Dehydrogenase	0.71±0.10	0.38±0.03*	0.56±0.04	0.64±0.03	0.54±0.05	0.48±0.36	0.61±0.03	0.58±0.03
9	Glucose 6-Phosphate dehydrogenase	0.54±0.01	0.27±0.08*	0.39±0.01	0.34±0.02	0.37±0.03	0.39±0.03	0.36±0.01	0.37±0.02

Units: SOD: units/mg protein; CAT: H_2O_2 Consumed/min/mg protein; LPO: nmoles of MDA formed/mg protein; GST: nmoles of CDNB-GSH complex formed/min/mg protein; GTP: µmoles of p-nitroaniline formed/min/mg protein; G-6-P: units/mg protein, GSH-µg/mg protein; LDH- mU/ml, Hydroxyradical - µmoles/min/mg protein. vValues expressed as mean±SD, *n*=6, Control: Group treated with vehicle alone; ED: treated with piperine 10 mg/kg body wt daily, E4D: Treated with piperine 10 mg/kg body wt on every 4th day; E7D: Treated with piperine 10 mg/kg body wt on every 7th day Phase I-Treatment period, Phase II-Withdrawal period, *=*P*<0.05, When compared with control group

Table 5: Effect of piperine on rat Leydig cells in phase I and phase II

S.No	Parameter	Phase 1				Phase II			
		Control	ED	E4D	E7D	Control	ED	E4D	E7D
1	3β-HSD	53.34±3.47	34.26±3.08*	40.14±4.02	44.34±3.56	69.27±6.45	57.74±4.8	65.77±4.57	61.58±4.64
2	17 β-HSD	30.75±2.14	18.71±2.04*	24.65±2.17	25.82±2.36	26.22±2.02	31.33±2.93	28.02±2.14	36.36±2.73
3	Super oxide dismutase	10.4±1.02	7.07±0.65*	8.20±0.58	8.50±0.68	8.42±0.64	7.75±0.65	7.90±0.52	8.19±0.65
4	Catalase	0.69±0.07	0.37±0.02*	0.47±0.05	0.61±0.07	0.75±0.13	0.77±0.05	0.80±0.05	0.86±0.06
5	Lipid peroxidation	1.29±0.08	2.52±0.19*	1.56±0.09	1.76±0.10	3.01±0.24	2.23±0.26	2.48±0.19	3.11±0.23

Units: 3β-HSD - nmoles of NAD reduced/min/mg protein; 17 β-HSD - nmoles of NADPH oxidized/min/mgprotein; SOD: units/mg protein; CAT- H_2O_2 Consumed/min/mg protein; LPO - nmoles of MDA formed/mg protein. Values expressed as mean±SD; *n*=6; Control: Group treated with vehicle alone; ED: treated with piperine 10 mg/kg body wt daily; E4D: Treated with piperine 10 mg/kg body wt on every 4th day; E7D: Treated with piperine 10 mg/kg body wt on every 7th day Phase I-Treatment period; Phase II-Withdrawal period; *=*P*<0.05; When compared with control group

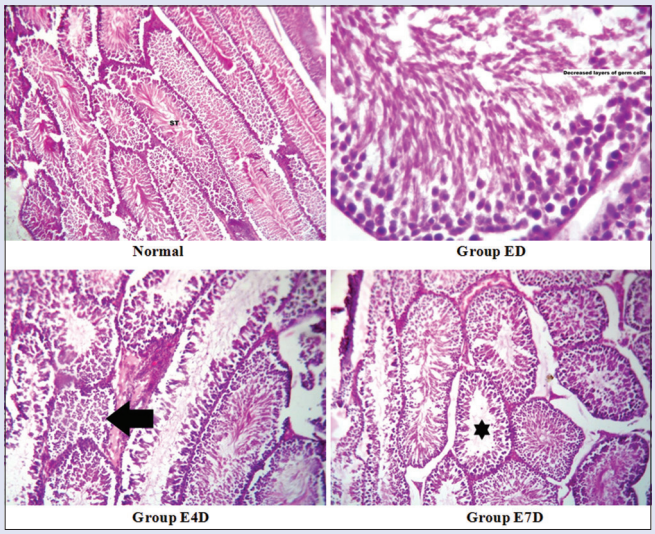

Figure 2: Histopathological sections of testis after the treatment period Legend: Control: Seminiferous tubules showing normal spermatogenesis (H and E, ×100), ED: Seminiferous tubules showing decreased thickness of germ cells due to fewer spermatocytes (H and E, ×400), E4D: Many seminiferous tubules show normal spermatogenesis while some others show no mature spermatozoa (hypospermatogenesis – left arrow) (H and E, ×100), E7D: Most seminiferous tubules show normal spermatogenesis while very few show no mature spermatozoa (mild hypospermatogenesis - star) (H and E, ×100)

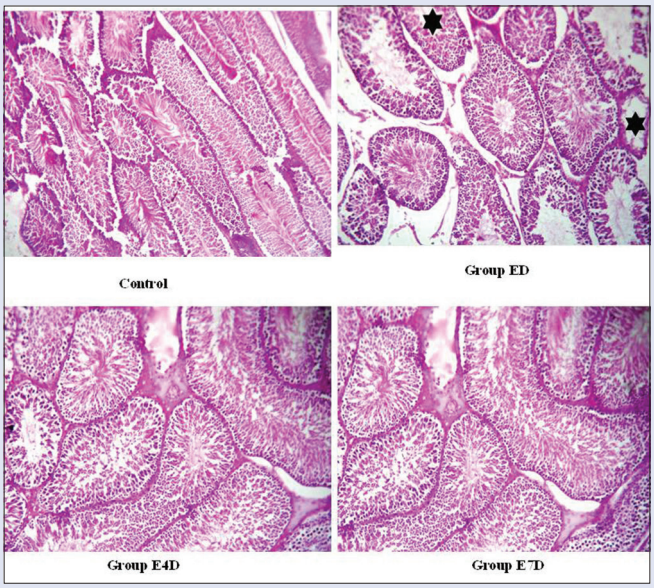

Figure 3: Histopathological sections of testis after the withdrawal period Legend: Control: - seminiferous tubules showing normal spermatogenesis (H and E, ×100), ED: Most seminiferous tubules show normal spermatogenesis while very few show no mature spermatozoa (mild hypospermatogenesis - star) (H and E, ×100), E4D: Seminiferous tubules showing normal spermatogenesis (H and E, ×100), E7D: Seminiferous tubules showing normal spermatogenesis (H and E, ×100)

The present study was aimed to evaluate the effect and reversibility of piperine mediated testicular toxicity in male albino rats. Various parameters related to male fertility such as weights of the testis and prostate, serum, and intra-testicular testosterone levels, and serum gonadotropin levels were measured. Furthermore, functional status of testis was ascertained by measuring the steroidogenesis-related parameters such as activity levels of enzymes 3β-hydroxy and 17β-HSD, ALP, ACPs, γ-GT, lactate dehydrogenase, and G-6PD. The extent of oxidative damage to testis caused by piperine treatment was monitored by measuring the activity levels of SOD, CAT, and GST enzymes; further, levels of MDA, ROS, and reduced glutathione levels were measured.

Our study result suggests that piperine disrupted the functional integrity of the testis by altering the germ cell markers, antioxidant status, and testicular hormones. These biochemical study results were further reinforced by histopathological observation of hypospermatogenesis on piperine treatment. However, the toxic effects of piperine were reversed after the drug-free withdrawal period of 60 days, suggesting reversible nature of piperine effect on male fertility. From the above results, it can be concluded that piperine can be used as a lead molecule for the development of reversible oral male contraceptive agent.

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Conflicts of interest

There are no conflicts of interest.

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