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Defensive Effects of Breberine against Cypermethrin Induced Male Reproductive System Toxicity in Rabbits

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ABSTRACT

Current research has determined the protective role of Berberine (BRB) in the reproductive toxicity induced by cypermethrin (CYP) in male rabbits, Twenty adult New Zealand white rabbits were randomized into four main groups: group I (control group) received (1 ml / kg b.w saline orally), group II received (CYP64mg / kg b. w./orally); group III (received BRB 50 mg / kg b. w./plus CYP64mg / kg b.w orally); and group IV (received BRB 100 mg / kg b. w plus CYP64mg.kg b. w orally) received all oral gavage therapies for 21 consecutive days. On the (22) day of the experiment, all the rabbits were scarified, and then samples were taken of the blood, testis and caudal epididymis. CYP exposures were found to have substantial adverse effects on the reproductive system of adult male rabbits as a result of decreased testicular and epididymal weight, a significant decrease ($p<0.05$) in serum testosterone (T) and increase significantly in follicle stimulating hormone (FSH), luteinizing hormone (LH), Changes and histopathological ones. BRB has a substantial improvement in T, FSH and LH ($p < 0.01$) and enhances tissue morphology in BRB (50 mg/kg), while BRB (100 mg/kg) has more reproductive system curative ability. These findings indicate that CYP-induced reproductive system toxicity in rabbits can be covered by BRB (100 mg/kg) antioxidant impact.

Keywords: Berberine, Cypermethrin, Reproductive system, Rabbits

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INTRODUCTION

Among the most widely used insecticides are synthetic pyrethroids. Due to their high efficacy against a wide range of insects, rapid biodegradation, low mammalian toxicity and target-oriented mechanism of action, the use of these insecticides has increased in recent years over organochlorines, organophosphates and carbamates. Cypermethrin is a popular synthetic pyrethroid used in agriculture, forestry, and programs for public and animal health. Recent studies have shown the adverse impact of cypermethrin on the nervous system,[1] the hepatic and renal systems[2] and the male reproductive system, while known to be non-toxic to mammals[3-5]. in laboratory animals. Reports of reproductive toxicity of cypermethrin are of significant concern since, at very low dosage, human spermatogenesis may be susceptible to chronic chemical exposure. Therefore, this research was planned to determine the cypermethrin-associated reproductive toxicity of male Wistar rabbits.

In order to ensure that spermatogenic and steroidogenic roles are not disrupted by repeated exposure to xenobiotics, the testicular tissue requires an elaborate collection of antioxidant enzymes and free radical scavenger. These antioxidant defence systems are of major importance since the most significant cause of impaired testicular function is considered to be peroxidative harmWhile there are plenty of endogenous antioxidants in tests to scavenge free radicals, chronic exposure to xenobiotics such as cypermethrin can lead to excessive lipid peroxidation (LPO) and oxidative injury. There is also a need for exogenous antioxidants to minimize oxidative stress in studies and to control the spermatogenic cycle and steroidogenic function positively [6].

Berberis vulgaris (barberry), and Berberis aristata (tree turmeric). The BRB alkaloid may be present in the plants' roots, rhizomes, and stem bark. BRB extracts and decoctions have shown important antimicrobial activity for the treatment of bacterial diarrhea against a range of is an over-the-counter medication. In 1988, species, including microbes, viruses, fungi, protozoa, helminths, and chlamydia, had hypoglycemic impact. BRB was first recorded in China when Berberine was administered to treat diarrhea in patients with diabetes [7]. This compound of alkaloids has a broad range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, anti-cancer, anti-hypertensive, renoprotective and anti-hyperglycemic impact [8, 9]. BRB functions as a scavenger for ROS and reactive nitrogen species and has a defensive effect against free radicals [10]. In addition, BRB increases the activity of enzymes with antioxidants such as SOD, CAT, GPx and GSH [11]. In addition, BRB has been shown to reduce oxidative stress parameters such as protein carbonyl (PC) content, activity of malondialdehyde (MDA), NO level, and activity of myeloperoxidase (MPO)[12].

MATERIALS AND METHODS

Chemicals

Garda Chemicals, Mumbai, India, obtained a technical grade of cypermethrin or alpha-cypermethrin (97 percent). 100% Natural was purchased from BULK SUPPLEMENT.com USA by Berberine HCL (BRB). Ketamine: 10% inj. BY KEPRO-HOLLAND. Xylazine, XYL-M2 and VMD-Belgium, respectively.

Animals

Male New slant Rabbits, obtained from the animal house of the College of Science, Babylon University, were used in this study at aged (10 months) and weighted (1800-2000

g) At controlled temperature (22 ± 2 ° C) and humidity (45 -55), the animals were maintained with a light-dark period of 12 h and given food and water ad libitum.

Experimental procedure

Twenty rabbits were distributed into four comparable classes and they got the rabbits

Therapy as if they were and the medications they obtained were as follows:

1. Group I: Control (1 ml/kg Saline orally) for (21) days.
2. Group II: CYP (64 mg/kg, orally) for (21) consecutive days [13].
3. Group III: CYP (64 mg/kg, orally) + BRB (50 mg/kg orally by stomach tube) for (21) consecutive days [14].
4. Group IV: CYP (64 mg/kg orally) + BRB (100 mg/kg orally by stomach tube) for (21) consecutive days [15].

Assessment of absolute testis and epididymis weight

With the aid of a Sartorius digital balance, the testes and epididymis of rabbits were dissected, cleared of fats and blood-free, and weighed [16].

Blood collection

Both animals were fasted overnight at the end of the trial, euthanized, and blood samples were taken from the heart in serum separation centrifuge tubes, centrifuged for 15 minutes at 3000 rpm (4oC) and processed at 3000 rpm (4oC) [17], Radioimmunoassay (RIA) kits Siemens ADVIA Centaur XP (Siemens; Erlangen, Germany), Serum FSH and LH were tested at-20oC as aliquots for further testosterone (T) determination using enzyme-linked immunosorbent assay (ELISA) kits Blue Gene Biotech (Shanghai, China) according to the prescribed manufacturers' instructions[18].

Histological analysis

Testis and epididymis have been separately excised, washed and weighed for each rabbit. The testicles have been dissected and fixed for 1 week in Bouin 's solution. Samples were treated by paraffin embedding and a rotary microtome (MICROM GmbH, Germany) was cut into the blocks and stained with hematoxylin-eosin (H&E) [19].

Statistical analysis

The data obtained was described as Mean ± SD. Full Randomized Design (C.R.D.) was used to analyze the homogeneity of variance for and variable [20]. The mean differences between the averages of the studied traits were calculated using the Duncan test [21] at the likelihood level of (0.01) and (0.05). The statistical data was analyzed using [22].

RESULTS

Reproductive organ weights

The treatment of adult male rabbits with CYP, as shown in Table (1), resulted in a statistically significant decrease in the weight of both right and left testis, right and left epididymis, relative to the control group. Co-administration of both BRB and CYP doses, however, showed a substantial elevation in test weights, epididymis, equivalent to that of rabbits receiving CYP alone. The weight values of the reproductive organs were thus restored by BRB to meet roughly the control group values.

Serum testosterone, LH and FSH levels

Compared to the corresponding control rabbits, the serum testosterone concentrations showed a substantial decrease in CYP animals ($p < 0.01$, while it showed a significant increase in the level of LH and FSH hormones. In both concentrations of CYP+BRB treated animals, an improvement has been observed in hormones level compare with CYP rabbits ($p < 0.01$) (Table 2).

The CYP-induced histopathological alteration effect of BRB in testicular and epididymal tissues

Histopathological section of both testis and epididymis examination in control group using H&E(100) stain shown normal histological structures **(Fig1:a ; Fig1:b).** The light microscopic examinations of group II which treated with CYP only shown atrophy of seminefrons tubules, irregular membranes and vaculation of spermatogonia (**Fig2:a)**, also light microscopic of epididymis shown decrease in amount of spermatozoa inside the tubules lumen with cribriform change in some tubules **(Fig2:b).** In group III (CYP plus BRB50 mg/kg b. w)shown congestion of testicular blood vessels, vaculation in some tubules **(Fig3:a)** while the histological section in epididymis shown good regeneration tubular epithelium which contain moderate amount of sperm cells in their lumen **(Fig3:b).** Group IV (CYP plus BRB100 mg/kg b. w) light microscopic of testis shown good regeneration of epithelial cells lining the seminefrons tubules with mild interstial edema **(Fig4:a),** light microscopic of epididymis of this group shown excellent regeneration of tubular epithelia and contain large amount of sperm cells **(Fig4:b).**

Table 1: Effect of CYP, CYP + BRB on the reproductive organs weight of male rabbit.

Group	Testosterone	LH	FSH
Control	1.7 ± 0. 2 A	1.6 ± 0.2 C	7.8 ± 0.8 C
CYP	0.7 ± 0.1 C	3.6± 0.3 A	14.2 ± 0. 6 A
CYP+ BRB 50mg/kg b.w	1.1 ± 0.2 B	2.2 ± 0.1 B	10.1 ±0. 7 B
CYP+BRB 100mg/kg b.w	1.5 ± 0.3 A	1.8 ± 0.1 C	8.0 ± 0.5 C

The values are expressed as ±SE-mean. Means followed by the same alphabetical letter at p < 0.05 do not vary significantly

Mean±SD serum testosterone (ng/ml), LH (ng/ml) and FSH (ng/ml) – in Control, CYP and CYP+ BRB groups. The average ages of characteristics that have vertically held distinct levels suggest high significance at 0.01

Table 2: The table compared among control group, group II, group III and group Iv from Where testosterone,LH and FSH hormones

Weight of organ Group	Right testis(g)	Left testis(g)	Right epididymis(g)	Left epididymis(g)
Control	2.42 ± 0.13 AB	2.40 ±0.13 AB	1.48 ± 0.05 AB	1.45 ± 0.0 4 AB
CYP	1.85 ±0.07 C	1.82 ± 0.10 C	0.96 ± 0.06 C	0.82 ± 0.03 C
CYP+ BRB 50mg/kg b. w	2.22± 0.12 B	2.20 ± 0.15 B	1.37 ± 0. 04 B	1.35 ± 0.0 5 B
CYP+BRB 100mg/kg b. w	2.65 ± 0.17 A	2.62 ± 0. 12 A	1.50 ± 0.0 3 A	1.47 ± 0.02 A

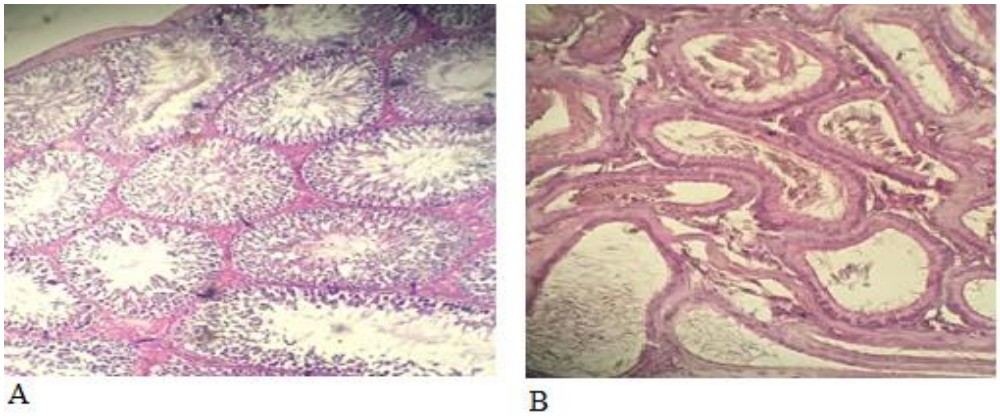


Figure 1: Histopathological section of both, A: testis and B: epididymis in control group with using H&E(100) stain and examined under light microscope

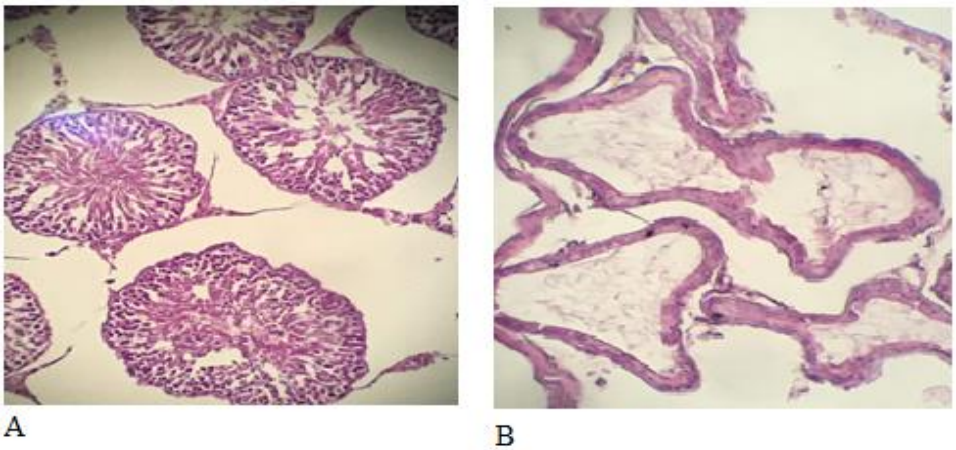


Figure 2: Histopathological section of both, A: testis and B: epididymis group II (treated with CYP only) with using H&E(100) stain and examined under light microscope

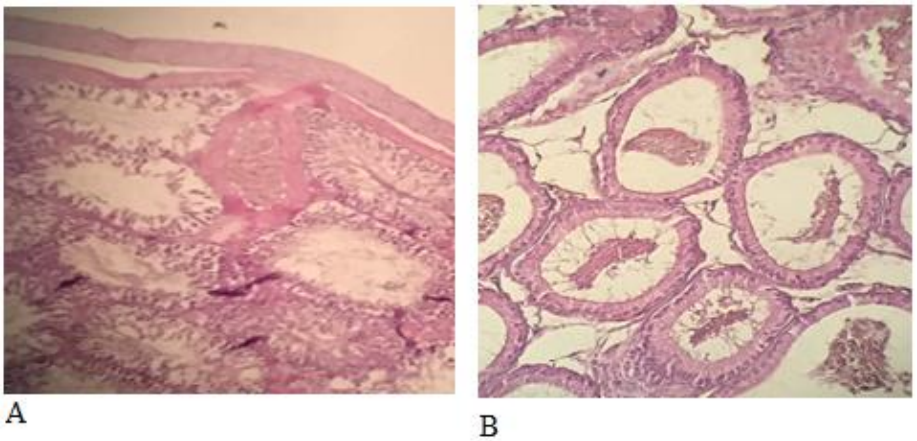
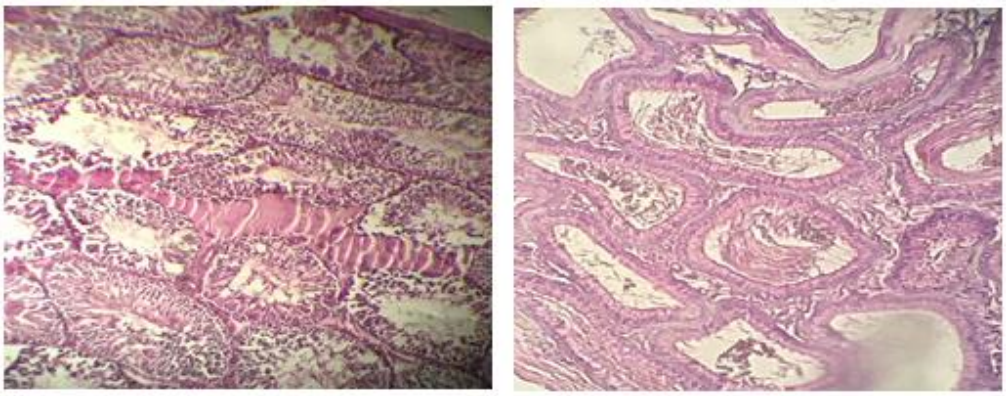


Figure 3: Histopathological section of both, A: testis and B: epididymis group II (CYP+ BRB 50mg/kg b.w) with using H&E(100) stain and examined under light microscope



A B

Figure 4: Histopathological section of both, A: testis and B: epididymis group II (CYP+ BRB 100mg/kg b.w) with using H&E(100) stain and examined under light microscope

DISCUSSION

An established factor in the weakening of the male reproductive system and infertility is exposure to environmental toxicants, including pesticides. A number of recent studies have shown its reproductive toxicity in mammalian and non-mammalian laboratories and wildlife species, initially thought to be healthy for household use. [23,24] The weight of the testicles and epididymis is a valuable reproductive health index. The decrease in testis weight when exposed to xenobiotics may be due to decreased tubule size, decreased number of germ cells, and elongated spermatids. [25] As observed in the study, the decrease in organ weight may be due to a decrease in serum T, FSH and LH levels, consistent with other findings. [26] The antiandrogenic effect of pyrethroids and other pesticides was primarily attributed to accumulation in the testicles. Co-administration of BBR to the CYP-given rabbits increased the weight of the testes and accessory sex organs. BRB revoked CYP's adverse effect on the weight of reproductive organs, these findings are in line with the results of other studies [27].

A decrease in serum testosterone level has been observed in cypermethrin exposed rabbits. The decrease in serum T level may be due to the direct effect of cypermethrin on testicular tissue. The results of this study indicate that the accumulation of cypermethrin in the testicular tissue increased oxidative stress. The high oxidative stress in testicular tissue has resulted in reduced cell viability of all cell types. Low cell viability and accelerated cell death have contributed not only to reduced cell and tissue mass, but also to numerous sperm structure and function abnormalities [6]. This result showed that CYP significantly increased LH and FSH while decreasing testosterone levels across the pituitary-testicular axis, in line with another research. In current research, FSH and LH levels have been shown to be elevated in exposed rabbits to CYP. FSH and LH interfere by influencing Sertoli and Leydig cells in spermatogenesis and steroidogenesis, respectively [28]. CYP decreased the resistance of Leydig cells and serum testosterone, leading to an increase in the level of LH. The increase in serum FSH levels indicates the destruction of spermatogenesis in experimental rabbits and represents the loss or damage of germ cells to Sertoli cells due to the disruption of FSH secretion control[29].An undamaged pituitary-testicular axis is also demonstrated by decreased serum testosterone levels with increased FSH and LH levels in experimental rats[30]. It was shown in our findings that BRB can increase the levels of reproductive hormones testosterone, reduce the FSH, and LH in CYP rabbits. This may be due to the defensive impact of BRB on cells of Leydig. Previous research has authorized the protective effects of BRB in support of our findings by using properties such as anti-apoptotic, anti-oxidative and antigenotoxic properties [31]. As preliminary details, we found that BBR significantly improves the VCL-reduced tubular differentiation and spermiogenesis ratios and significantly upregulates the synthesis of testosterone. It would be more rational to assume that the enhancement of testosterone in BBR-treated animals could partially boost spermatogenesis and spermiogenesis ratios, given the promotional role of testosterone on Sertoli cell-related niches as well as spermatogenesis [32,33]. Histopathological changes atrophy of tubules of seminefrons, abnormal membranes and spermatogonia vaculation, with constant findings[34] Recent studies that indicated the production of behavioral and testicular structure abnormalities in sub-lethal doses of CYP included atrophic and twisted seminiferous tubules and multi nucleated formation of sertoli cell vacuolization spermatids with atrophy with ledig cells, The decrease in the diameter of the seminiferous tubules in sham rats suggests that atrophy of these tubules contributes to disruption of morphology and spermatogenesis in the testis. There is actually a positive relationship between the diameter of the seminiferous tubules and the operation of spermatogenesis.[35] Testicular toxicity, however, is more proportional to the time of treatment than the amount of dosage used. There is also a decrease in the amount of spermatozoa within the epididymis tubules lumen, these results suggest that CYP is responsible for increasing oxidative stress that induces plasma membrane alteration by increasing lipid peroxidation and causing sperm viability inhibition due to testicular and epididmal dysfunction, These findings are consistent with several studies [36] which explained that CYP could induce impairment in male rats of the structure of seminiferous tubules and spermatogens, and impairment could be attributed to decreased expression of the androgen receptor,[37] also found that a single oral LD50 dose of alpha-CYP in rats reduced the activity of the catalase enzyme and increased serum (AST, ALT and ALP) associated with a cytotoxic effect on testes characterized by seminiferous tubule hyalinization and vacuolation. Our findings show the ameliorative effect of BRB (100 mg / kg) on CYP for [21] days mediated epithelial cell regeneration lining the seminefron tubules and containing significant quantities of sperm cells, which can be explained by the

BRB antioxidant [33]. As for BRB, it had less impact than (100 mg / kg) (50 mg / kg). It can be deduced that, based on the advanced reports, CYP had adverse effects on the testis and epididymis. Administration of BRB (100 mg / kg) showed a marked protective effect on male reproductive organs due to barberry root and stem bark containing different alkaloids, such as; Berberine, oxyacanthine, bermamine, palmatine, jatrorrhizine, columbamine, and berberubine They have antioxidant, medicinal, and anti-inflammatory properties. The most important antioxidant compounds are plant alkaloids. Given that barberry roots are abundant in alkaloids and since barberry root antioxidant properties have been confirmed in several studies, we may assume that barberry root antioxidant activity is likely related to its alkaloid compounds [16,38].

CONCLUSION

The BRB could protect the testes and epididymis of rabbits against CYP-induced toxicity based on our findings. Furthermore, the effect of BRB (especially at 100 mg / kg bw concentration) was greater than that of BRB (50 mg / kg bw concentration). BRB (100mg / kgb.w) can also be recommended to protect the testes and epididymis of the rabbit against the toxic effects of Cypermethren due to its antioxidant properties.

CONFLICT OF INTEREST

None of the writers was expected to report any conflicts of interest.

SOURCE OF FUNDING

The research was conducted independently, there was no funding, impact on the design of the study, review, preparation of manuscripts or scientific publication.

ETHICAL CLEARANCE

The local ethical committee (College of Veterinary Medicine/ Al-Qasim Green University) approved the project. C413/12).

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