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Protective Role of Black Berry Juice Against Hepatotoxcity and Reproductive Toxicity of Chlorpyrifos in Male Rats

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Chlorpyrifos (CPF) is causing different sorts of toxicity and black berry juice is a new species of Black berry juice and it is the first time to study its effect against the toxicity of CPF. Therefore, the present study is aimed to elucidate the possible protective effects of Black berry juice in alleviating the toxicity of chlorpyrifos on reproductive performance, testosterone levels, enzyme activities and lipids profile in serum of male rats. Animals were divided into four groups; one orally administered (o.d.) CPF at a dose of 9 μg/kg b.w. for consecutive 80-days, *Black berry juice* group (50 mg/kg b.w.), *Black berry juice* and chlorpyrifos group and a control group. Results showed that there was a correlation between CPF administration and significant decrease of the sperm counts, spermatozoon survival and testosterone level as well as increase of sperm aberrations. CPF increased significantly the lipid profile and the levels of various serum liver marker enzymes. In contrast, co-administration of *Black berry juice* to CPF-treated rats restored almost most of these biochemical parameters to normal levels. On the other hand, CPF resulted in histopathological alterations in testes of male rats. However, pre-administration of *Black berry juice* to CPF-treated animals improved the testicular damage and alleviates the toxic effects of CPF on reproductive functions and neural functions in male rats

Key words: Chlorpyrifos, Black berry juice, Stress, Biochemical, Reproductive toxicity, Tetsosterone Testes, Spermatozoa.

During the last decades the use of pesticides has increased steadily in developing countries in an effort to increase food production and control vector-borne diseases. At present, there are more than 65,000 chemicals that are classified as pesticides. Because large amounts of these chemicals are released into the environment daily and many of them affect non-target organisms unfortunately, this has resulted in some negative side effects on human health and the environment[1,2].

Organophosphates are among the most widely used synthetic insect pesticides. The widespread use of organophosphates has stimulated research into the possible existence of effects related with their reproductive toxic activity[3]. Occupational exposures to pesticides could diminish or destroy the fertility of workers sparked a concern about the effects of hazardous substances on male reproductive health. The issue of testicular toxicity is of growing concern as a large number of Organophosphates viz., diazinon[4], and methyl parathion[5] adversely affect the testicular functions in experimental animals. Owing to the extensive use of organophosphate pesticides in agriculture there is a high risk of human exposure to these chemicals[6].

Chlorpyrifos (CPF), (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphoro-thioate) is a conventional organophosphorous insecti- cide, that was first registered as a broad spectrum insecticide in 1965. It was used widely to control a variety

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of pests in agriculture and animal farm[7]. CPF interferes with the acetyl cholinesterase enzyme, which is necessary for normal nerve transmission[8]. It has been reported that it is linked to male and female genital deformities[9,10]. Additionally, the exposure of laboratory animals to CPF elicits a number of effects including hepatic[11,12] and testicular damage[3].

The testes of humans and other mammals are highly susceptible to damage produced by genetic to chemical or other means. It has been reported that pesticides have been shown to cause overproduction of reactive oxygen species (ROS) in both intra and extra cellular spaces, resulting in a decline of sperm count and infertility in wildlife human[13].

One of the most important natural diets with anti-oxidant properties is black berries. Berries are among one of the most widely consumed fruits in the human diet. Berry fruits, wild or cultivated,are proved as a traditional and rich source of bioactive compounds, possessing important biological activities such as flavonoids (anthocyanin), some minerals (Na, K, Ca, Se, Zn and P), vitamins (vitamin A, B complex, C and E) phenolic acids(galic, p coumaric,caffeic, ferulic) and phenolic polymers (ellagic acids)[14]. The anti-oxidant capacity of these berries was related to their constituents' particularly total phenolics and anthocyanins and[15]. Furthermore, anthocyanin is one of the most important water-soluble flavonoid pigments that give black berries their characteristic red to blue color[16].

It was found that, the contents of vitamin C, vitamin E, selenium and zink in fresh fruit of blackberry has a good effect on human body through protection the integrity of cells and internal structure of cells, avoiding some enzymes and internal components of cells from being destructed. These contents have anti-oxidant and it can improve immunity, play an antagonistic role of protective agent from toxic substances[17].

The actual capacity of the constituents of plants which have antioxidant properties is that they are essential for health protection especially against cancer and heart diseases[18].

Present study was therefore, undertaken to assess the effects of CPF on testes, the main organ of male reproduction in rats. More- over, the present study was conducted to compare and evaluate the protective effects of Black berry juice in restoring the altered biochemical and histopathological alterations in the testes of male rats exposed to CPF, which may have implications in managing humans with accidental exposures to such compounds.

MATERIALS AND METHODS

Test insecticide

CPF technical grade (98%) was obtained from El-Helb Company for pesticides and chemicals, Egypt.

Drugs

Methanolic extract of *Black berry juice* was prepared by drying 150 gm of plant after grinding by using electrical mixer and then the plant powder was soaked in methanol for 24 hours and then we take the filtrate and obtain the extract by using Heidolph rotatovapour .

Animals

Healthy adult male albino rats of the Wistar strain (Rattus norvegicus) with proven fertility, 4–5 months of age and weighing 150–180 g, were supplied from the Animal Breeding House of the Medical Research Institute (MRI), Cairo University, Egypt. Animals were maintained at the animal care facility in the Zoology Department, Faculty of Science, Zagazig university in plastic cages under controlled temperature (23 ± 2 C), 12-h light/dark cycle and 50 ± 5% relative humidity. Water and food were available ad labium. Rats were acclimatized to the laboratory environment for two weeks prior to the start of experiments.

EXPERIMENTAL

After the period of acclimation, animals were divided into four groups with 25 animals in each. The first group was used as control. The animals of control group were orally given corn oil (4 ml/kg). The second male group was orally treated with CPF (9 mg/kg b.w.); about 1/25 LD50. The third male group was orally treated with Black berry juice (50 mg/kg b.w.) and fourth group was treated with combination of CPF (9 mg/kg b.w.) and Black berry juice (50 mg/kg b.w.). The selected dose of the CPF was based on studies of McCollister *et al.,*[19]. The duration of the oral administration during the experiments lasts for

80-day for completion of the spermatogenic cycle and maturation of sperms in epididymis[19].

Mating and fertility indexes

After the end of the treatment course, males of control and experimental groups of treated rats (n = 25/group), were mated 1:1 with untreated proven fertile, with regular estrus cycle, females for 5 days (complete one estrous cycle)[20]. Mating was confirmed by the presence of vaginal plugs or deposition of spermatozoan at the vaginal orifice upon vaginal examination. The day that a vaginal plug was found was considered day 0 of gestation. Then mating and fertility indexes were estimated and recorded.

Sperm quantity and quality

The right caudal epididymis was used for sperm motility and left caudal epididymis was used for sperm counts and morphology. Right caudal epididymis was first weighed, placed in a Petri dish. An appropriate dilution (1:20) was made with physiological saline (0.9% NaCl). Caudal epididymis was nicked in few sites with a scalpel blade and kept at 37°C to release the spermatozoa from the tubules. The sperm suspension was examined within 5 min after their isolation from epididymis. The counting of both motile and immotile sperms was done at 40 magnification. The calculated results were finally expressed as percent motility[21]. Left caudal epididymis was weighed, diluted in 1:20 with physiological saline (0.9% NaCl) solution in a petri dish and minced with a scalpel blade in the mid-to-distal region. Suspension was kept at 37°C for 5 min for the dispersion of sperm into medium. Sperm suspension was pipated very gently 20 times and placed in a hemocytometer and total number of the sperm head counted[22] at 40 magnification. Each sample was counted twice and means value was taken for calculation.

Body and genital organ weight

Initial and ûnal body weights of male rats were recorded and subsequently weight changes were calculated. After fertility study, the rats were sacrificed by cervical dislocation. The testes and accessory sex organs (seminal vesicles, prostates and epididymis) were dissected out, trimmed off the attached tissues and weighed individually. Then, the organ/body weight ratio was calculated. Specimens of the testes were fixed immediately in Bouin's fluid for histological study.

Biochemical assays

At the end of the 80th day of the treatment course, blood samples were collected from anaesthetized males of all groups by puncturing the retro-orbital venous plexus with a ûne sterilized glass capillary tube into heparin-coated and dry tube. The gathered blood left for 20 min at room temperature, then centrifuged at 4000 rpm (600 g) for 15 min for the separation of sera. The sera were kept in a deep freezer (-20º C) until analyses of certain biochemical parameters. The biochemical measurements were performed according to the details given in the kit's instructions.

Enzymes activity determination

Activities of serum lactate dehydrogenase (LDH), alanine trans- aminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (AlP), and the levels were measured spectrophotometery using commercial kits (Boehringer Mannheim, GmbH, Mannheim, Germany).

Testosterone determination

Serum samples of the treated male rats were used for estimating testosterone concentration using radioimmunoassay (RIA) method. After incubation, the liquid contents in the tubes were withdrawn and the bound radioactivity was determined using gamma counter according to method described by Shalaby *et al.,*[23].

Determination of serum lipid profile

Serum concentrations of total lipids (TL) were assayed by the method of Knight *et al.,*[24] and that of cholesterol and triglycerides (TG) were determined by the method Carr *et al.,*[25]. High density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) were determined according to the methods of Warnick *et al.,*[26] and Bergmenyer[27], respectively. Very low-density lipoprotein (VLDL) was calculated mathematically by dividing the values of TG by a factor of 5 according to Friedewald *et al.,*[28].

Histopathological examination

Testes of the treated rats were taken and fixed in Bouin's solution. The fixed specimens were then trimmed washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in parafûn, sectioned at 4–6 lm thickness and stained with Hematoxylen and Eosin (H&E) then examined microscopically according to Lillie[29].

Statistical analysis

Data are expressed as mean values ± SD (n = 10). Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. For each significant effect of treatment, the post hoc Turkey's test was used for comparisons. The criterion for statistical signiûcance was set at $P < 0.05$. All statistical analyses were performed using SPSS statistical version 8 software package (SPSS Inc., USA).

RESULTS

Morbidity and mortality

Male rats orally administered to CPF (9 mg/kg b.w./day) for 80 days have shown signs of toxicity such as salivation, diarrhea, nose and eye bleeding, tremor. No death was recorded throughout the experimental groups.

Body and organs weights

Body and organs weights of experimental male rats are presented in Table 1. No significant change in body weight gain was observed in male rats treated with *Black berry juice* as compared to controls. However, a significant decrease in the body weight gain was observed in groups that treated with CPF alone or in combination with *Black berry juice* as compared to control or CPF rat, respectively. Absolute and relative reproductive organ weights (testes, epididymides, prostate, and seminal vesicles) were reduced dramatically in the groups treated with CPF or its combination with *Black berry juice* as compared to the control or CPF group, respectively. However, no statistically significant differences in absolute and relative genital organs weights were noted in Black *berry juice* treated group as compared to the control rats.

Sperm characteristics

Data of sperm characteristics (Table 2) showed that treatment of male rats with CPF significantly ($P < 0.01$) decreased sperm count and progressive motility (%), while increased immotile and morphologically abnormal sperms as compared to control and *Black berry juice* groups (Table 2). Treatment with *Black berry juice* alone showed no significant effects on sperm concentration and motility, while caused significant decrease in non-viability and abnormal sperm compared to control group. On the other hand, treatment with *Black berry juice* in combination with CPF caused significantly alleviated the decline in sperm concentration and motility, and significantly increased the percent of viability but decrease abnormality of sperms compared to CPF group, and this means that *Black berry juice* minimized the reproductive toxicity of CPF.

Table 1. Effect of oral administration of chlorpyrifos and/or Black berry juice for 80 days on the body and relative sexual organs weight of male rats

	Control	CPF	Black berry juice	CPF + Black berry juice
Body weights (g)				
Initial	192.7±1.9	190.6 ± 2.55	193.8 ± 2.06	193.5 ± 2.80
Final	222.5±3.25	199.3 ± 2.62a	221.4 ± 3.70	200.4 ± 4.10b
Body weight change (%)	25.1 ± .48	8.6 ± 0.216a	25.8 ± 0.43	16.9 ± 0.39b
Organs weights (g)				
Testes	2.88 ± 0.04	1.89 ± 0.07	2.89 ± 0.16	2.11 ± 0.042
Epididymides	0.83 ± 0.01	0.67 ± 0.02	0.93 ± 0.01	0.75 ± 0.02
Prostate	0.56 ± 0.02	0.26 ±0.02a	0.56 ± 0.01	0.45 ± 0.01b
Seminal vesicles	1.82 ±0.07	0.98 ± 0.01a	1.96 ± 0.12	1.57 ± 0.01b
Relative organ weights				
Testes	1.24 ± 0.02	0.98 ± 0.03	1.22 ± 0.02	1.15 ± 0.03
Epididymides	0.38 ± 0.01	0.35 ± 0.01	0.37 ± 0.01	0.37 ± 0.01
Prostate	0.26 ± .01	0.17 ± .01a	0.25 ± 0.01	0.24 ± 0.01
Seminal vesicles	0.83 ± .012	0.42 ± .01a	0.84 ± 0.02	0.73 ± 0.02b

The data are presented as means ± SE, n = 25.
a Significant difference as compared with control group (P 6 0.05).
b Significant difference as compared with chlorpyrifos group (P 6 0.05).

Mating and fertility indices

Fertility indexes of the male rats given CPF at doses of 9 mg/kg/ b.w. for 80 consecutive days were 44% compared to 100% in the control normal group. Rats given *Black berry juice* at 50 mg/kg/b.w. have fertility index of 100% while it was 60% in male rats given combination of *Black berry juice* and CPF as shown in Table 3.

Table 2. Sperm characteristics in male rats after oral administration of chlorpyrifos and/or Black berry juice for 80 days

Sperm parameters	Control	CPF	Black berry juice	CPF + Black berry juice
Count/epididymis (106)	93.2 ± 1.97	54.1 ± 0.93a	92.4 ± 1.736	78.3 ± 1.14b
Motility (%)Progressive	58.2 ± 0.94	25.2 ± 0.21a	56.6 ± 0.93	45.2 ± 0.78b
Non-progressive	17.5 ± 0.42	28.3 ± 0.53a	17.1 ± 0.41	22.7 ± 0.53b
Immotile	24.3 ± 0.33	46.5 ± 0.79a	27.3± 0.58	32.1 ± 0.65b
Morphology (%)Normal	87.4 ± 1.59	60.4 ± 1.214a	86.3 ± 1.57	77.7 ± 1.54b
Abnormal head	5.2 ± 0.01	21.0 ± 0.21a	5.1 ± .12	10.2 ± .41b
Abnormal tail	6.6 ± 0.15	19.1 ± 0.24a	7.1 ± .41	9.9 ± 0.16b
Other abnormalities	0.7 ±0.01	1.5 ± 0.02	0.7 ± 0.01	1.1 ± 0.12
Total abnormalities	13.5±0.4	37.6 ± 0.59a	14.7 ± 0.21	21.2 ± 0.48b
Viability (%)	88.5±1.62	46.5 ± 1.24a	87.5 ± 1.39	75.1 ± 1.59b

The data are presented as means ± SE, n = 25.
a Significant difference as compared with control group (P 6 0.05).
b Significant difference as compared with chlorpyrifos group (P 6 0.05).

Table 3. Functional fertility parameters of male rats after oral administration of chlorpyrifos and/or Black berry juice for 80 days

	Control	CPF	Black berry juice	CPF + Black berry juice
Number of males that used for mating	25	25	25	25
Mating index (%)	25/25 (100)	11/25a (44)	25/25 (100)	20/25b (80)
Fertility index (%)	25/25 (100)	5/13a (38)	25/25 (100)	15/25b (60)

Mating index (%) = Number of males inseminated females/total number of males cohabited with females 100.
Fertility index (%) = Number of cohabited females becoming pregnant/number of non pregnant with evidence of vaginal plug 100.
a Significant difference as compared with control group (P 6 0.05).
b Significant difference as compared with chlorpyrifos group (P 6 0.05).

Table 4. Effect of chlorpyrifos alone and its combination with Black berry juice on serum ALT, AST, ALP and LDH activities of male rats after 80 days of oral administration

Enzymes activities (U/L)	Control	CPF	Black berry juice	CPF + Black berry juice
ALT	89.51 ± 2.72	125.36 ± 4.50a	85.58 ±2.20	90.14 ± 2.28b
AST	156.82 ± 5.14	188.41 ± 7.24a	155.97 ± 3.56	163.50 ± 3.95b
ALP	140.06 ± 6.21	175.92 ± 7.78a	139.92 ± 5.31	167.73 ± 5.44b
LDH	1259.25 ± 34.36	2494.36± 34.90a	255.47 ± 31.20	1976.73 ± 33.79b

The data are presented as means ± SE, (n = 10).
AST, aspartate transaminase; ALT, alanin transaminase; AlP, alkline phosphatase; LDH, lactate dehydrogenase.
a Significant difference as compared with control group (P 6 0.05).
b Significant difference as compared with chlorpyrifos group (P 6 0.05).

Table 5. Biochemical changes of serum lipids proûle after oral administration of male rats with chlorpyrifos and/or Black berry juice for 80 days

Parameters (mg/dl)	Control	CPF	Black berry juice	CPF + Black berry juice
TL	279.98 ± 7.15	346.01 ± 8.70a	278.23 ± 8.38	321.34 ± 9.87b
TG	124.60 ± 6.93	156.71 ± 5.31	125.13 ± 5.12	139.92 ± 4.49b
TC	102.19 ± 3.11	135.50 ± 3.22a	103.25 ± 3.11	121.47 ± 3.35b
HDL-c	46.31 ± 1.26	32.43 ± 0.94a	45.64 ± 1.25	39.86 ± 1.27b
LDL-c	35.44 ± 0.90	75.25 ± 2.52a	36.76 ± 1.02	54.63 ± 1.97b
VLDL	22.92 ± 0.76	27.34 ± 0.91a	23.03 ± 0.94	21.78 ± 0.83b

The data are presented as mean ± SE, (n = 10).
TL, total lipid; TG, triglycerides; TC, total cholesterol; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol; VLDL, volatile low density lipoprotein.
a Significant difference as compared with control group (P 6 0.05).
b Significant difference as compared with chlorpyrifos group (P 6 0.05).

Biochemical changes

Serum ALT, AST, ALP and LDH activities

The results of enzymes activities of male rats are shown in Table 4. Male rats exposed to CPF (9 mg/kg/day) showed an increase in the serum enzyme activities of ALT, AST, ALP and LDH levels comparable to control. In addition, *Black berry juice* treated group did not show any changes as compared to control. Combination treatment (CPF with Black berry juice) decreased the enzymes activity as compared with rat that treated with CPF only.

Serum lipids profile

The lipid profile in serum of male rats was assayed showing significant ($P < 0.05$) increases in total lipids, triglycerides, total cholesterol, VLDL-C and low density lipoprotein cholesterol (LDL-c) in CPF treated group compared to the control group (Table 5), while the content of high density lipoprotein cholesterol (HDL-c) showed a significant ($P < 0.05$) decrease in CPF treated group compared to the control group (Table 5). On the other hand, no significant difference ($P > 0.05$) was showed in the lipid profile of *Black berry juice* group compared to the control rats (Table 5). Treatment the rats with CPF and *Black berry juice* showed a significant ($P < 0.05$) decrease in the lipid content compared to the CPF group (Table 5).

Serum testosterone

Data presented in Fig 1 showed significant decrease in serum testosterone concentration ($P < 0.05$) in rats treated with CPF compared to control. While, *Black berry juice* significantly increased testosterone and mitigated the negative effects for CPF treated group on this parameter. Nevertheless, CPF administration alone lowered the level of testosterone without *Black berry juice*, but a combination of CPF and *Black berry juice* recovered the testosterone levels

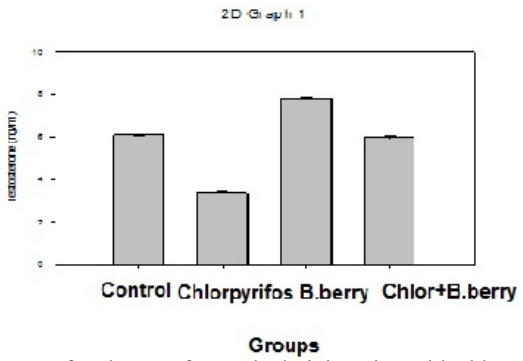


Fig. 1. Serum testosterone of male rats after oral administration with chlorpyrifos and/or Black berry juice for 80 days. The data are presented as means ± SD, (n = 10). a Signiûcant difference as compared with control group (P 6 0.05). b Significant difference as compared with chlorpyrifos group (P 6 0.05)

Histopathology

Fig. 2A–D shows photomicrographs of testes from the different experimental groups. Histopathological examination of the testes of normal rats revealed active mature functioning seminiferous tubules associated with complete spermatogenital cell series (Fig. 2A). Moreover, some germ cells had small and darkly stained nuclei. Sections of testes of rats treated with Black berry juice alone showed that they are less or more similar to the control sections (Fig. 2B). Testis of rats treated with CPF plus Black berry juice revealed that it regained nearly its normal structure and remarkable restoration of the normal picture of seminiferous tubules was attained. The germ cells appeared regular in shape with

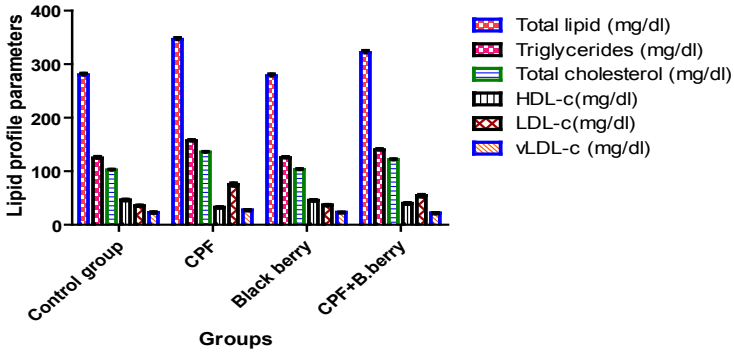
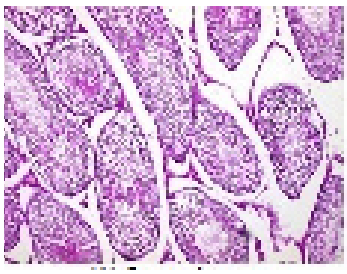
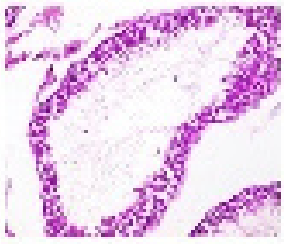
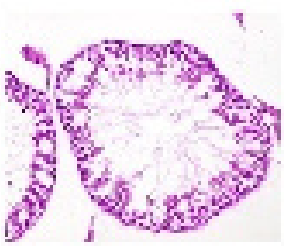


Fig. 2. Serum Lipid profile of male rats after oral administration with chlorpyrifos and/or Black berry juice for 80 days. The data are presented as means ± SD, (n = 10). a Signiûcant difference as compared with control group (P 6 0.05). b Significant difference as compared with chlorpyrifos group (P 6 0.05)

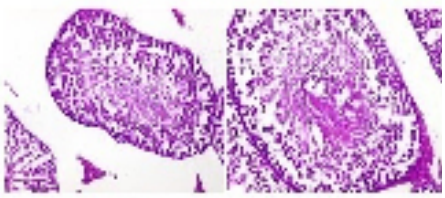


A) Control group

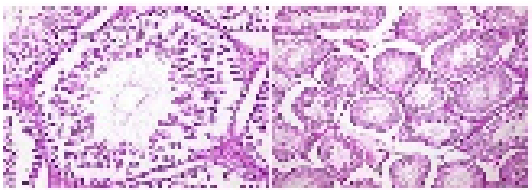




b) Chloropyrifos treated group



c) Black berry treated gropu



d) CPF + Black berry treated group

Fig. 2. Photomicrograph of testicular histology of the (A) control rats showing normal structure of germinal epithelium with primary spermatocytes, secondary spermatocytes, spermatids are seen. (B). Testis of rats treated with 9 mg/kg b.w. CPF, showing vacuolation and deterioration the germinal cells are abnormal and complete destruction is seen in all components of the cells. The structures of the seminiferous tubules were severely damaged seminifeous tubules were lined by few layers of spermatogenic cells and few sperms (hypospermatogenesis). (C) Testis of rats treated with 50 mg/kg b.w. *Black berry juice*, showing the normal architecture of the seminiferous tubules lined by layers of spermatogenic cells up to sperm formation and surrounded by thin basement membrane. (D) Testis of rats treated with combination of CPF and Black berry juice, showing mild changes in the cellular components of germinal epithelium.

disappearance of most cytoplasmic vacuolation. Most nuclei became vesicular (Fig. 2C). However, the histological architecture of the testicular pathology of CPF treated rats showed depression of spermatogenesis, Sertoli cell toxicity and degeneration of somniferous tubules (Fig. 2D). The general architecture of some seminiferous tubules was disorganized. They were characterized by the accumulation of exfoliated germ cells within the affected tubules and appearance of cytoplasmic vacuolation (Fig. 2D).

DISCUSSION

The administration of chlorpyrifos brought about marked alteration in the weight of testes. The reduction in the testicular weight reûects regressive changes in seminiferous tubules. The change in testicular weight has also corresponded to the presence or absence of post meiotic germ cell[3]. Reduction in the number of spermatogenic elements and spermatozoa leads to reduction in the weight of testes. Similar reduction in the weight of testes was also observed by Joshi *et al.,*[3] with different doses and exposure time from the present study. Decrease in testicular weight was accompanied by necrotic changes[30].

Sperm motility is an important functional measurement to predict sperm fertilizing capacity. Any negative impact on motility would seriously affect fertilizing ability. In the present study, marked inhibition of sperm motility in CPF treated group may be because of low level of ATP content[30]. Sperm motility may be affected by altered enzymatic activities of oxidative phosphorolytic process. Oxidative phosphorolytic process is required for ATP production, a source of energy for the forward movement of spermatozoa[31]. Full ATP pool is crucial for normal spermatozoa movement and a slight deprivation of ATP leads to reduction in motility, which may cause infertility. Sperm count is considered to be one of the important factors that affect fertility[31]. Suppression of gonadotrophins might have caused decrease in sperm density in testes[3]. Also, toxicants have direct effect on sertoli cell function, which appears to be involved in the control of spermiation, and when disturbed caused epithelial disorganization and subsequent tubular atrophy[32]. The negative fertility test may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milieu of caudal epididymis. The present study considers the first study that used *Black berry juice* against the toxicity of CPF. The male rats that were given combination of *Black berry juice* and CPF have fertility index 60% while the fertility index in the rat treated with CPF only was 44% as compared to control group.

In the present findings, increased level of cholesterol is attributed to decreased androgen concentration, which resulted in impaired spermatogenesis[29]. Similar effects by other insecticides have also been reported[5,7,30]. Reduction in the serum testosterone, clearly demonstrated the inhibitory effect of insecticides on the secretion of pituitary gonadotrophins (FSH and LH) and in turn on the testosterone biosynthesis in the testes of rat[31]. Marked reduction in testosterone content in association with highly reduced circulating levels of this hormone confirmed alteration in the reproductive physiology of rat. The decrease in testosterone production may be induced by the stimulation of P450 aromatase (P450arom), which catalyzes estrogen production from androgen, thereby decreasing androgen levels[35]. These results suggest that chlorpyrifos exerts suppressive effects on testicular function and leads to infertility in rats which in turn could be enhanced by using *Black berry juice*.

The decrease in plasma testosterone levels, body weight, relative testes and epididymis weights observed here confirms earlier results of Grote *et al.,*[36] in rats and Sarpa *et al.,*[37] in mice. Sarpa *et al.,*[37] found that treatment with triphenyltin chloride during gestation days 6–17 of mice caused a decrease in weight gain and food intake. The decrease in relative testes and epididymis weights of rat treated with CPF agreed with results obtained by Yousef *et al.,*[38], who found that treatment with triphenyltin chloride caused a decrease in relative weight of testis and epididymides of rabbits. The present study declared that CPF caused a decrease in epididymal sperm count and sperm viability of rat. These results could be suggested that CPF impair male reproduction in rat by decreasing circulatory testosterone. The decline in ejaculate volume, sperm concentration, and total sperm output and semen initial fructose concentration (Table 2) can be partly attributed to the CPF-induced reduction in testosterone (Fig. 1).

The observed decrease in sperm motility could be attributed in part to the con- comitant abnormality of the sperms, decrease their viability (Table 2) and the decrease in body weight (Table 1). Moreover, CPF has been reported that it was able to generate reactive oxygen species (ROS) in different tissue organs[11,39] which coincides with the elevation in lipid peroxidation induced in seminal plasma (not published data). Overproduction of ROS can be detrimental to sperm as it is may be associated with male infertility[40]. Thus, the sperm toxic effect of triphenyltin chloride might be due to induced free radicals.

The transaminases and phosphatase in semen play an important role in transamination and phosphorylation processes in sperm metabolism[43]. The present results revealed a significant ($P < 0.05$) decrease in the activities of serum AST, ALT, LDH and AlP of rats treated with CPF. El-Kashoury and Tag El-Din[41] reported that CPF in different local manufactures (chlorozan, pestpan and pyriban) at dose of 23.43, 21.40 and 17.43 mg/kg b.w., respectively significant decrease the activities of ALP and LDH. This fact is a conventional indicator of liver injury due to CPF-treatment[44]. Moreover, propetamphos (15 mg/kg b.w./ day) was determined to cause harmful effects in rats by increasing the levels of glucose and TG, and the activities of AST, ALP and ALT after 28 of the treatment.

The serum content of lipid profile showed remarkable increase due to CPF-treatment (Table 5); significant increases in TL, TG, TC and LDL-cholesterol were shown in CPF-treated rats compared to the control group. Results of the present study clearly indicate that previous administration of the *Black berry juice* marked changes in lipoproteins and marked fall in the total cholesterol and LDL-c in rats induced by CPF in the rat blood serum.

In the current study we evaluated the protective role of *Black berry juice* against the toxicity and the histological changes resulting from the administration of CPF in rats. Many environmental, physiological, and genetic factors have been implicated in defective sperm function, the most common cause of infertility. Free radical-induced oxidative damage to spermatozoa is one such condition which has recently gained a considerable attention for its role in inducing poor sperm function and infertility[44]. Factors that can offer spermatozoa protection are, therefore, of great importance. In the present study, we found evidence suggesting that *Black berry juice* possesses the capacity to protect sperm, testes and enzymes from deleterious actions of CPF. *Black berry juice* increased ($P < 0.05$) the levels of testosterone, relative testes and epididymis weight, and alleviated the negative effects of CPF, and this is in accordance with Nutritiondata and Peter[42,43] who suggested that the increase in weight gain and feed intake of animals treated with Black berry juice due to high content of flavonoids .

Black berry juice leaves are highly nutritious and are rich in vitamins D, K, A, C, B6, Manganese, Magnesium, Lysine, Riboflavin, Calcium, Thiamin, Potassium, Iron, Protein and Niacin. Black berry juice also contains all 8 essential amino acids and is rich in flavonoids, including Quercetin, Kaempferol, Beta-Sitosterol, Caffeoylquinic acid and Zeatin so it give reason for improving the quality of the sperms and increasing the ATP content needed for spermatogenic formation and thus alleviating the negative effect of chlorpyrifos on reproductive performance.

The main benefit and basic property of antioxidant compounds is that they are closely related to the inhibition of lipid molecules oxidation[47] and the main compounds that have antioxidant properties are phenolic acids and flavonoids which is found extensively in Black berry juice species and thus it is the cause of alleviating the capacity of male rats against oxidative stress[48].

The present study showed that the administration of *Black berry juice* alone caused significant improvements in sperm characteristics and male fertility of rats and also ameliorates hepatic function parameters.

CONCLUSION

The present results showed that exposure to CPF caused deterioration in semen quality, decrease of fertility indexes as well as alternation in lipid profile and decreased the enzyme activities of serum. Moreover, the testosterone decreased and there were many histological changes in the tests. The combination treatment of *Black berry juice* with CPF had beneficial effects in improving the reproductive performance of male rats and improved

all the biochemical parameters. Therefore, the present study elucidated the therapeutic effects of Black berry juice administered in combination with CPF to minimize its reproductive toxicity.

Confiict of interest

The authors declare that there is no conûict of interest.

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Abbreviations: CPF:Chlorpyrifos , b.w., body weight; AST, aspartate transaminase; ALT, alanine transaminase; AlP, alkaline phosphatase; LDH, lactate dehydrogenase.