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Antioxidant activity of crud extracts of *Brassica juncea* L. seeds on experimental rats

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Abstract
The present study was aimed to investigate the potential effect of crude extract of *Brassica juncea* L. seeds in alleviating hematological and serum biochemical alterations induced by cadmium chloride toxicity in rats. For this purpose, 32 adult male rats were selected and divided in to 4 groups (8 rats for each group), Group (1): received distilled water by i.p injection and served as a control, Group (2) was daily administered with mustard oil at a dose of 112 mg/Kg B.W orally, Group (3) was daily administered with cadmium chloride at a dose of 225 mg/Kg B.W by i.p injection and Group (4) was daily administered with cadmium chloride at a dose of 225 mg/Kg B.W by i.p injection and after one hour of cadmium chloride administration, the treated rats were given with the mustard oil extract at a dose of 112 mg/Kg B.W. The obtained results of the present research indicated that the cadmium chloride possesses a deleterious effect on blood cytology, induce oxidative damage, hepatic-renal dysfunction, increase of MDA and thyroid hormone defect. The administration of mustard oil with cadmium chloride minimized the hazard effects of cadmium chloride. It improved the RBCs count, PCV, Hb concentration, total & differential WBCs count and blood indices, and diminished the level of serum malondialdehyde (MDA). Moreover, it ameliorates the activities of AST, ALT, ALP, cholesterol, TG, LDL, VLDL, HDL, urea, creatinine, glucose and various thyroid hormones like TSH, T4 and T3.

Keywords: *Brassica juncea* mustard oil hematology toxicity antioxidant biochemical toxicity

1. Introduction
Mustard is an annual herb that belongs to the division Magnoliophyta, class Magnoliopsida, order Brassicales and family Brassicaceae [1]. The family Brassicaceae consists of 350 genera and about 3500 species like *Sinapis*, *Thlaspi* and *Brassica* [2]. The genus *Brassica* is the most important one than the remaining two genus which includes some crops and species of great worldwide economic importance such as *Brassica juncea* L. The *B. juncea* is also known as Indian Oriental or Brown mustard, mustard greens, Chinese mustard and leaf mustard which was widely believed to be one of the earliest domesticated plants as well as a condiment since early times [3, 4]. *Brassica juncea* was widely used to reduce the severity of various health problems like asthma, lower high blood pressure and restore normal sleep pattern in women having difficulty with the symptoms of menopause which reduces the frequency of migraine attacks and prevent heart attack in patient suffering from atherosclerosis or diabetic conditions [5]. Anticancer activity has been reported by various researchers on isolation of new phytochemical bioactive compounds from leaf extracts of *Brassica juncea* [6]. Cadmium is an ubiquitous non- essential metal and an environmental pollutant which was emerged from the activities of various industrial wastes. It is one of most dangerous occupational and environmental toxins and was reported as a highly cytotoxic heavy metal which can causes heavy damage for humans, animals and plants in trace amount. Long term exposure of cadmium from water, air, soil and food leads to its manifestation in the form of various diseases and disorders. Induction of cancer, mild anemia, damaged renal tubules, osteoporosis and hypertension was highly induced by cadmium toxicity [7]. Cadmium with age accumulates preferentially in the liver, kidney, lungs and reproductive organs which was considered as the major target of cadmium toxicity as well as in other tissues and organs causing many metabolic and histopathological changes, membrane damage and apoptosis [8, 9].

2. Materials and Methods
2.1. Determination of *In vivo* Antioxidant activity
2.1.1. Animal and experimental design
Experimental Rats are divided into 4 groups (8 rat in each group) as following:

Group 1 (G1) (Control group): In this group, 8 male rats were injected i.p with 0.5 ml of 0.9% normal saline (N.S) daily for 21 days.

Group 2 (G2) : This group consisted of 8 male rats which were treated orally with mustard oil at a dose of 0.5 ml daily for 21 days.

Group 3 (G3) : This group consisted of 8 male rats which were injected i.p with 225 mg/Kg of cadmium chloride daily for 21 days.

Group 4 (G4) : In this group, 8 male rats were treated with 112 mg/Kg mustard oil daily after one hour of cadmium chloride administration for 21 days.

Blood samples were collected from the heart of experimental rats by heart puncture by the use of the disposable syringes of 5 cc capacity. After giving anesthesia for the rats, blood samples were collected and analyzed according to the procedure proposed by Sood [10].

A volume of 0.4 ml of blood was poured into a tube containing the ethylene diamine tetra acetic acid (EDTA) as an anticoagulant for RBC, Hb, PCV and WBC, differential WBC analysis.

A volume of 0.6 ml of blood was poured into the test tubes which were free from the anticoagulants to get the blood serum to estimate the various biochemical parameters such as glucose, total cholesterol, HDL- cholesterol, LDL-cholesterol and triyglycerol, VLDL, GOT, GPT, ALP urea, creatinine and thyroid hormones.

2.1.2. Hematological tests

Erythrocytes count, Hb, PCV, WBC and blood indices were estimated according to the method of Schalm *et al*. [11].

2.1.3. Biochemical tests

Serum glucose, total cholesterol and triglyceride levels were estimated by the enzymatic method were measured according to the method described by Wahlefeld [12]. The HDL-cholesterol content was estimated by following the Burstine method [13]. The presence of Serum LDL was calculated according to Burstine method, formula and serum VLDL level were calculated according to the procedure of Friedewald *et al*. [14].

The MDA content was estimated according to Yagi method [15]. Serum urea and creatinine was also measured *via* Wahlefeld method, while the liver enzymes ALT, AST and ALP were estimated according to the procedure contributed by Reitman *et al*. [16]. In addition to the thyroid hormones, the presence of TSH, T4 and T3 was also studied.

2.1.4. Statistical analysis

Computerized SPSS (statistical package for social sciences) (V.13) program were used for the analysis of the results the present study. The data were expressed as mean± standard deviation (mean± SD). Least significant difference test (LSD) was used to test the difference between means (groups); $p \leq 0.05$ was considered significant [17].

3. Results and Discussion

3.1. Hematological results

3.1.1. Red blood corpuscles count (R. B.C.)

The effect of mustard oil (*Brassica juncea* L.) on blood parameters of cadmium chloride treated male rats was investigated and the obtained results were given in Table - 1. When the mustard oil was administrated alone to the experimental rats, there was no effect on blood picture parameters values like R.B.C, Hb and P.C.V. Injection of cadmium chloride into the experimental rats caused the significant decrease in the R.B.C count, Hb and P.C.V values when ($p \leq 0.05$) compared to the control group value. When mustard oil (b.j) was offered as treatment, it caused the R.B.C, Hb and P.C.V values to elevated significantly compared to cadmium chloride group value at ($p \leq 0.05$) but the values were still less significantly from those of control value.

Table 1: The effect of mustard oil (*Brassica juncea* L.) on blood parameters of cadmium chloride treated male rats.

Parameters / Groups	RBC ($n \times 10^6/mm^3$)	Hb (g/dl)	PCV (%)
Control (0.9 % N.S)	A 8.10 ±0.34	A 15.21 ±1.31	A 42.33 ±1.53
Mustard oil (112 mg/ Kg)	A 7.97 ±0.20	A 14.45 ±0.53	A 41.65 ±1.54
Cadmium chloride (225 mg/Kg)	C 5.25 ±0.32	C 11.77 ±0.67	C 32.10 ±1.95
Cadmium chloride and Oil (225 mg + 112 mg /Kg)	B 7.57 ±0.23	B 13.22 ±0.51	B 37.06 ±0.96
LSD	0.52	1.22	4.58

Different letters refer to significant differences among groups ($P \leq 0.05$)

3.1.2. Total and differential leukocytes count

The effect of Mustard oil (*Brassica juncea*) on total and differential leukocyte count on cadmium chloride treated male rats was studied and the results were showed in Table - 2. From Table 2, it was observed that the total WBC was not affected by Mustard oil treated normal rats when compared to the control group experimental rats. Cadmium chloride treatment caused a significant increase in the WBC count ($p \leq 0.05$) when compared to the WBC count of the control value. It was also seems that the Neutrophils, Lymphocytes, Acidophils, Basophils and Monocytes percentages were elevated due to the cadmium chloride treatment, whereas no basophils were observed in the blood rat serum. Treatment with Mustard oil after one hour of Cadmium Chloride injection caused a significant decline ($p \leq 0.05$) in total WBC count. In addition to Neutrophils, Lymphocytes, Basophils, Acidophil and Monocytes percentages were still higher significantly in their values ($p \leq 0.05$) when compared to control group values.

Table 2: The effect of Mustard oil (*Brassica juncea*) on total and differential leukocyte count of cadmium chloride treated male rats.

Parameters / Groups	WBC ($n \times 10^3/mm^3$)	Neutrophils (%)	Lymphocytes (%)	Acidophils (%)	Basophils (%)	Monocytes (%)
Control (0.9 % N.S)	D 6.26 ±0.38	B 21.53 ±8.61	C 68.50 ±1.06	C 2.25 ±0.46	0.00 ± 0.00	C 3.75 ± 0.46
Mustard oil (112 mg /Kg)	C 6.99 ±0.46	A 24.50 ±0.53	C 69.12 ±0.64	C 2.75 ±0.46	0.00 ± 0.00	C 3.62 ± 0.51
Cadmium chloride (225 mg/Kg)	A 11.53 ±0.97	D 17.75 ±1.28	A 72.87 ±1.35	A 3.62 ±0.51	0.00 ± 0.00	A 5.75 ± 0.70
Cadmium chloride and Oil (225 mg+ 112 mg/Kg)	B 9.01 ± 0.48	C 20.87 ±0.83	B 70.62 ± 0.51	B 3.37 ±0.51	0.00 ± 0.00	B 5.12 ± 0.64
LSD	0.65	2.12	1.12	0.62	0.00	0.62

Different letters refer to significant differences among groups ($P \leq 0.05$).

3.1.3. Red Corpuscles Indices

MCH, MCHC and MCV.

The effect of Mustard oil (*Brassica juncea*) on blood indices of cadmium chloride treated male rats was tested and the results were tabulated in Table - 4. It was clear from the Table 3 the mustard oil was administrated alone to the experimental rats, no effect was observed on MCH, MCV and MCHC values. Injection of cadmium chloride caused significant increase in the MCH, MCHC and MCV value when compared to the control group value. It was also seems from the results of the table (3) that offering mustard oil as treatment caused an elevation in the MCH, MCHC and MCV when compared with cadmium chloride but it was still significantly higher than the MCHC and less than in MCH and MCV as compared to the control group rats ($p \leq 0.05$).

Table 3: The effect of Mustard oil (*Brassica juncea*) on blood indices of cadmium chloride treated male rats.

Parameters / Groups	MCH (pg)	MCHC (g/dl)	MCV (FI)
Control (0.9 % N.S)	***C***18.30 ±0.97	C 35.02 ±1.02	C 51.21 ±5.34
Mustard oil (112 mg /Kg)	***c***17.78 ±0.51	C 34.92 ±0.58	C 51.38 ±1.72
Cadmium chloride (225 mg/ Kg***)***	***a***21.27 ±0.91	A 36.73 ±2.67	A 58.07 ±2.85
Cadmium chloride and Oil (225 mg + 112 mg/Kg)	B 17.42 ±0.91	B 36.00 ±3.51	B 48.95 ±2.45
LSD	0.87	0.16	0.87

Different letters refer to significant differences among groups ($P \leq 0.05$).

3.1.4. Biochemical results

TCh, TGS, HDL, LDL and VLDL

The effect of Mustard oil (*Brassica juncea*) on Lipid profile of cadmium chloride treated male rats was analyzed and the findings were given in Table - 4. From table (4), it was observed that the administration of mustard oil alone to the rats caused the decrease in TCh, TSG, and LDL values, while there was a significant increase in the HDL when compared to the control group rats. Cadmium chloride treatment caused a significant increase in the all biochemical parameters such as TCh, TGS, LDL and VLDL values except the HDL value when compared to the control value. Treatment with mustard oil after one hour of cadmium chloride injection caused a significant decline ($p \leq 0.05$) in all the above biochemical parameters. The HDL value was increased significantly due to the treatment with mustard oil after one hour of cadmium chloride injection, but was still significantly less than the control group value.

Table 4: The effect of Mustard oil (*Brassica juncea*) on Lipid profile of cadmium chloride treated male rats.

Parameters / Groups	TCh (mg/dl)	TGS (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control (0.9 % N.S)	C 77.46 ±1.04	C 63.20 ±1.67	B 55.92 ±1.52	C 16.94 ±0.51	D 12.64 ± 0.33
Mustard oil (112mg /Kg***)***	D 70.11 ±1.69	D 61.15 ±3.18	A 66.72 ±4.41	D 15.93 ±1.12	C 15.03 ± 0.63
Cadmium chloride (225 mg /Kg)	A 98.41 ±1.11	A 93.40 ±3.16	D 36.90 ±1.96	A 31.02 ±0.74	A 18.68 ± 0.63
Cadmium chloride and Oil (225 mg +112 mg/ Kg)	B 87.76 ± 1.69	B 79.68 ±1.63	C 46.06 ± 2.89	B 24.25 ±0.95	B 15.93 ± 0.32
LSD	7.35	0.10	1.01	1.01	0.90

Different letters refer to significant differences among groups ($P \leq 0.05$).

3.1.5. AST, ALT and ALP

The effect of Mustard oil (*Brassica juncea*) on liver enzymes of cadmium chloride treated male rats was estimated in the present research and the findings were presented in Table - 5. The data given in table (5) showed that the mustard oil alone was given to the normal rats and it does not have significant effect on the AST, ALT and ALP values compared to the control group value. The biochemical parameters were significantly increased in the Cadmium chloride treatment when compared to the experimental rats present in the control group. Mustard oil treatment after one hour of cadmium chloride injection results in significantly decrease of the AST, ALT and ALP values but it was significantly higher than that the control group rats ($p \leq 0.05$).

Table 5: The effect of Mustard oil (*Brassica juncea*) on liver enzymes of cadmium chloride treated male rats.

Parameters / Groups	AST (IU/ L)	ALT (IU/ L)	ALP (IU / L)
Control (0.9 % N.S)	***c***51.51 ± 2.15	C 28. 80 ± 0.62	C 13. 13 ±1. 15
Mustard oil (112 mg / Kg)	C 50. 38 ±2.47	C 28.97 ± 0.77	C 14.34 ± 1.17
Cadmium chloride (225 mg /Kg***)***	***a***83.71 ±5.03	A 55.15 ±3.69	A 38.60 ±1. 76
Cadmium chloride and Oil (225 mg + 112 mg/ Kg)	B 68.00 ± 3.10	B 44.61 ±1.73	B 21.37 ± 1.85
LSD	15.71	10.53	8.02

Different letters refer to significant differences among groups ($P \leq 0.05$).

3.1.6. TSH, T4 and T3

The effect of Mustard oil (*Brassica juncea*) on thyroid hormones (TSH, T4 and T3) of cadmium chloride treated male rats was investigated in the present study and the results were furnished in Table – 6. It was clear that the mustard oil caused the significant increase ($p \leq 0.05$) in the hormones like TSH, T4 and T3 and the values were compared to control group value. Cadmium chloride injection caused the significant increase in the TSH and significant decrease in T4 and T3 hormone values ($p \leq 0.05$). Treatment with mustard oil after one hour of cadmium chloride injection caused significant increase in the TSH, T4 and T3 values but they were still significantly less than that of the control group values ($p \leq 0.05$).

Table 6: The effect of Mustard oil (*Brassica juncea*) on thyroid hormones (TSH, T4 and T3) of cadmium chloride treated male rats.

Parameters / Groups	TSH (µg IU/ mL)	T4 (µg / dl)	T3 (µg / dl)
Control (0.9 % N.S)	***c***0.50 ± 0.07	B 1.50 ± 0.20	B 1.40 ±0.18
Mustard oil (112 mg/Kg)	B 0.73 ± 0.14	A 1.91 ± 0.11	A 2.06 ± 0.28
Cadmium (225mg/Kg***)***	A 0.83 ±0.05	D 0.28 ±0.11	d0.27 ±0.10
Cadmium and Oil (225 mg + 112 mg/Kg)	D 0.40 ± 0.07	C 1.07 ±0.29	C 1.16 ± 0.26
LSD	0.10	0.41	0.23

3.1.7. Urea, Creatinine, Glucose and MDA

The effect of Mustard oil (*Brassica juncea*) on antioxidant enzyme (MDA) and serum level of Urea, Creatinine and Glucose on cadmium chloride treated male rats was studied in the present research and the results were tabulated in Table - 7. The Table (7) showed that the administration of mustard oil alone has no effect on the urea, creatinine concentration and MDA enzyme values. But, it was caused significant decrease ($p \leq 0.05$) in the glucose concentration value when compared to the control group. It was also noticed from the table (7) that the all above parameters were affected by cadmium chloride injection and caused the significant increase in all parameters when compared to the control group rats. Whereas, the animals treated with mustard oil after one hour of cadmium chloride injection caused significant decline ($p \leq 0.05$) in all the parameters but it was still significantly higher than that of the control group.

Table 7: The effect of Mustard oil (*Brassica juncea*) on antioxidant enzyme (MDA) and serum level of Urea, Creatinine and Glucose on cadmium chloride treated male rats.

Parameters / Groups	Urea (mg/dl)	Creatinin (mg/dl)	Glucose (mg/ dl)	MDA (µm/ L)
Control (0.9 % N.S)	C 56.57 ± 2.13	C 128.06 ± 0.99	C 144.33 ± 2.14	C 1.20 ±0.07
Mustard oil (112 mg/Kg***)***	C 54.04 ±2.25	C 123.30 ± 1.16	D 137.79 ± 1.79	1.17 ± 0.06
Cadmium (225 mg/Kg)	***a***136.28 ± 3.77	A 342.12 ± 51.42	A 339.60 ± 6.76	A 5.50 ±0.48
Cadmium and Oil (225mg + 112mg/Kg)	B 84.79 ± 2.63	B 203.19 ± 8.94	B 222.83 ± 2.61	B 2.63 ±0.03
LSD	28.21	75.13	6.54	1.43

Effect of cadmium chloride on blood parameters and use of mustard oil as amelioration agent

A significant decrease ($p \leq 0.05$) in RBC count, hemoglobin concentration and hematocrit ratio in the rats administered with cadmium chloride was compared with control group. The results of the current study was in agreement with that of Sakar *et al*. [18] who showed that the cadmium stimulates the formation of metallothioneines and reactive oxygen species, thus causing oxidative damage to erythrocytes and various tissues resulting in loss of membrane functions. Preeti *et al*. [19] treated Swiss mice with a single oral dose of cadmium chloride 50 mg/Kg/animal for 16 day and found a marked decline in RBC count, haemoglobin concentration and induction of anemia. It was seems that the cadmium stimulated synthesis of metallothionein in the liver and store there which results in damage to liver tissue, then metallothione in moves to blood and finally to the kidney and other tissues where it can cause damage [20]. The decrease in RBC count, hemoglobin concentration and packed cell volume (P.C.V.) may be due to anemia which was resulted from the inhibition of erythropoietin hormone from the kidney that very important in production of RBCs. It was reported that inhibition in this values was also due to the toxic effect of cadmium chloride on bone marrow and this effect leads to inhibition in erythropoiesis process as well. Improvement of blood values by mustard oil could be attributed to inhibition of hemolysis of RBCs and toxic effects of cadmium chloride on the hemopoietic organs. The significant increase was recorded for the PCV and Hb levels at 112 mg/Kg of methanolic extract of *Brassica juncea* suggested that the extract contains some bioactive constituents or phytochemical constituents such as glucosinolates which have been imposed by hematopoietic activities. It was also supported by the fact that *Brassica juncea* seed was rich in terms of nutritional value. Various studies have showed that the mustard seeds contain protein, carbohydrates, fibers, calcium, phosphors, iron, potassium and vitamins such as vitamin A, thiamin, niacin and riboflavin [21].

No effect on the blood parameters *viz*., MCH, MCV and MCHC values were observed due to the administration of mustard oil alone to the animals. The present results were similar to the findings reported by other authors [22-24]. They reported that the seeds of mustard *Brassica juncea* did not cause any effect on red blood cell count, hemoglobin and PCV percentage in rabbit. The effect was obvious due to inject cadmium chloride which caused the significant increase in the MCH, MCHC and MCV values when compared to the control group rats. The elevated values means the animals suffered from anemia in cadmium chloride treated animals were indicated by a significant decreased in RBCs, Hb concentration and PCV ratio.

This effect could be attributed to decrease in lipid peroxidation in the membranes of erythrocytes and increase membrane

resistance to spontaneous hemolysis of the toxic effect of cadmium chloride on the bone marrow. The results of this present study also showed improvement in blood parameters due to the administration of mustard oil. The result was agreed with that of Ekanem and Yusuf [25] who found that there was a significant increase in the hemoglobin concentration, packed cell volume, red blood cell in oil treated rats group when compared with the cadmium chloride treated rats.

The present study was demonstrated that the oral administrated of the crude methanol seed extract of *Brassica juncea* possibly affect on bone marrow, thereby leading to production of red blood cells and improvement of all blood parameters.

Effect of cadmium chloride on blood parameters and use of mustard oil as amelioration agent**.**

The seeds of mustard *Brassica juncea* did not cause any effect on white blood cells count, differential counts [26].

Data obtained in the present study showed the significant increase in leukocytes count and lymphocytes percentage and a significant decrease in neutrophils percentage in rats treated with cadmium chloride compared with control group (Table 2). Preeti *et al*. [27] reported that the treated Swiss mice with a single oral dose of cadmium chloride 50 mg/Kg for 16 day found a marked an increased in leukocytes count. The exposure to cadmium has been linked to the many disease such as leukemia, osteoporosis and classified as group 1 human carcinogen. It has been known that a sufficient evidence for carcinogenesis has been found in both human and animals, such cancer of the lung, kidney, pancreas, breast and urinary bladder [28, 29].

The increase in the lymphocytes percentage occurs due to increase in the gene expression to the chemokines [30]. The increase in the monocytes percentage may be occur due to increase in the inhalation of cadmium oxides or due to internal as form chlorides *via* digestive system with food in the pollution areas with cadmium [31]. The changes in total and differential leukocytes count may be attributed to the inflammatory response induced by cadmium chloride which leads to the release of a large number of leukocyte count from bone marrow [32]. Modulation of these changes and improvement by oral administration of mustard oil might probably due to some of the phytochemical constituents of *Brassica juncea* seed extract like isothiocyanate and flavonoids which have been reported to have antiviral, anti-allergic, anti-inflammatory, anti-tumor and anti-oxidant activities [33]. These results were in agreement with Mesembe *et al*. [34] who observed that the white blood cell count of the oil treated group was significantly higher than that of control group.

Effect of cadmium chloride on lipid profile and use of mustard oil as amelioration agent.

The results of the present study pointed out that there was a significant elevation of lipid profile in cadmium treated animals apart of high density lipoprotein. Injection of mustard oil only led to reduction in serum total cholesterol and low density lipoprotein and triglycerides. Whereas, a significant increase in the high density lipoprotein was recorded when compared to the cadmium chloride treated animals groups. These results were in agreement with the results of Yoshimasa and Yoko [35] who reported that the using of Brassicacea vegetable and *Brassica juncea* reduced the serum cholesterol levels in human.

According to Gupta *et al*. [36] and Shyni and Kanchan [37] sinapic acid and phytosterols of the plant have the beneficial effect against the metabolic disorders that are associated with the hyperglycemia and hyperlipidemia in animals. The omega3 fatty acids (linolenic acid) in oils can increase the level of circulating good cholesterol [38].

The beneficial effects of Alpha- linolenic acid on plasma lipid and lipoproteins are more controversial. It has been reported to decrease the total cholesterol and low density lipoprotein cholesterol [39]. A study by Abd EL-Hamid [40] indicated that the seed oil has a hypocholesterolemic effect and this may be due to the high percentage of unsaturated fatty acid.

The results of the present study showed little effect on most haematobiochemical parameters. The data recorded in the present study was similar to the findings of Hosain *et al*. [41] results who reported that the male rats treated with edible oils such as mustard oil have a little effects in rats haematobiochemical parameters.

Mustard oil contained high concentration of phytosterols which was considered as one of the most important anti-oxidant compounds that reduced the oxidative damage of reactive oxygen species (ROS) in the human and animals bodies retarding the progress of many chronic disease as well as the oxidation of low density lipoproteins (LDL) which plays an important role in atherosclerosis [42].

The results of the present research was in concordance with the study of Kurde [43] who had showed that the cadmium chloride caused damage to the liver and the esterification mainly occurs in the liver. The proportion of the esterified cholesterol was decreased which leads to the hyper cholesterolemia and this may be due to the impairment of the liver and inhibition of various enzymes that converts the cholesterol into bile acid.

Agrawal and Sharma [44] concluded that the cadmium caused hypercholesterolemia due to the reduced lipoprotein lipase activity that plays an important role in the increment of plasma lipid.

Long term exposure of the heavy metal cadmium leads to the increase in lipid peroxidation that attributed to alteration in the anti-oxidant defense system which includes an enzymatic and non-enzymatic molecules such as glutathione that normally product against reactive oxygen species toxicity [45].

According to the lipid profile of the current study, it seems that the cadmium chloride induced the changes in lipid profile which were ameliorated by the mustard oil. This hypolipemic effect of mustard oil could be related to free radical scavenging activities of the phytochemicals present in the mustard oil such as glucosinolates.

Effect of cadmium chloride on alanine amino transferas, aspartate amino and alkaline phosphatase and use of mustard oil as amelioration agent.

Data recorded in the present study revealed that the serum liver enzymes (AST, ALT and ALP) were not affected when the animal was treated with the mustard oil only. There was no significant effect on these values compared to the control group value. The results of the present study showed that there was a significant increase in the levels of ALT, AST and ALP on treated animals with cadmium chloride. Whereas, mustard oil takes the enzymes level almost to the normal value compared with control group.

Reports of various previous researchers suggested that the cruciferous vegetables can act as a good source of natural antioxidants due to the high levels of Carotenoids and phenolic compounds. Strong epidemiological evidence showed that these compounds may helps to protect the human body against

various physiological damages. Most of the anti-oxidative effects were related to the phenolic compounds which reduced the levels of enzymes due to the free radicals scavenging activities [46].

It was known that the AST, ALT and ALP enzymes were found in the liver at high concentration. These enzymes are normally found in circulation as small amounts because of the hepatic growth and repair. Liver plays a major role in detoxification of toxic materials. It was eliminated by the liver after their metabolism and degradation. This process may lead to the disruption in cell membrane and elevation in serum transaminases [47]. High levels of AST and ALT are usually indicative of liver damage in animals [48].

Similar finding was reported by AL- Hashem *et al*. [49]. They found that the transaminases are the most sensitive biomarkers of cellular damage and toxicity because they are cytoplasmic in location and after that released into the circulation after damage.

Rikans and Yamano [50] found that the oxidative stress intensification after cadmium administration in the liver are responsible for the increase of the AST, ALT and ALP activity.

Koyuturk *et al*. [51] revealed that the cadmium hepatotoxicity was probably affected by two ways firstly *via* occurrence of inflammatory state, secondary *via* direct toxic action of cadmium on liver cell. The present study showed that the administration of the mustard oil extract after cadmium chloride restored the levels of the enzymes in the serum of the animals as an indication of productive effect of mustard oil extract against liver damage which was induced by cadmium due to its presence of high levels of Carotenoids and phenolic compounds.

Effect of cadmium chloride on thyroid hormone levels and use of mustard oil as amelioration agent.

The present results showed that the administration of mustard oil in rats caused the significant increase in the hormones like TSH, T4 and T3 values when compared to the control group value.

Glcosinolates whose degradation products such as thiocyanate and isothiocyanate are well known to suppress the thyroid uptake of iodine and lead to reduce the levels of the thyroid hormones [52].

Ibrahim [53] used the thirty adult male rabbits were treated with 2 g/d of the mustard oil, amiodarome 8 g/kg and combined mustard oil 2 g and amiodarome 8 mg for two weeks. The results showed that the combination of mustard oil and amiodarone caused the significant increase in the thyroid hormones like T4 and T3, and this effect was supported by significant increase in the thyroid gland weight and reduction in the body weight. In addition, mustard oil in combination form reduced the liver function enzymes especially SGOT.

The present study also showed that the injection of cadmium chloride to the animals caused significant increase in the TSH and significant decrease in the T4 and T3 values. This results indicated the dysfunction of thyroid hormones clearly by cadmium injection. Yoshizuka *et al*. [54] concluded that the accumulated cadmium in the mitochondria of the thyroid gland might disturb the oxidative phosphorylation as well as the loss of energy supply possibly caused the inhibition of the synthesis and release of T4 and T3.

It was also indicated that the increase of TSH level may be due to the cadmium interference with pituitary regulation of thyroid hormones production and secretion [63].

Gupta and Kar [55] showed that the cadmium inhibited the enzyme 5D-1 activity by decreasing selenium which play a vital role to form the deiodinase enzyme which converted the hormone T4 to T3 which resulting in low T3 and hypothyroidism. Therefore, less selenium reduced the glutathione peroxidase enzyme which was considered as one of the body's prime antioxidant. This lead to increased level of reactive oxygen species which caused the damage of thyroid glands.

It was cleared previously that the reduction in concentration of serum T3 might be due to decrease of transformation rate from T4 and T3 because inhibition of type- 1 iodothyronine 5-monodeiodinase (5-D) activity through binding to sulfhydryl groups of this enzyme [56]. Yousif and Ahmed [57] reported that the decrease of T4 level in the serum could be due to interference of cadmium in the synthesis or the secretion of T4 by the thyroid follicular cell. Therefore, it lead to the damage of follicular cell structure of the thyroid glands.

Administration of mustard oil extract after cadmium chloride for 21 days improved the thyroid function as it caused the significant elevation in TSH, T4 and T3 levels.

Effect of cadmium chloride on urea, creatinine, Glucose and MDA enzyme and use of mustard oil as amelioration agent.

Data in the present study showed the administration of mustard oil alone has no effect on the urea, creatinine concentration and MDA enzyme values but it caused significant decrease ($p \leq 0.05$) in the glucose concentration values as compared to the control group. The results were in agreement with the studies of Khan *et al*. [26] and Srinivasan [58] who reported that the Black mustard caused hypoglycemia in rats. In addition, Srinivasan [58] indicated that the mucilage (soluble fiber) of mustard at different dosages improved glucose concentrations and insulinaemia in normal rats. The results of glucose concentration obtained in the present study was in line with the findings of [2] who pointed out that the administration of aqueous extract of *Brassica nigra* to streptozotocin induced diabetic rats for two months decreased the serum glucose level and increased the serum insulin level.

In another studies, they found no significant alterations in the content of bilirubin, urea and creatinine levels after giving mustard oil, corn oil and sun flower oil in the diet for 3, 6 and 12 month respectively in rats [59].

Treatment with *Brassica* seed extract on radiation induced hematological and biochemical changes in Swiss albino mice caused the significant decrease in malondialdehyde (MDA) in the liver [60].

The present results showed that the administration of cadmium chloride in rats caused significant increase in urea, creatinine, Glucose levels and MDA enzyme when compared to the control which was in agreement with the studies of Moshtaghie *et al*. [61] and AL-Rikaby [62].

The present results showed that the reduction of serum urea, creatinine, Glucose and MDA values by administration of mustard oil after one hour of cadmium chloride injection might be attributed to active phytochemical present in Brassicaceae family which includes indole, glucosinolates, aromatic and phenols that are responsible for the alterations in the level of enzymes.

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