

Comparative Effects of Scorpion Venom and Aqueous Basil (*Ocimum basilicum*) Leaves Extracts on Ccl4-induced Toxicity in Albino Rats

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Abstract

The present study aims to compare the therapeutic effects between to extract scorpion venom (bradykinin potentiating factor; BPF) and aqueous extract of Sweet basil (*Ocimum basilicum*) on CCl₄-induced toxicity of liver and kidney in albino rats. Rats were divided into 8 groups. Group (1) was served as normal group; Group (2) was injected intraperitoneal (i.p.) once a week for 3 weeks with BPF at a dose (1 µgm/g). Group (3) was received orally *O. basilicum* extract, twice a week for 6 weeks at dose (20 ml/kg). Group 4 was treated with the same doses of BPF and *O. basilicum*. Group (5) was injected i.p. with CCl₄ (1 ml/kg), 3 times weekly, for 2 weeks and served as control group. Groups 6, 7 and 8 were injected i.p. with CCl₄ then treated i.p. with BPF, orally with *O. basilicum* and BPF plus *O. basilicum* respectively. The results of the present study cleared that normal group (1) and groups (2, 3 and 4) showed no significant difference in all liver and kidney functions, besides antioxidant enzymes except creatinine and GSH, were highly significant increase in group (4). CCl₄ caused a highly significant decrease in serum albumin, uric acid, besides Catalase, GSH, SOD activities in liver tissue, while there are an elevation in serum AST, ALT, ALP, γ-GT, creatinine and Urea, besides MDA and NO levels in liver tissue. While, groups (6, 7 and 8) revealed reverse effect in all parameters and recorded a remarkable improvement, comparing with normal group. It can be concluded that the treatment with the extract from the scorpion venom *Buthus occitanus* (BPF) is more effective than those of extract from the plant (*O. basilicum*) against the toxicity of liver and kidney-induced by CCl₄ in albino rats. In addition, the hepato-ameliorating and antioxidant effects of two extracts were found to be better than those of extract of BPF or *O. basilicum* indently.

Keywords: BPF; *O. basilicum*; *Buthus occitanus*; CCl₄; Antioxidant; Albino rats

Introduction

Carbon tetrachloride (CCl₄) is one of the aliphatic hydrocarbons (chlorinated hydrocarbons) that has wide spread uses in different industrial sectors as in production of chlorofluorocarbon refrigerants, foam-blowing agents, cleaning compounds, organic solvents industry [1,2]. CCl₄ is a highly toxic chemical agent [3,4]. It is considered as one of the environmental pollutants which mainly cause hepatotoxic effect. Also, CCl₄ is considered as one of the most commonly used toxins in the experimental study of liver diseases and induction of hepatic cirrhosis [5,6], liver necrosis and liver fibrosis [7]. CCl₄ can induce the oxidative stress beside the inhibition of the activity of the antioxidant enzymes in renal tissue [8]. Oxidative stress has been shown to play a very crucial role in some diseases including liver disease. Free radical that generate inside the body is responsible for oxidative stress and antioxidant compounds that can scavenge free radicals have great potential in ameliorating these disease processes [9,10]. Various studies have demonstrated that carbon tetrachloride intoxication causes free radical generation in many tissues such as liver, kidney, heart, lung, testis, brain and blood [11-13].

Bradykinin potentiating factor (BPF) fractions have many physiological effects, Nassar et al. [14] isolated BPF1, BPF2 and BPF3 from the Egyptian scorpions, *Buthus occitanus* and *Leiurus quinquestriatus* as well as the Cobra; *Naja haje* respectively. These fractions considerably enhance urea and creatinine clearance. They elevate enzyme activities in different organs (especially liver and heart) and creatine kinase and alkaline phosphatase in blood [15,16]. Egyptian scorpion *Buthus occitanus*, contains a strong BPF that was proved to enhance pharmacological effect of bradykinin (BK) on guinea pig ileum [17]. Moreover, topical on burnt application of BPF on skin of guinea pigs accelerate the healing and stimulate kidney and liver functions [18].

Many natural and synthetic agents possessing antioxidative properties have been proposed to prevent and treat hepatopathies induced by oxidative stress [19]. There is increasing evidence of the hepatoprotective role of hydroxy and polyhydroxy organic compounds, particularly from vegetables, fruits and some herbs [20,21]. Sweet basil (*O. basilicum*) is a plant that belongs to the family Labiatae and is known as Holy Basil in English and Rehan in Egypt. It was employed traditionally as a folk remedy for a wide spectrum of ailments. Basil is known to have numerous pharmacological activities. Basil leave extracts have potent antioxidant, anti-aging, anticancer, antiviral, and antimicrobial properties [22,23].

The aim of this work was to highlight the reverse effect of BPF and *O. basilicum* against the toxicity of CCl₄ on albino rats, and also to compare the curative effect of bradykinin potentiating factor on CCl₄-induced toxicity in albino rats with that of *O. basilicum* extract.

Materials and Methods

Materials

Animals: This experiment was carried out on 56 adult male albino rats weight about (250-280 g). The animals were obtained from the

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animal house of the Egyptian Organization for Biological products and vaccines Helwan, Cairo, Egypt. Rats were maintained in animal house of department of Zoology, Faculty of Science, South Valley University, Qena, Egypt. The rats were divided into eight groups housed in controlled suitable plastic cages with natural day and night periods and kept at room temperature and fed on balanced stable commercial diet for drinking tap water was provided *ad libitum*. Animals were examined daily for two weeks, before starting the experiment.

Carbon tetrachloride (CCl₄): CCl₄ is a colorless non-flammable liquid, of molecular weight 153.84 was obtained from El-Nasr Pharmaceutical Chemical Company.

Bradykinin potentiating factor (BPF): Bradykinin and its related peptides are widely distributed in venomous animals, including scorpion. A peptide fraction isolated from the venom of the Egyptian scorpion *Buthus occitanus* was proved to have a bradykinin-potentiating activity. *Buthus occitanus* contains a strong BPF that was proved to enhance pharmacological effect of BK on guinea pig ileum.

Ocimum basilicum (*O. basilicum*) known as Rehan in Arabic and sweet basil in English belongs to the Lamiaceae family. It is an Egyptian plant used as a folkloric remedy in Egyptian traditional medicine. In the current study, the leaves of this plant were used [24,25].

Biochemical kits: All biochemical kits except (γ -Glutamyltransferase " γ -GT", Glutamic-Pyruvic Transaminase "GPT", Glutamic-Oxaloacetic transaminase "GOT" and Creatinine) were brought from bio-diagnostic co. Giza, Egypt. γ -GT was brought from Spectrum co. Cairo, Egypt. While GPT and GOT were brought from Human company. And Creatinine was brought from Diamond Company.

Methods

Isolation of BPF: Firstly the crude venom was collected from scorpion, *Buthus occitanus* gathered from the Aswan area. The scorpions were milked in the physiology laboratory at the Faculty of Science in Qena, South Valley University with a specific device using electrical shocks (6 volts) at the articular membrane of the telson. The collected droplets were received into a clean dry glass container. The venom was lyophilized and freeze dried after which it was kept at -10°C (in the dark) until it was used. The venom fraction (BPF) separated from *Buthus occitanus* was isolated, purified and detected according to the method of Ferreira et al. [25].

Preparation of *Ocimum basilicum* extract: Fresh leaves of *O. basilicum* were collected from a garden within the gardens of south valley university, Qena, Egypt. The leaves were rinsed with clean water to remove any foreign matter. Leaves were blended with distilled water. The mixture was strained, pressed and the mixture was filtrated using filter paper. The aqueous extract was used at a dose level of 20 ml/kg *O. basilicum* [26].

Experimental design

The experimental animals were randomly assigned into 8 groups, 7 rats for each group.

Group 1: Rats were injected i.p. with 0.9% isotonic saline solution at dos (10 mg/kg b.wt) and used as normal group.

Group 2: (BPF treated) rats were injected i.p. with BPF (1 μ gm/1 g b. wt.), once a week for 3 weeks.

Group 3: (*O. basilicum* treated) rats received oral doses of *O. basilicum* (20 ml/kg b. wt.) twice a week for 6 weeks.

Group 4: (BPF+*O. basilicum*) rats were treated i.p. with BPF (1 μ gm/g b.wt.) once a week for three weeks plus oral administration of *O. basilicum* (20 ml/kg b. wt) twice a week for six weeks.

Group 5: Rats were injected i.p. with CCl₄ (1 ml/kg), 3 times weekly, for two weeks and served as control group.

Group 6: (CCl₄+BPF) animals were injected i.p. with CCl₄ (1 ml/kg b.wt.), 3 times weekly for 2 weeks, following with i.p. injection of BPF (1 μ gm/1 g body weight) once a week for 3 weeks.

Group 7: (CCl₄+*O. basilicum*) rats were injected i.p. with CCl₄ (1 ml/kg per b. wt.), 3 times weekly for 2 weeks, following with oral administration of *O. basilicum* (20 ml/kg b. wt.) twice a week for 6 weeks.

Group 8: (CCl₄+BPF+*O. basilicum*) rats were injected i.p. with CCl₄ (1 ml/kg b. wt.), 3 times weekly for 2 weeks, following with i.p. injection of BPF (1 μ gm/1 g b.wt.), once a week for 3 weeks; plus oral administration of *O. basilicum* (20 ml/kg b.wt.), twice a week for 6 weeks. All experimental animals were sacrificed at the end of the experiment. The serum and liver tissue were collected from the experimental groups for biochemical analysis.

Biochemical assays

Biochemical analysis in serum: For biochemical assays blood was collected and centrifuged at 3000 rpm for 30 minutes and stored at -20°C. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the kinetic method which described by Fischbach et al. and Schumann et al. [27,28], alkaline phosphatase (ALP) was measured according to the method of Belfield and Goldberg [29], albumin was determined by the colorimetric method which described by Doumas et al. [30], γ -Glutamyltransferase (γ -GT) was determined by the kinetic colorimetric method which described by Szasz [31], creatinine was determined by the method of Murray et al. [32], urea was determined by using the method of Fawcett et al. [33], uric acid was determined by the enzymatic colorimetric method which described by Barham et al. [34].

Biochemical analysis of liver tissue homogenate: Prior to dissection, perfuse liver tissue with phosphate buffered saline (PBS) solution pH=7.4 containing 0.16 mg/ml heparin to remove any red blood cells. Then homogenize the tissue in 5-10 ml cold buffer per gram tissue. After that, Centrifuge at 4000 rpm for 15 min at 4°C. Finally, remove the supernatant for biochemical assay and store on ice (If not as saying on the same day, freeze the sample at -80°C) until used for biochemical assays.

In liver homogenate, reduced glutathione (GSH) was determined by colorimetric method described by Beutler et al. [35], Superoxide dismutase (SOD) was measured using the method described by Nishikimi et al. [36]. Catalase (CAT) was carried out according to the method of Aebi [37], Malondialdehyde (MDA) was analyzed according to the method of Ohkawa et al. [38] and nitric oxide was carried out according to the method of Montgomery et al. [39].

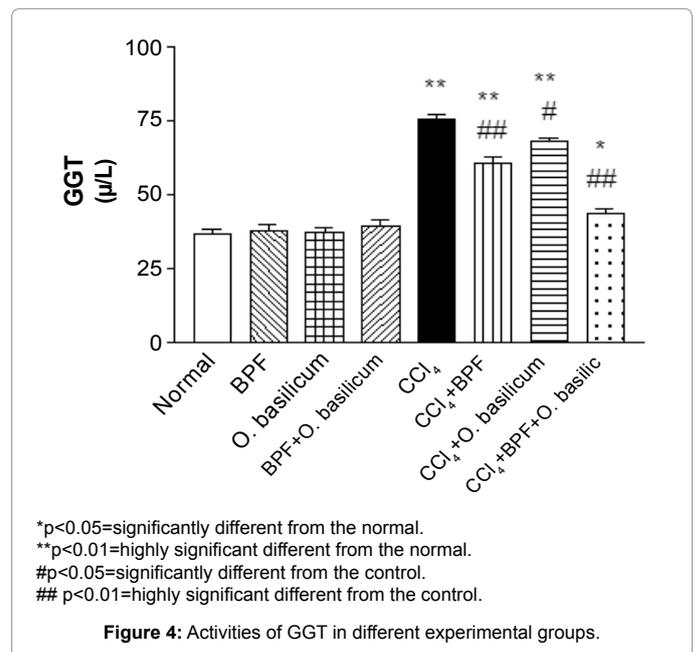
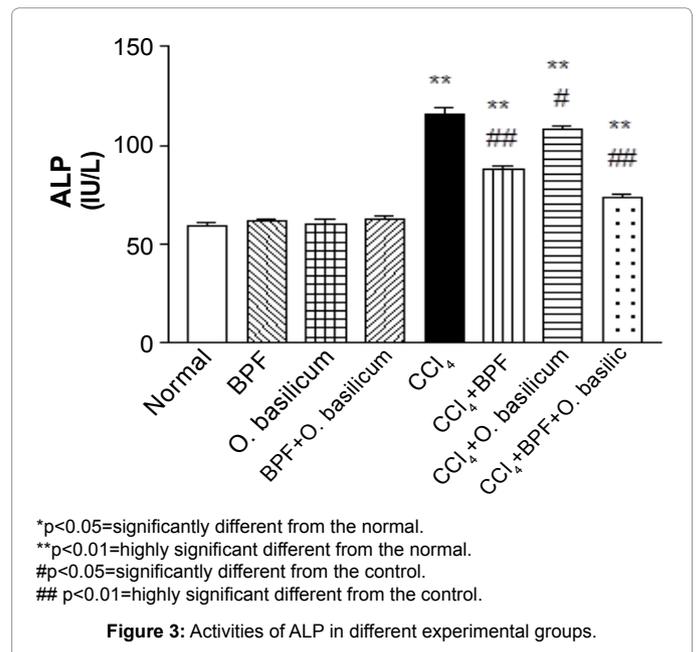
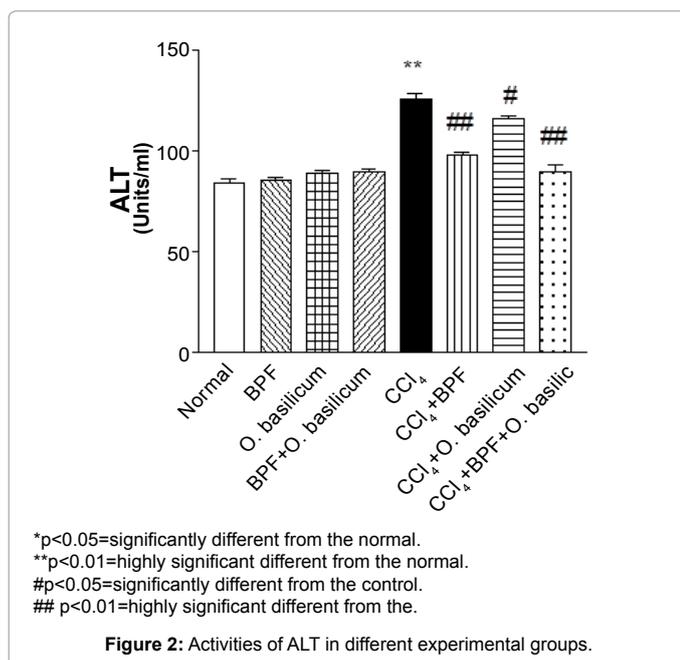
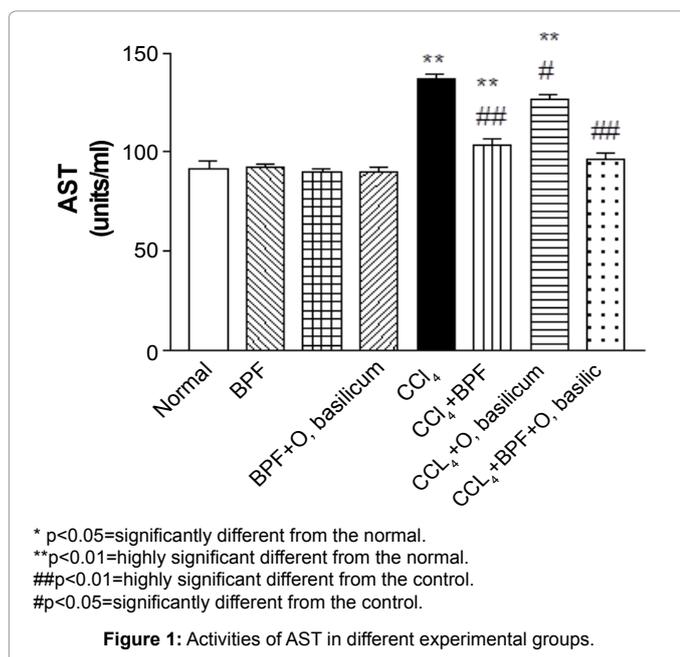
Statistical analysis

The results were expressed as means \pm S.D. The differences between the mean values were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test using Graph Pad Prism 03n software. Statistical significance was set at p<0.05.

Results

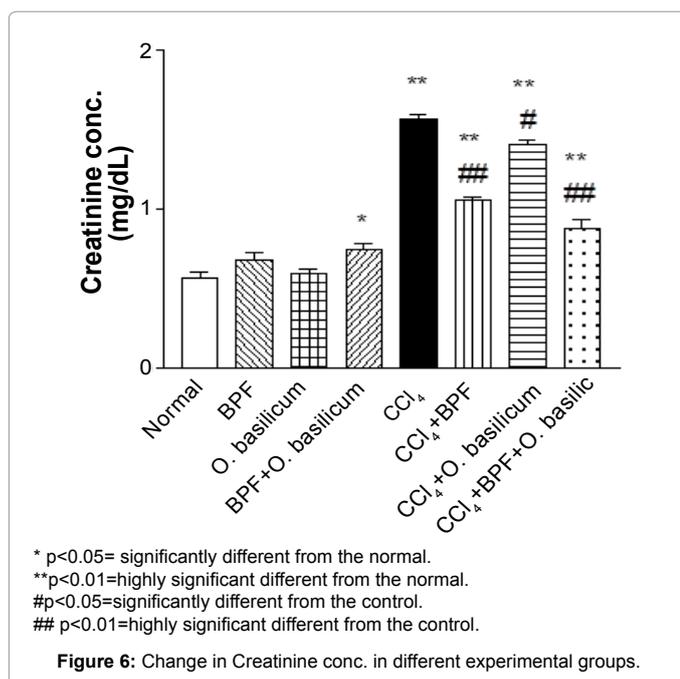
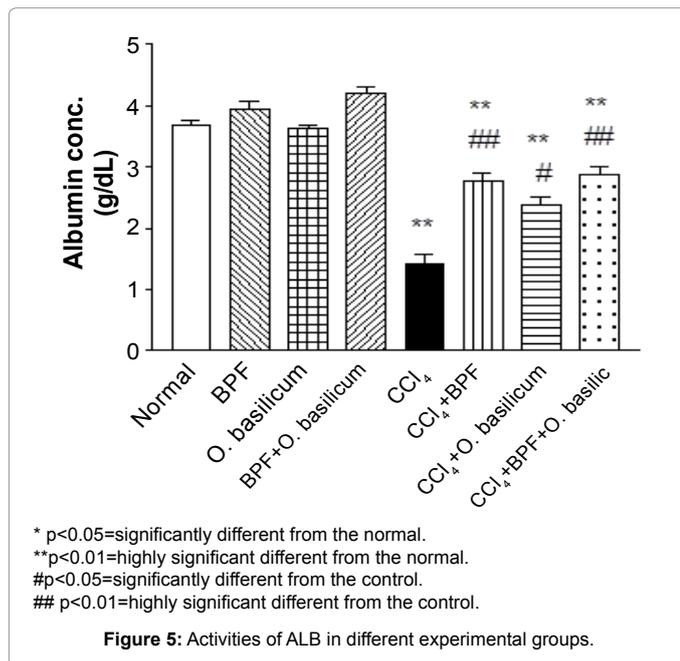
Biochemical results

Liver function enzymes: Results in Figures 1-5 indicated that normal animals G. (1) and G. (2, 3 and 4) showed no significant difference in the activities of serum AST, ALT, ALP, γ -GT and albumin. Moreover, treatment with CCl_4 for 2 weeks G.(5) showed a highly significant increase at ($p < 0.01$) in the activities of serum ALT, AST, ALP and γ -GT with a highly significant decrease at ($p < 0.01$) in serum albumin concentration as compared with the corresponding normal values. Activities of serum ALT, AST, ALP and γ -GT were significant decreased at ($p < 0.05$) in G. (7) and were highly significant decreased at ($p < 0.01$) in G. (6) and G. (8). While, serum albumin



concentration showed a significant increase at ($p < 0.05$) in G. (7) and highly significant increase at ($p < 0.01$) in G. (6) and G. (8) as compared to the control group G. (5).

Renal function: Urea and uric acid levels in serum of normal group G.(1) and G. (2, 3 and 4) showed no significant differences. While, serum creatinine showed no statistical differences in G.(2 and 3) and a significant increase in G.(4). Those findings recorded in Figures 6-8. In addition, the concentration of serum creatinine and urea in control group G. (5) were highly significantly increase, while serum uric acid concentration was highly significant decrease as compared to the normal group G.(1). Creatinine and urea levels showed a significant decrease at ($p < 0.05$) in G. (7) and a highly significant decrease at ($p < 0.01$) in G. (6 and 8). While, uric acid level recorded a significant increase at ($p < 0.05$)



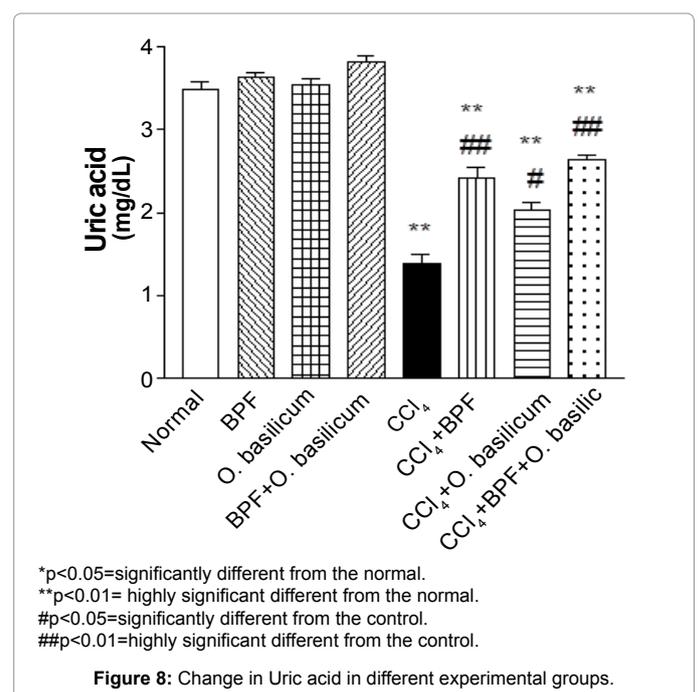
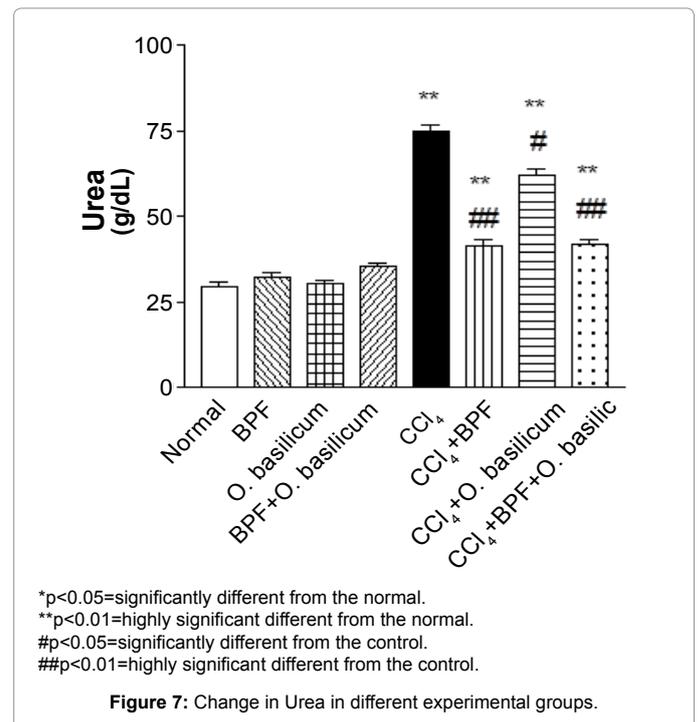
in G. (7) and a highly significant increase at (p<0.01) in (G. 6 and 8) as compared to control group G. (5).

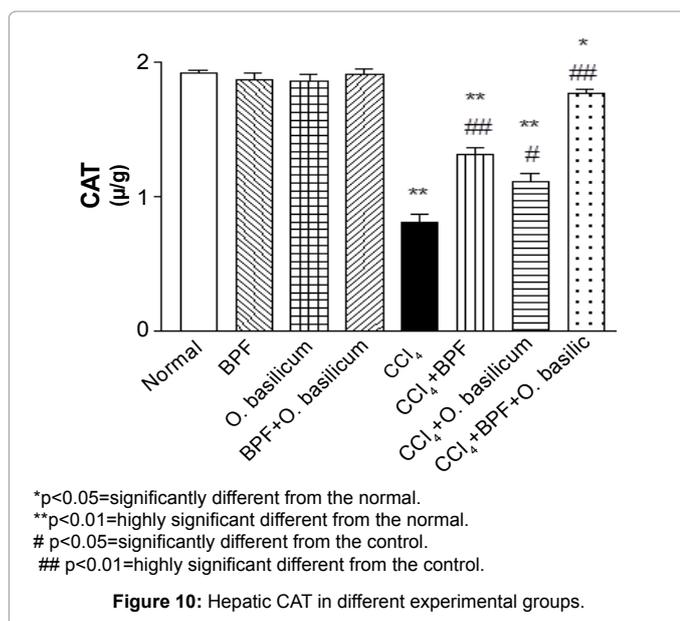
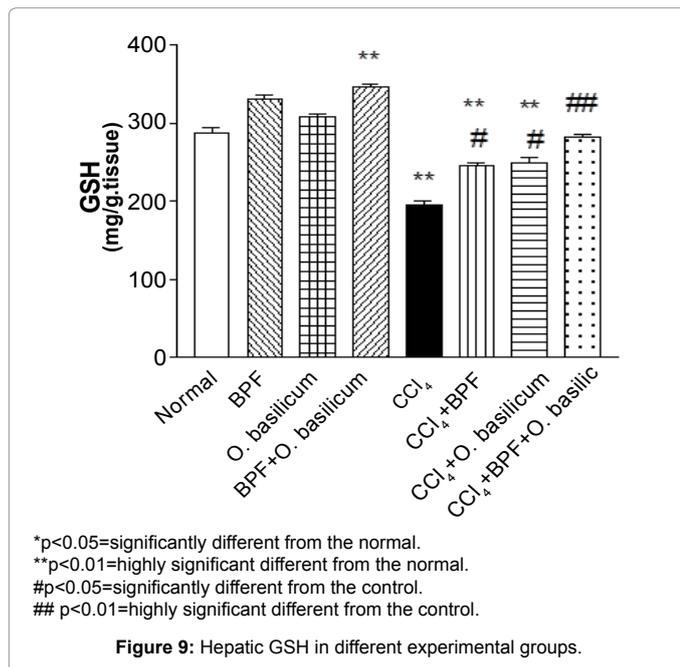
Liver homogenate biochemical results: Lipid Peroxidation and liver antioxidant enzyme activities results in Figures 9-13 showed no significant (p>0.05) changes in the level of CAT, SOD, MDA and NO in G. (2, 3 and 4) as compared to the normal group G. (1). Also, there was no significant difference observed between G. (1) and G. (2 and 3) in the level of liver GSH but in G. (4) GSH was highly significant increase at (p<0.01). Besides, CCl₄ treated group G. (5) resulted in a highly significant increase in the level of liver MDA and NO, with marked reduction in GSH, CAT and SOD activities when compared with normal group. The activity of liver GSH was significantly increase in G.

(6 and 7) and highly significantly increase in G. (8) as compared to the control group G. (5). Moreover, the levels of liver SOD and CAT were highly significantly increase in G. (6 and 8) and significantly increase in G. (7) as compared to the control group G. (5). The activities of liver MDA and NO were highly significant decrease in G. (6), significant decrease in G. (7) and highly significant decrease in G. (8) as compared to the control group G. (5).

Discussion

The present study confirmed the beneficial effect of bradykinin



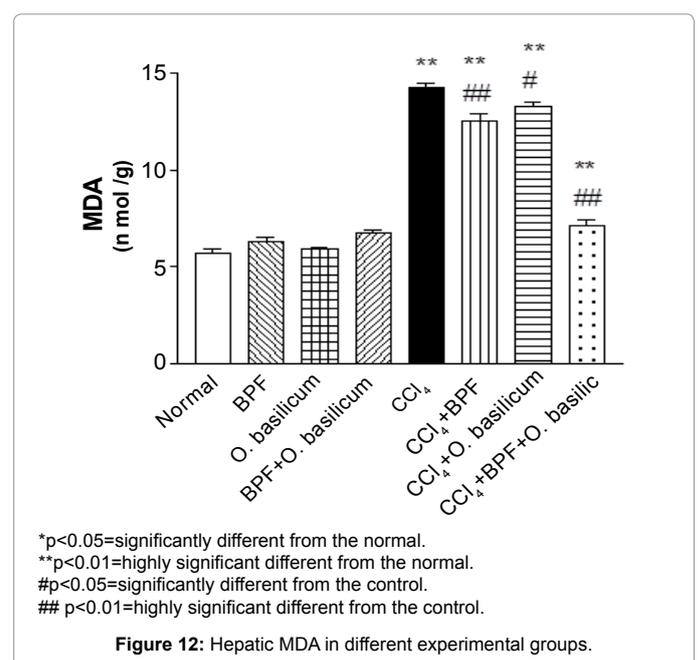
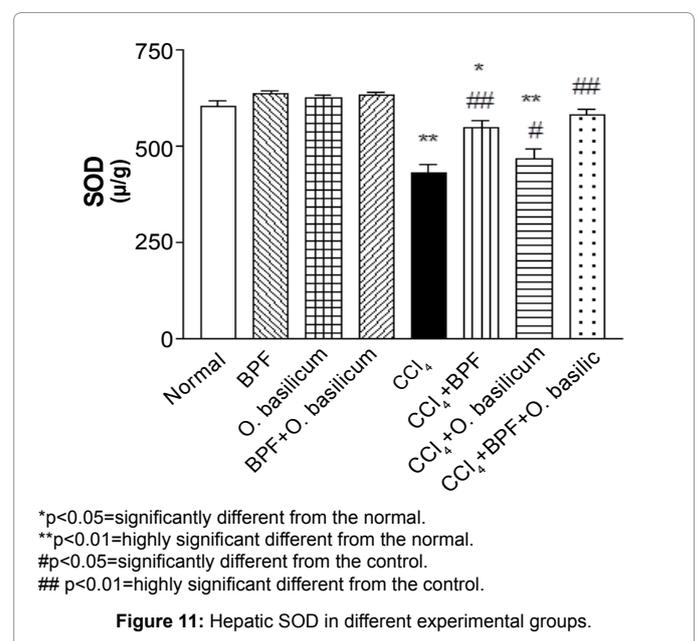


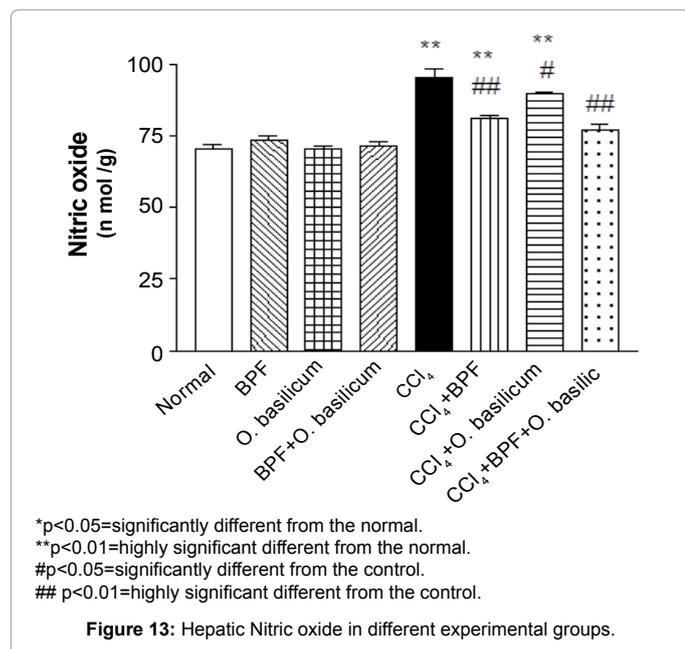
potentiating factor (BPF) and basil (*O. basilicum*) in the prevention of liver and kidney toxicity produced by CCl₄. It also, showed a comparison between the treatment of liver and kidney toxicity by BPF or by basil, as a drug already used for the treatment of liver and kidney toxicity. Results obtained in the present work indicated that carbon tetrachloride (CCl₄) induced biochemical alterations in albino rats.

The present study revealed that, injection of CCl₄ induced a highly significant increase in serum ALT, AST, ALP and γ-GT levels while albumin stated a highly significant decrease. The status of these marker enzymes are sensitive indices of hepatocellular necrosis as cell damage results in the leakage of these enzymes into the systemic circulation [40]. These elevations in the serum liver marker enzymes could be attributed to the free radicals which caused structural integrity damage of the liver cell membrane and hence a leakage of the cellular enzymes

into the blood [41]. The reduction in serum albumin is due to the hepatic injury which caused by CCl₄ [42]. Highly Significant reduction in ALT, AST, ALP and γ-GT levels and highly significant increase in serum albumin of serum of BPF treated rats was recorded in the present study. This effect may be attributed to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity [43]. In addition, treatment with *O.basilicum* remitted the decreased serum albumin and the increase in serum ALT, AST, ALP and γ-GT levels. The mechanism of action may be due to its free radical scavenging (antioxidant) activity [44].

Elevated creatinine, urea and uric acid levels were observed in rat's injected i.p. with CCl₄. These increments could be considered as impairment in renal functions. The increase in serum creatinine





and urea levels may indicate a reduction in the glomerular filtration rate (GFR) as a result of acute renal dysfunction [45]. The reduction in serum uric acid level in the present study may be attributed to the increased utilization of uric acid against increased production of the free radicals since it has a capable especially of reacting with free radicals [46]. Treatment with BPF (G. 6) and basil (G. 7) ameliorated the elevation of kidney functions parameters. BPF induced prostaglandin biosynthesis which as a cytoprotective regulatory substance may trigger enhanced glomerular filtration in guinea pigs [47]. The ameliorative effect of *O. basilicum* against renal toxicity may be attributed to the antioxidant activity of one or more of its flavonoids which have been shown to possess various biological properties related to antioxidant mechanisms [48].

The present study showed that i.p. injection with CCl₄ produced marked oxidative impact as evidenced by a highly significant increase in MDA and NO levels in liver tissue homogenate. This increase may be due to excessive formation of free radicals and activation of lipid peroxidation of cell damage in liver of rats treated with CCl₄ [49,50]. Furthermore, it was reported that a high level of nitric oxide (NO) is associated with CCl₄-induced acute liver injury [51]. NO can protect hepatic cells against oxidative damage and lipid peroxidation caused by carbon tetrachloride and H₂O₂-mediated oxidative stress.

The marked elevation of MDA and NO levels associated with a highly significant decrease in the activities of hepatic SOD, CAT and GSH were recorded after i.p. injection of CCl₄. It has been reported that exposure to CCl₄ induces oxidative stress in rats [52]. Oxidative stress is a state of redox imbalance caused by increased reactive oxygen species (ROS) generation and decreased antioxidant capacity. This process is the main and primary step in CCl₄ toxicity contributing to both onset and progression of fibrosis [53]. Hepatotoxicity produced by CCl₄ seems to be mediated by reactive metabolite trichloromethyl free radical (CCl₃) formed by the hemolytic cleavage of CCl₄ or even by more reactive species trichloromethylperoxy free radical (Cl₃COO) formed by the reaction of CCl₃ with O₂. The reactive trichloromethyl radical binds covalently to the macro-molecules and induces peroxidative degradation of membrane lipids of endoplasmic reticulum

rich in polyunsaturated fatty acids. This peroxidative degradation of membrane lipids leads to the formation of lipid peroxides which probably causes damage to cell membrane of hepatocytes and resulted in centrilobular necrosis [54].

In rats treated with BPF, hepatic SOD, Catalase activities and GSH levels were highly significant increased while MDA and NO levels were highly significant decreased. This result was in accordance with who reported that BPF showed an improvement in the levels of antioxidant enzymes (GSH, CAT and SOD) in liver. This improvement may be due to the antioxidant effect of BPF. Furthermore, the antioxidant activity of BPF may be attributed to the scavenging of CCl₃• radical. Furthermore, the decreased level of MDA, observed after bradykinin administration, may point to a reduction in free radicals production. BPF also can regulate the production of nitric oxide by activation of bradykinin while, bradykinin stimulates nitric oxide [55]. The treatment with *O. basilicum* induced significant increase in GSH, Catalase and SOD activities and significant decrease in MDA and NO levels in liver tissue of treated rats. This may be attributed to the presence of numerous compounds with high antioxidant activities that scavenge the produced superoxide anion and hydroxyl radicals [56]. Rats treated with BPF plus *O. basilicum* (G. 8) showed marked improvements in all biochemical parameters. This may be attributed to the antioxidant effect and free radical scavenging properties of the both treatments.

It could be concluded that treatment with the extract from the scorpion venom *Buthus occitanus* (BPF) is more effective than those of extract from the plant (*O. basilicum*) against the toxicity of liver and kidney-induced by carbon tetrachloride (CCl₄) in albino rats. In addition, the hepato-ameliorating and antioxidant effects of two extracts were found to be better than those of extract of BPF or *O. basilicum* independently.

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