

Antioxidant Effects of Bradykinin Potentiating Factor (BPF) Isolated from Scorpion Venom in Liver Injury Induced by Carbon Tetrachloride (CCl₄) in Male Albino Rats

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Abstract

The main purpose of this study is to evaluate the ability of bradykinin potentiating factor (BPF) isolated from scorpion venom (*Leiurus quinquestriatus*) in treatment of liver injuries which induced by injection of CCl₄ in male Albino rats. Male Albino rats (250 20 g body weight) were divided into four groups. In the control group; Albino rats were interaperitoneally (i.p) injected with 100 L saline solution. The second group (i.p) injected with BPF in 100 L saline solutions (1 gm/g. b. w. per 5 days). Third and fourth groups were i.p. injected with 0.5 ml/kg body weight (b. w.) twice weekly of CCl₄ for fifteen days, after that only the fourth group was treated by BPF in 100 µL saline solutions (1 µgm/g. b. w. per 5 days). The results indicated that, CCl₄ injection induced a significant decrease in serum catalase (CAT), Superoxide Dismutase (SOD), reduced glutathione (GSH), total protein and albumin, within thirty days post-injection of CCl₄ as compared to the normal control group. In contrast, CCl₄ induced a significant increase in Malondialdehyde (MDA), Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), and Alkaline Phosphatase (ALP) compared to normal control animals. The efficiency of BPF treatment is alleviation the effects of CCl₄ on these parameters. The improvement of these parameters may be attributed to the release antioxidant and cytokines and/or amelioration of the toxic effects of CCl₄ on the liver.

Keywords: Scorpion venom; *Leiurus quinquestriatus*; BPF; CCl₄; Liver injury; Albino rats.

Introduction

The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. In spite of the tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise [1,2]. It is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [3]. Carbon tetrachloride (CCl₄) is widely used for modeling liver injury in rats. Hepatotoxicity is connected with severe impairment of the cell protection mechanisms. CCl₄ is a heavy compound that may act as a nonflammable liquid [4]. It is widely used in the dry-cleaning industry although it is a highly toxic chemical agent. Thus, CCl₄ is most widely used for experimental induction of hepatic cirrhosis [5,6]. CCl₄ can induce the oxidative stress beside the inhibition of the activity of antioxidant enzymes in renal tissue [7]. The liver injury is induced mainly by the bio- transformation of CCl₄, which is cytochrome p-450 dependent free radicals initiate the process of lipid peroxidation, which is generally caused an inhibition of enzyme activity [3]. Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cell membranes [8]. It is known that the Reactive Oxygen Species (ROS) would lead to oxidative damage of biological macromolecules, including lipids, proteins, and DNA [9]. Against these types of oxidative injuries, tissues have a variety of defense mechanisms including the non-enzymatic glutathione (GSH), the enzymatic SOD scavenger systems and CAT [10,11]. However, a few

hepatoprotective drugs from natural sources are available for the treatment or ameliorating the liver disorders. A bradykinin potentiating factor BPF extracted from *buthus occitanus* venom was shown to enhance the cellular growth of the uterus and development of the ovarian follicle in female mice [12]. Similarly, injection of this BPF enhanced the spermatogenesis. Moreover, topical on burnt application of BPF skin of Guinea pigs accelerated its healing that attributed to a direct effect of a growth like activity or indirectly by stimulating the endogenous prostaglandin E₂, both in turn, stimulates collagen and elastin synthesis and skin epithelialization [13]. Moreover, Salman [14] declared that injection of BPF in sublethally-irradiated and non-irradiated Guinea pigs accelerated the generation of thymus and spleen cellularity and completely recovered; the normal platelets, WBCs, RBCs and blood globulins picture without noticeable toxic effects in non-irradiated control animals. It is worthy to mention that, the bradykinin-stimulated release of several cytokines important in proliferation and differentiation of various blood cell progenitors, is implicated in achieving the forementioned effects. These cytokines include interleukin-1 (IL-1) IL-3; IL-6, IL-11, IL-12, tumor necrosis factor (TNF α) and thrombopoietin [15]. The activation of Kallikarin-Kinin System (KKS) may regulate the progression of chronic liver diseases by inducing hepatoprotection and reducing fibrogenesis [16]; the KKS also possess anti thrombotic, anti-inflammatory, and anti-apoptotic effects [17], which suggesting its beneficial effect in reducing liver damaging cell. Kinin may attenuate inflammatory responses and renal fibrosis by inhibiting oxidative stress and Mitogen-Activated Protein Kinase (MAPK) activation [18]. Therefore, the aim of this study is to investigate the possible prophylactic effect of BPF that isolated from *leiurus quinquestriatus* venom against oxidative damage of CCl₄ in male Albino rats.

Materials and Methods

Carbon tetrachloride (CCl₄)

CCl₄ is a colorless non-flammable pleasant smelling liquid, of molecular weight 153.84 was obtained from El-Nasr pharmaceutical chemical Co., A.R.E.

Bradykinin potentiating factor (BPF)

BPF was previously isolated from the venom of the scorpion *Buthuthus occitanus* [13,14,19,20] according to the chemical method of Ferreira [21]. LD₅₀ crude venom was determined as described by Meier and Theakston [22]. The LD₅₀ of BPF was found to be 1.25 mg/kg b. w. of Albino rats.

Animals

Adult male Albino rats of approximate weight (about 250 ± 20 g body weight each) were selected from the animal house of the Egyptian organization for Biological products and vaccines (VACSERA), Helwan, Cairo, Egypt. The animals were housed in the animal house of the faculty of science, South Valley University, Qena, Egypt, for two weeks under natural day and night periods and with a balanced diet and water *ad libitum*.

The animals divided into three groups:

Group1: The animals (16 animals) were i.p. injected with 0.9% isotonic saline solution at a dose (100 ml/kg body weight) per 5 days along the experimental period and served as a normal group.

Group2: The animals (16 animals) were (i.p.) injected with CCl₄ (0.5 ml/kg body weight), and left without any treatment.

Group3: The animals (16 animals) were injected with CCl₄ (0.5 ml/kg body weight) and then (i.p.) injected with BPF dissolved in saline solution 1 gm/g b.w. per 5 days. Animals were sacrificed after 15 and 30 (8 animals each), when received 3 and 6 successive doses of BPF, respectively.

Sample collection

Peripheral blood was collected from each animal and divided into two portions; part was taken in EDTA containing tubes for monitoring reduced blood Glutathione (GSH) and the other portion of blood was collected in clean tubes at room temperature. After an hour, serum was separated by centrifugation for 15 minutes at 3000 rpm [23]. The sera were collected in aliquots in labeled Eppendorf's tubes and stored at -20°C until used for biochemical assay.

Prior to dissection, the liver tissue is perfused with a cold BPS (Phosphate buffered saline) solution, pH 7.4 containing 0.16 mg/ml heparin to remove any blood cell and clots. Hardening the dissected tissue by liquid nitrogen then crushed and homogenized in 5-10 ml cold buffer (i. e., 50 mM potassium phosphate, pH 7.5, 1 mM EDTA) per gram tissue. The tissue is centrifuged at 4000 rpm. For 15 minutes and then taken supernatant for assaying or kept frozen at -20°C until assayed.

Assessment of biochemical parameter of blood, serum and tissue of liver

Biochemical parameters; Alanine amino transferase, aspartate amino transferase [24], alkaline phosphatase [25], total protein [26], albumin [27], were analysed according to the reported method, Malondialdehyde [28], reduced blood Glutathione [29], Catalase [30] and super oxide dismutase [31], were analyzed using available kits according to the reported method.

Statistical analysis

The results are expressed as mean S.E. The means comparisons were made by using one-way analysis of variance (ANOVA) using Graph Pad Prism 03n software, where appropriate. Statistical significance was set at p<0.05

Results

Effect of the bradykinin potentiating factor (BPF) isolated from *Leirus quinquestriatus* venom on the (ALT), (AST) and (ALP), 1 µg/g b.w. per 5 days in Albino rat injected with CCl₄ (0.5 ml/kg body weight) post 15 and 30 days of treatment respectively.

As shown in Figure 1, the ALT, AST, and ALP recorded a significant increase in group 2 post 15 and 30 days of injection when compared with normal animals.

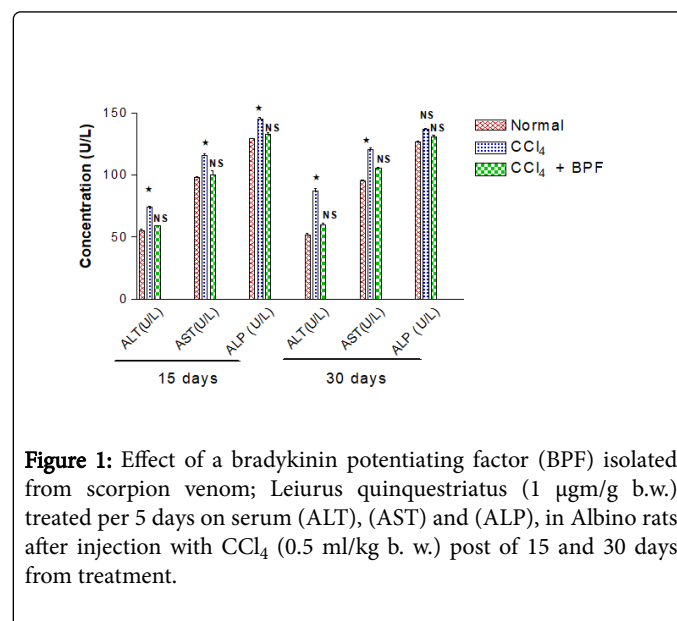


Figure 1: Effect of a bradykinin potentiating factor (BPF) isolated from scorpion venom; *Leirus quinquestriatus* (1 µgm/g b.w.) treated per 5 days on serum (ALT), (AST) and (ALP), in Albino rats after injection with CCl₄ (0.5 ml/kg b. w.) post of 15 and 30 days from treatment.

In group 3 when the animals treated with BPF (i. e. 3 doses within 15 days and 6 doses within 30 days), the serum ALT, AST, and ALP decreased significantly compared with group 2, and almost recorded to normal level.

Effect of a *serjanja erecta* bradykinin potentiating factor (BPF) isolated from *Leirus quinquestriatus* venom on the albumin and total protein, 1 µgm/g b.w. per 5 days in Albino rat injected with CCl₄ (0.5 ml/kg body weight) post 15 and 30 days of treatment respectively.

Total protein and albumin levels were significantly decreased in group 2 when compared with normal animals as shown in Figure 2. In group 3 when treated with BPF (3 doses within 15 days and 6 doses within 30 days), total protein and albumin recorded a significant

decrease, when compared with group 2, and almost recorded the normal level.

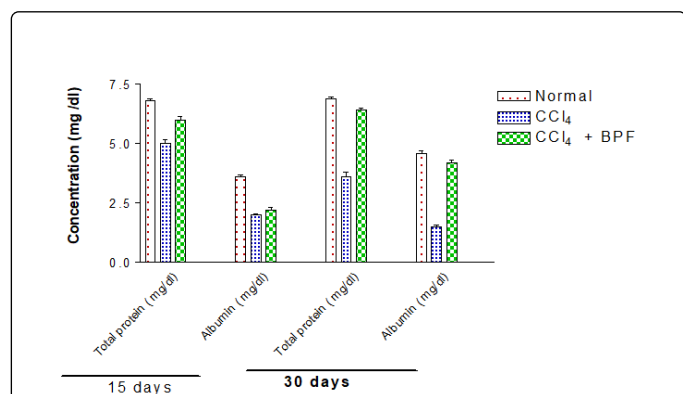


Figure 2: Effect of a bradykinin potentiating factor (BPF) isolated from *leiurus quinquestriatus* venom on the Serum proteins (T. protein and albumin) 1 gm/gm b.w. per 5 days in Albino rats injected with CCl₄ (0.5 ml/kg body weight) post 15 and 30 days of treatment.

Lipid peroxidation

As shown in Figure 3, the MDA level was significantly increased in group 2 when compared with normal animals. On the treatment, in group 3 which injected with BPF (3 doses within 15 days and 6 doses within 30 days), MDA recorded a significant decrease, when compared with the group 2 and almost recorded the normal level.

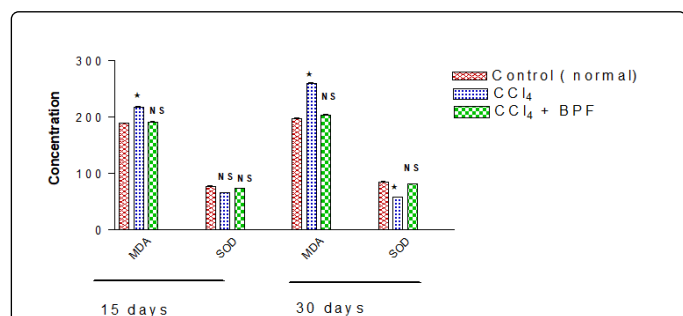


Figure 3: Effects of a bradykinin potentiating factor (BPF) 1 µgm/gm b.w. per 5 days isolated from *leiurus quinquestriatus* on MDA and SOD of liver tissues of Albino rats injected with CCl₄ (0.5 ml/kg body weight) post 15 and 30 days of treatment

Hepatic antioxidant enzyme activities

GSH, CAT and SOD levels were significantly decreased in group 2 when compared with normal animals as shown in (Figures 3 and 4). With treating, the animals which injected with BPF (3 doses within 15 days and 6 doses within 30 days) recorded a significant increase, when compared with group 2, and almost recorded the normal level.

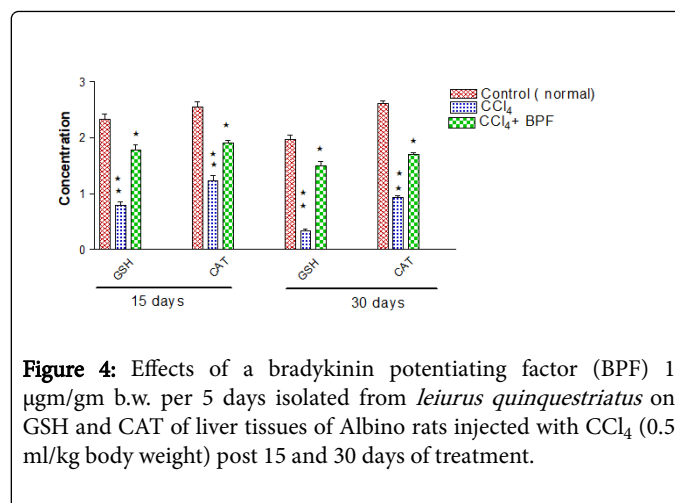


Figure 4: Effects of a bradykinin potentiating factor (BPF) 1 µgm/gm b.w. per 5 days isolated from *leiurus quinquestriatus* on GSH and CAT of liver tissues of Albino rats injected with CCl₄ (0.5 ml/kg body weight) post 15 and 30 days of treatment.

Discussion

The hepatic injury produced by carbon tetrachloride in Albino rats is well-known as hepatotoxic agent [2,32]. The changes associated with CCl₄-induced liver damage are similar to that of acute viral hepatitis. An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma [33] due to the disturbance caused in the transport function of hepatocytes. When plasma of liver cell is damaged a variety of enzymes located normally in cytosol is released into the blood. The estimation of enzymes in the serum is a useful quantitative marker of the extent and types of hepatocellular damage [34]. In the present investigation, the injection of CCl₄ caused liver injury of Albino rats and developed significant hepatic damage, which was observed through a substantial change in the concentration of serum parameters. Liver enzymes such as ALT, AST and ALP are marker enzymes for liver function and integrity [35]. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. A high level of AST indicates liver damage [36]. AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. ALT is more specific to the liver, and is thus a better parameter for detecting liver injury [37]. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [38]. Serum ALP, albumin and total protein levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [39].

In the present study injection with CCl₄ caused a significant elevation of serum enzyme levels such as AST, ALT and ALP, and significant decrease in total protein and albumin, when compared to normal animals. There was a significant restoration of these enzymes and protein levels in animals injected with the BPF as a treatment in injured animals when compared to the group which injected only CCl₄. The reversal of increased serum enzymes in CCl₄-induced liver damage by the venom fraction (BPF) may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [40]. Additionally, endogenous potentiating by the venom fraction (BPF) on the bradykinin, induces cellular activation and hence proliferation [41]. On the other hand, there are interactions between bradykinin and a classical hormonal transmitter, example of such interactions is that

bradykinin, stimulates the synthesis or release of prolactin and growth hormone [42]. Furthermore, the growth hormones and growth factors increase protein synthesis and stimulate the proliferation of mammalian cells [43]. Additionally, bradykinin stimulates the release of several cytokines as important molecules in cellular proliferation and differentiation of various blood cell progenitors [44]. These cytokines include: interleukin-1 (IL-1), IL-3, IL-6, tumor necrosis factor- α (TNF- α) and interferon- γ (IF- γ), that known to affect recovery from radiation-induced hemopoietic injury [45-47]. These findings thus establish a therapeutic role to the venom animals.

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl₄ are largely due to its active metabolite, trichloromethyl radical [48]. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. It is well known that MDA is a terminal product of lipid peroxidation [37]. So the content of MDA can be used to estimate the extent of lipid peroxidation. The latter can indirectly reflect the status of the metabolism of free radicals, the degree to which the tissue cells are attacked by free radicals and the degree to which lipid is peroxidated [49]. The superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and the hydroxyl radical (OH⁻) are the major reactive oxygen species in the body. Free radicals are produced as a consequence of normal metabolism and their activities are controlled by enzymatic defense mechanisms, such as the SOD, GPx and CAT, and non-enzymatic defense mechanisms, such as ascorbic acid, Vitamin E and GSH [11,50,51]. Furthermore oxidative damage arises when an imbalance occurs in this system, i.e. over-production of free radicals and/or a decrease in antioxidant defenses mechanisms [52]. In fact, the increase of some antioxidant enzymes activities such as SOD, GPx and CAT, which are the main antioxidants in the body, may be indicative of the failure of compensating the induced oxidative stress [53,54]. These enzymes may scavenge excess O₂⁻ and H₂O₂, and peroxides ROOH produced by free radicals. For example, SOD catalyzes the conversion of super oxide anion radical to H₂O₂. The resulting hydrogen peroxide in turn is decomposed by the enzymes GPx and CAT [55,56]. It is worthy to mention that, the exogenous bradykinin causes a decrease of hydrogen peroxide and malondialdehyde levels and an increase of antioxidative enzyme activity in hyperglycaemic rats [55], which indicates the important role of kinins in the development of oxidative stress. Furthermore, the decreased level of hydrogen peroxide and malondialdehyde, observed after bradykinin administration, may point to a reduction in free radicals production. Additionally, NADPH is a cofactor required for the resynthesis of reduced GSH. Reduced GSH regulates glutathione peroxidase activity and indirect activity of other antioxidative enzymes [57-59]. Therefore, the increase in NADPH level may lead to the activation of all examined antioxidative enzymes, additionally the increase of SOD, CAT and GSH-Px activity may also be connected with the increase in kinin-mediated transport of proteins and amino acids [55]. In the present study, the recorded results indicated that, the levels of GSH and MDA approached the normal in all animals treated with BPF exposed to CCl₄. Restoration of MDA to nearly normal levels by this fraction may be due to an enhancement of antioxidant enzyme, such as SOD, CAT and reduced GSH. Consequently, it could be suggested that the potentiated endogenous bradykinin due to the used venom fraction enhanced the activity of antioxidant enzymes

In conclusion BPF that isolated from *leirus quinquestriatus* venom normalized the hepatic injury induced by CCl₄. This normalizing was indicated by the increase of liver GSH content as well as CAT, SOD, total protein and albumin activities, and decrease in ALT, AST, and ALP. Therefore, BPF may have therapeutic values in treatment of CCl₄-induced hepatic injury.

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