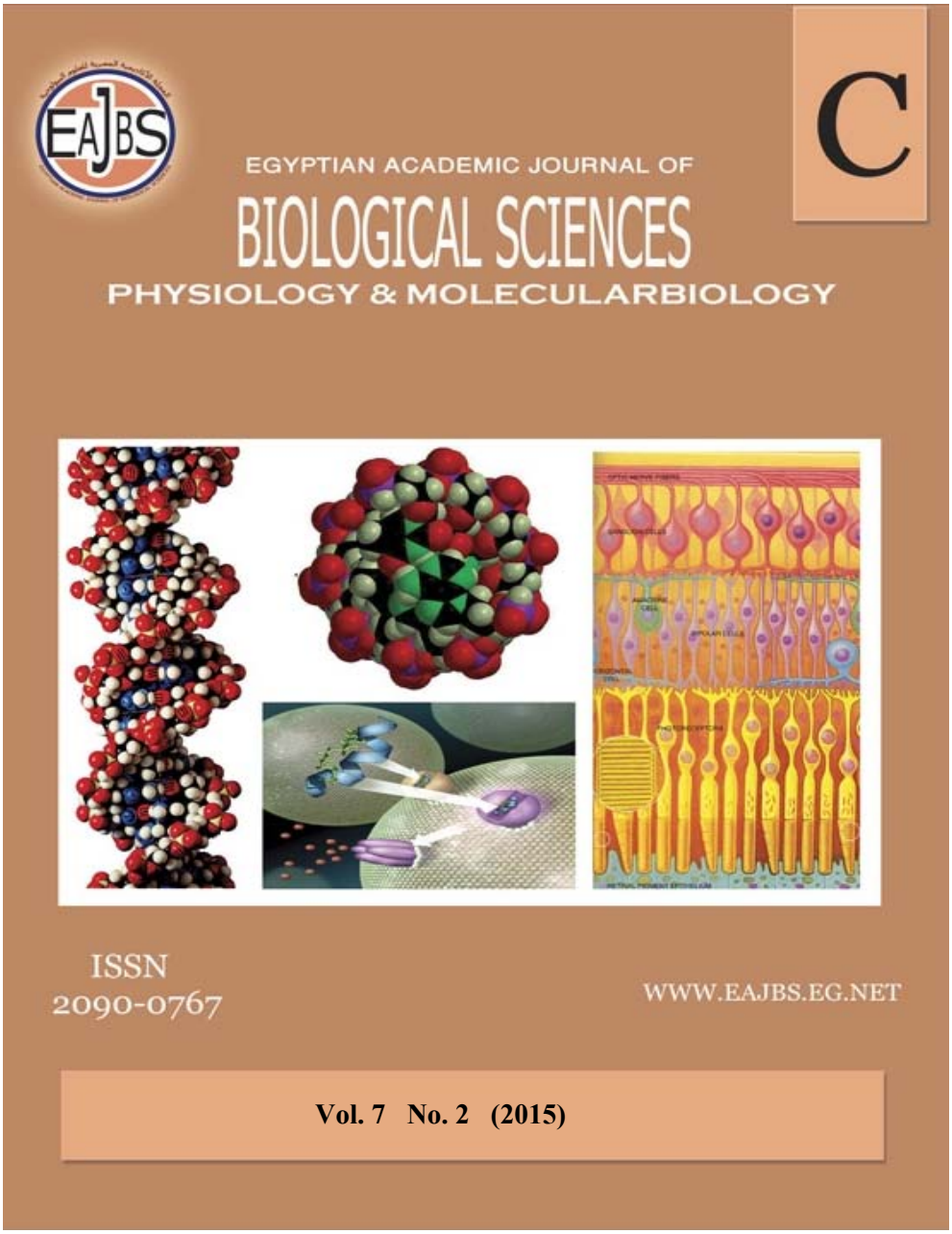

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Biochemical Effects of Bradykinin Potentiating Factor (BPF) Isolated from Scorpion Venom (*Leiurus quinquestriatus*) against CCl_4- Liver Injury in Male Albino Rats.

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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 CCl_4 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 µL saline solution. The second group (i.p) injected with BPF in 100 µL saline solutions (1µgm/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 CCl_4 for fifteen days, after that only the fourth group was treated by BPF in 100 µL saline solutions (1µgm/g. b. w. per 5 days). The results indicated that, CCl_4 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 CCl_4 as compared to the normal control group. In contrast, CCl_4 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 CCl_4 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 CCl_4 on the liver.

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 (Pang *et al.*, 1992 and Al-Jumaily *et al.,* 2014 and Abhilash,*et al*., 2013 and 2014). 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 (Wards and Daly, 1999;). Carbon tetrachloride (CCl_4) is widely used for modeling liver injury in rats. Hepatotoxicity is connected with severe impairment of the cell protection mechanisms. CCl_4 is a heavy compound that may act as a nonflammable liquid (Cetin *et al*., 2011and Mnaa *et al*., 2015). It is widely used in the dry-cleaning industry although it is a highly toxic chemical agent. Thus, CCl_4 is most widely used for experimental induction of hepatic cirrhosis (Abd-El-Dayem and Moawad, 2001 and Lin *et al.,* 2005).

CCl_4 can induce the oxidative stress beside the inhibition of the activity of antioxidant enzymes in renal tissue (Basu, 2003). The liver injury is induced mainly by the bio-transformation of CCl_4, which is cytchrome p-450 dependent free radicals initiate the process of lipid peroxidation, which is generally caused an inhibition of enzyme activity (Ward and Daly, 1999). Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cell membranes (Wang and Salahudeen, 1995). It is known that the reactive oxygen specious (ROS) would lead to oxidative damage of biological macromolecules, including lipids, proteins, and DNA (Das and Chainy, 2001). 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 (Tirkey *et al.,* 2005 and Subudhi *et al.,* 2008). Recently, a few hepatoprotective drugs from natural sources are available for the treatment or ameliorating the liver disorders. The bradykinin potentiating factor BPF extracted from *Leiurus quinquestriatus* venom was shown to enhance the cellular growth of the uterus and development of the ovarian follicle in female mice (Abd-El-Reheim, 1995). 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 (Salman, 1995). Moreover, Salman (2002) 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 (1L-1), lL-3, lL-6, lL-11, lL-12, tumor necrosis factor$_\alpha$ (TNF_α) and thrombopoietin (Neben *et al*., 1996). The activation of Kallikarin-kinin system (KKS) may regulate the progression of chronic liver diseases by inducing hepatoprotection and reducing fibrogenesis (Sancho-Bru *et al.,* 2007); the KKS also possess anti thrompotic, anti-inflammatory, and anti-apoptotic effects (Kouyoumdjian *et al.*, 2009), 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 (Chao *et al.*, 2007). 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 CCl_4 in male Albino rats.

MATERIALS AND METHODS

Carbon tetrachloride (CCl_4): CCl_4 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 *Leiurus quinquestriatus* (Salman, 1995; 2002; 2009 and 2010) according to the chemical method of Ferreira (1965). LD_{50} crude venom was determined as described by Meier and Theakston (1986). The LD_{50} 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 CCl_4 (0.5 ml/kg body weight), and left without any treatment.

Group3: The animals (16 animals) were injected with CCl_4 (0.5 ml/kg body weight) and then (i.p.) injected with BPF dissolved in saline solution 1 µgm/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. (Dacie and Lewis, 1975). The sera were collected in aliquots in labeled Epindorff's tubes and stored at −20° C until used for biochemical assaying.

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 -20ºC until assayed.

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

Biochemical parameters; Alanine amino transferase, aspatate amino transferase (Young *et al.,* 1972), alkaline phosphatase (El-Aaser and El-Merzabani, 1975), total protein (Peters,1968), albumin (Doumas *et al.,*1971), were analysed according to the reported method, Malondialdehtde (Ohkawa, *et al.,* 1979), reduced blood Glutathion (Beutler *et al.,* 1963), Catalase (Fossati *et al.,* 1980) and super oxide dismutase (Nishikimi *et al.,* 1972), were analyzed using available kits according 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 *Leiurus quinquestriatus* venom on the (ALT), (AST) and (ALP), 1 µg/g b.w. per 5 days in Albino rat injected with CCl_4 (0.5 ml/kg body weight) post 15 and 30 days of treatment respectively.

As shown in (Fig. 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.

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.

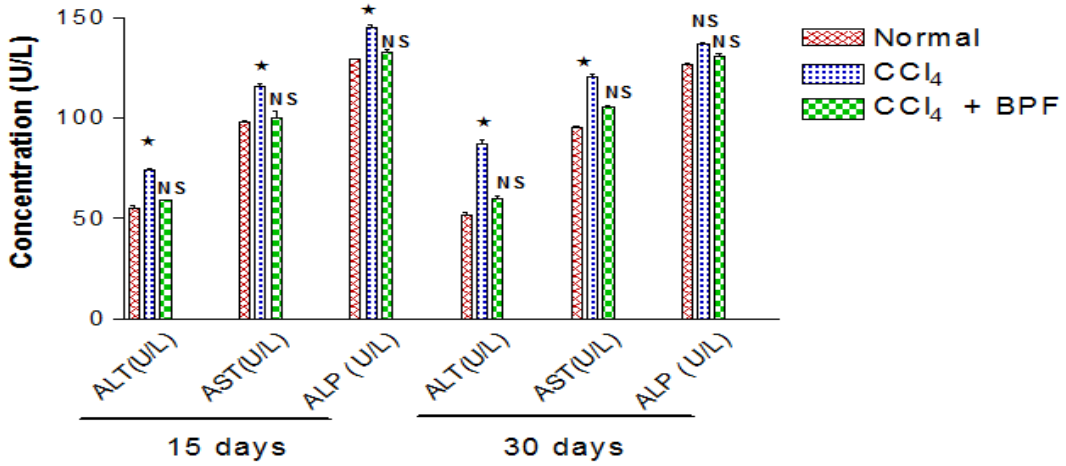

Fig.(1) : Effect of a bradykinin potentiating factor (BPF) isolated from scorpion venom, *Leiurus quinquestriatus* (1µgm/g b.w.) treated per 5 days on serum (ALT), (AST) and (ALP), in Albino rats after injection with CCl_4 (0.5 ml/kg b. w.) post of 15 and 30 days from treatment.
P < 0.05 = significant different from the control **NS**=Insignificant different from the control

Effect of the bradykinin potentiating factor (BPF) isolated from *Leiurus quinquestriatus* venom on the albumin and total protein,1 µgm/g b.w. per 5 days in Albino rat injected with CCl_4 (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 (Fig. 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.

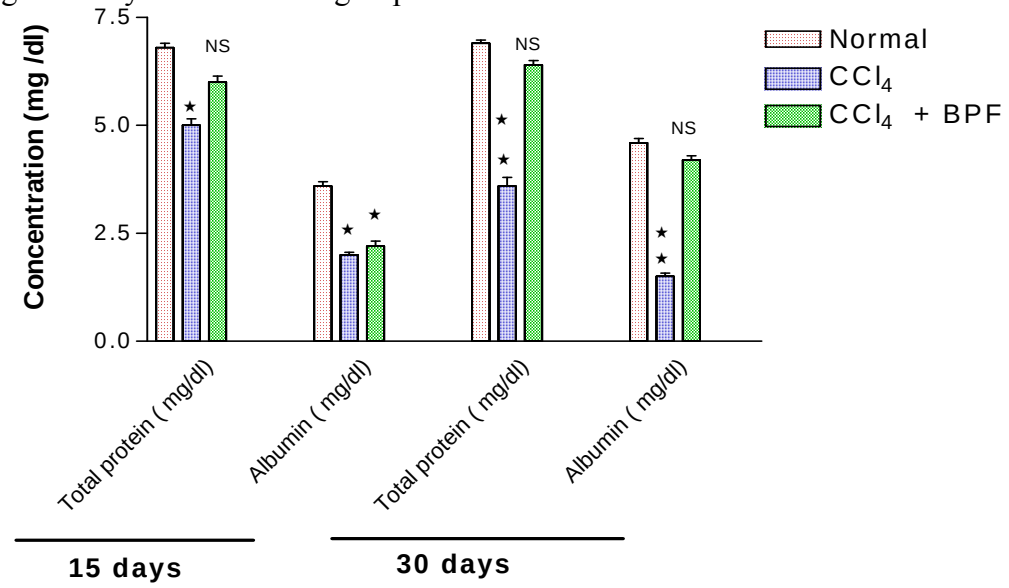

Fig. (2): Effect of a bradykinin potentiating factor (BPF) isolated from *Leiurus quinquestriatus* venom on the Serum proteins (T. protein and albumin) 1 µgm/g b.w. per 5 days in Albino rats injected with CCl_4 (0.5 ml/kg body weight) post 15 and 30 days of treatment.
P < 0.05 = significant different from the control **NS**=Insignificant different from the control

Lipid peroxidation:

As shown in (Fig. 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.

Hepatic antioxidant enzyme activities:

GSH, CAT and SOD levels were significantly decreased in group 2 when compared with normal animals as shown in (Fig. 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.

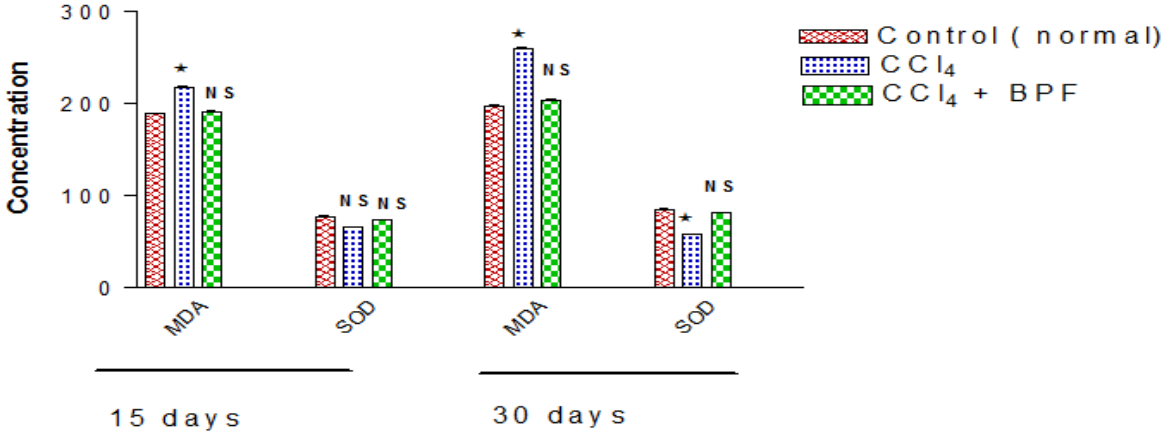

Fig. (3): Effects of a bradykinin potentiating factor (BPF) 1 µgm/gm b.w. per 5 days isolated from *Leiurus quinquestriatus* on MDA and SOD of liver tissues of Albino rats injected with CCl_4 (0.5 ml/kg body weight) post 15 and 30 days of treatment.

P < 0.05 = significant different from the control **NS**=Insignificant different from the control

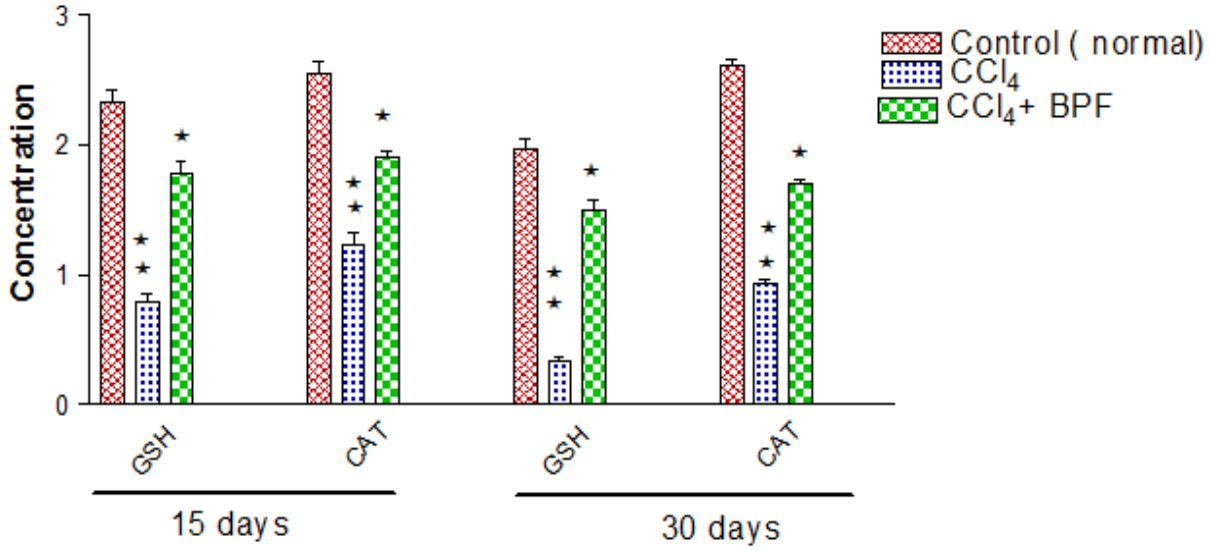

Fig.(4) : Effects of a bradykinin potentiating factor (BPF) 1 µgm/gm b.w. per 5 days isolated from *Leiurus quinquestriatus* on GSH and CAT of liver tissues of Albino rats injected with CCl_4 (0.5 ml/kg body weight) post 15 and 30 days of treatment.

P < 0.05 = significant different from the control **P < 0.0 1 = highly** significant different from the control **NS**=Insignificant different from the control

DISCUSSION

The hepatic injury produced by carbon tetrachloride in Albino rats is well-known as hepatotoxic agent (Thrall *et al.*, 2000 and Al-Jumaily *et al*., 2014). The changes associated with CCl_4-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 (Kumar *et al*.,

2005) 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 (Jadon *et al.,* 2007). In the present investigation, the injection of CCl_4 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 (Adaramoye *et al*., 2008). 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 (Rosa *et al.,* 2009). 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 (Palanivel *et al.,* 2008). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhan 1978). 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 billiary pressure (Muriel and Garcipiana, 1992).

In the present study injection with CCl_4 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 CCl_4. The reversal of increased serum enzymes in CCl_4-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 (Thabrew *et al.,* 1987and Bekheet, *et al.,* 2013). Additionally, endogenous potentiating by the venom fraction (BPF) on the bradykinin, induces cellular active ation and hence proliferation (Abu-Amra and Abd-El-Rehim, 2000). 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 (Chihara *et al.,* 1982). Furthermore, the growth hormones and growth factors increase protein synthesis and stimulate the proliferation of mammalian cells (Montgomery *et al*., 1980). Additionally, bradykinin stimulates the release of several cytokines as important molecules in cellular proliferation and differentiation of various blood cell progenitors (Özotürk, 2001).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 (Neta, 1997 a and b and Straub *et al*., 2002). 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 CCl_4 are largely due to its active metabolite, trichloromethyl radical (Johnson and Kroening, 1998). 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 (Palanivel *et al.,* 2008). 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 (Messaraha *et al*., 2010). The superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) 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 (Neradilova´ *et al.,* 1973; Benzie, 1996 and Subudhi *et al.,* 2008). 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 (Favier, 2003). 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 (Fernandez *et al.,* 2005; Iwuanyanwu *et al.,* 2007). These enzymes may scavenge excess O_2^- and H_2O_2, and peroxides ROOH produced by free radicals. For example, SOD catalyzes the conversion of super oxide anion radical to H_2O_2. The resulting hydrogen peroxide in turn is decomposed by the enzymes GPx and CAT (Ferna´ndez and Videla, 1989 and Venditti *et al.,* 2003). It is worthy to mintion that, the exogenous bradykinin causes a decrease of hydrogen peroxide and malondialdehyde levels and an increase of antioxidative enzyme activity in hyperglycaemic rats (Mikrut *et al.,* 2001) which indicates the important role of kinins in the development of oxidative stress. Furthermore, the decreased level of hydrogen peroxide and malondial-dehyde, 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 (Togashi *et al.,* 1999 and Ramasamy and Agarwal, 2008). 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 (Mikrut *et al.,* 2001). 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 CCl_4. 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 *Leiurus quinquestriatus* venom normalized the hepatic injury induced by CCl_4, 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 CCl_4-induced hepatic injury.

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ARABIC SUMMERY

تأثيرات بيوكيماوية لعامل منشط للبراديكينين معزول من سم العقرب (ليرس كوين كويستراتس) ضد أضرار رابع كلوريد الكربون الكبدية فى الجرذان البيضاء

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لقد أكدت البحوث العلمية الحديثة أن استخلاص مستخلصات من السموم الطبيعية مثل سموم العقارب والثعابين وغيرها لها دور واعد في علاج كثير من الأمراض ومن بين تلك المستخلصات عامل منشط للبراديكينين الذي تم عزله وتعريفه في دراسات عديدة سابقة علي سموم العقارب والثعابين (Bradykinin potentiating factor BPF). بيد أن تلك الدراسات في حاجة الي المزيد من البحث و الاستقصاء عن دور ذلك المستخلص في علاج أمراض الكبد في الجرذان

و من ثم كان هذا البحث علي دراسة تأثيرت بيوكيماوية لعامل منشط للبراديكينين فى علاج الكبد من التسمم برابع كلوريد الكربون في الجرذان البيضاء. ولذا فقد تم تقسيم ذكور الحيوانات الى ثلاث مجموعات كالتالى: المجموعة الأولى اعتبرت مجموعة ضابطة فقد تم حقنها بمحلول فسيولوجي (كلوريد الصوديوم) ٠.٩ % فقط. بينما حقنت المجموعة الثانية برابع كلوريد الكربون (٠.٥ مل/كجم) مرتين أسبوعيا. وأما المجموعة الثالثة فقد تم حقنها كالمجموعة الثانية و تم حقنها بجرعات متتالية بعامل منشط البراديكينين كل خمسة ايام (١ ميكروجرام لكل جرام) من وزن الجسم. ثم تم ذبح الحيوانات وكذلك تم جمع عينات الدم وجزء من نسيج الكبد طبقا لتصميم التجربة (أي بعد ١٥ يوما ممن العلاج و كذلك بعد ٣٠ يوما من العلاج .)

فلقد أدى الحقن برابع كلوريد الكربون الى زيادة معنوية فى مستويات وأنشطة انزيمات الكبد مثل الألانين ترانسفيريز، الأسبارتيت ترانسفيريز، والألكاين فوسفاتيز. كما ارتفع مستوى البروتين الكلى والألبومين فى مصل الدم بينما ارتفع تركيز انزيم مالون داى ألداهيد فى أنسجة الكبد للفئران البيضاء المجموعة الثانية بالمقارنه بالمجموعة الاولى(الضابطة). وقد سجلت النتائج السابقة بعد الاسبوع الثانى حتى وصلت مداها بعد الاسبوع الرابع. أما المجموعة الثالثة فقد سجلت انخفاضا معنويا فى تركيز كل من انزيما ت الكبد، البروتين ، الألبومين فى مصل الدم وكذلك المالون داى ألداهيد فى أنسجة الكبد مقارنة بالمجموعة الثانية المسممة برابع كلوريد الكربون. ومن ناحية أخرى، فقد اظهرت المجموعة المسممة برابع كلوريد الكربون انخفاضا معنويا لمعدل الجلوتاثيون المختزل فى الدم وكذلك نشاط انزيمى الكاتاليز وسوبر أكسيد دسميوتيز فى أنسجة الكبد لحيوانات المجموعة الثانية بالمقارنه بالمجموعة الاولى (الضابطة) .و مما جذب الانتباه أن حقن حيوانات المجموعة الثالثة بعامل منشط البراديكينين أدي الى تحسن فى تركيز القياسات السالفة الذكر و ذلك عند مقارنتها بالمجموعة التى حقنت برابع كلوريد الكربون (ألمجموعة الثانية). و اذا ما قورنت نتائج المجموعة الثالثة بالمجموعة الأولى (الضابطة) لوحظ أن التحسن فى النتائج يقترب من المعدل الطبيعى. وهذا يدل على أن للمستخلص السمي (BPF) أثر فعال فى علاج التأثيرات السامة لرابع كلوريد الكربون والتي نجم عنها اضرارا حادة للكبد .