

## Possible Hepatoprotective Effects of Mustard Seed Extract Against Paracetamol-Induced Liver Injury in Male Albino Rat

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### ABSTRACT

The evaluation of hepatoprotective effect of Mustard seed (MS) extract was assessed against paracetamol induced hepatic injury in albino rats. Animals were divided into 4 groups, each of 5 rats. Group I served as control group; group II treated with ethanolic extract from Mustard seed at a dose level of 500 mg/kg body weight orally once for 10 days. Group III represents induced liver injury animals using single dose of paracetamol (PCT). Group IV combined treatment of MS extract and PCT. Elevated serum marker enzymes such as ALT, AST, ALP, GGT and LDH were observed due to paracetamol treatment. Combined administration of MS extract with PCT to rat partially normalized the altered hepatic biochemical markers caused by PCT. Moreover, PCT caused histological changes in livers of rat including swelling and dilation in sinusoidal space, inflammation in portal tract and hepatocytes, as well as, hyperplasia in Kupffer cells. However, co-administration of MS extract with PCT alleviated to some extent the changes caused by PCT but not as the normal liver. The results of this study strongly indicate that Mustard seed has possible potent hepatoprotective action against paracetamol induced hepatic damage in rats.

**Keywords :** liver injury, paracetamol, mustard seed.

### INTRODUCTION

The liver has a vital significance in metabolism as well detoxification and removal of toxic substances. It is frequently affected by a multitude of chemicals and drugs, which can harm and weaken it, eventually leading to diseases like hepatitis or cirrhosis (Zimmerman *et al.*, 1994; Nassini *et al.*, 2010). Paracetamol's hepatotoxicity is triggered by its reactive metabolite *N*-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. Paracetamol toxicity is due to the construction of toxic metabolites when a part of it is metabolized by cytochrome P450 (Walubo *et al.*, 2004). In view of unwanted side effects of synthetic agents, there is growing interest in estimating traditional herbal medicines that are demanded to have hepatoprotective activity (Anantha *et al.*, 2012).

There has been a revival of attention in plant-based medicines due to the increase responsiveness of the limited ability of synthetic pharmaceutical product to control major disease and the need to determine new molecular structures as the lead compounds from other sources, comprising the plant kingdom (Hussain *et al.*, 2011). Mustard seed is a well-known medicinal plant in traditional medicinal system and new scientific studies have highlighted the possible use of mustard seed modern medicine (Lai, p. and Roy, 2004). The present work aims to observe the effect of mustard seed extract for protection of prompted liver injury in male rat; using the histological and biochemical changes as an indicator.

### MATERIALS AND METHODS

#### Animals

Twenty male albino rats weighing 120-135g were obtained from the Animal House of Faculty of veterinary

(Ismailia, Egypt). The animals were fed a standard diet (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and allowed free access to water. The rats were kept under standard conditions of temperature ( $22\pm 1.1^{\circ}\text{C}$ ) with 12-h light/12-h dark cycle.

#### Drugs and chemicals

Paracetamol was provided from El-Nile Pharmaceutical Company (Cairo, Egypt). All the reagents used were of analytical grade. The dried mustard seeds were purchased from local market, identified and authenticated by botanists in the department of Botany, Faculty of Science, Suez University, Egypt.

#### Preparation of seed extracts

The seeds were grounded to a fine powder using a clean mortar and pestle, their powder was stored in a clean bottle at room temperature in dark place. The powdered seed samples (100g) were weighed by using a sensitive balance and extracted with 300 ml of 70% ethanol. Then shaken for about 3 days. The extracts were filtered through Whatman No. 1 filter paper followed by evaporation of alcohol. The extract were transferred to glass vials which stored at  $4^{\circ}\text{C}$  until using (Amarowicz *et al.*, 1996)

#### Experimental design

Animals were divided into 4 groups, each of 5 rats. Group I: The rats were injected orally with (1ml /100 g.bw) saline once daily for 10 days. Group II: The rats were injected orally with Mustard seed extract in a dose of 500 mg/kg bw once daily for 10 consecutive days (Muriel *et al.*, 1992). Group III: The rats were injected orally with Paracetamol in a single dose of 300 mg/kg at the fourth day of experiment (Mcgill *et al.*, 2012). Group IV: The rats were injected orally with mustard (500 mg/kg) for 10 consecutive days. A single dose of PCT (500mg/kg) was administrated orally at the fourth

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day of experiment. On the tenth day, rats were anaesthetized with ether and

### Serum preparation

At the end of the experiment, rats were anaesthetized with light diethyl-ether. The blood samples were obtained directly from the ventricle of the heart and transferred immediately into centrifuge tubes for the separation of serum. The collected blood, were allowed to stand in slanting position for about 45 minutes at 4°C. The serum was separated by centrifugation at 2500 rpm for 10 min. The obtained serum was used to estimate the intended biochemical parameters.

### Determination of liver function

#### A. Alanine Aminotransferase (ALT)

ALT was measured using commercially available kit provided by SPINREACT, S.A. Ctra. Santa Coloma, Spain. Alanine aminotransferase (ALT) catalyses an amino group from alanine to  $\alpha$ -ketoglutarate forming pyruvate and glutamate. The pyruvate produced is converted to lactate by lactate dehydrogenase (LDH) and NADH. The rate of reduction in concentration of NADH, measured photometrically, is proportional to the catalytic of ALT present in the sample (Reitman and Frankel, 1957).

#### B. Aspartate Aminotransferase (AST)

AST was measured using commercially available kit provided by SPINREACT, S.A. Ctra. Santa Coloma, Spain. Aspartate aminotransferase catalyses the formation oxalacetate and glutamate from  $\alpha$ -ketoglutarate. The oxalacetate produced is converted to malate by malate dehydrogenase (MDH) and NADH. The rate of reduction in concentration of NADH, measured photometrically, is proportional to the catalytic of AST present in the sample (Reitman and Frankel, 1957).

#### C. Alkaline phosphatase

Alkaline phosphatase activity was estimated by the method of AACC using Point Scientific, INC kit (Tietz *et al.*, 1986).

#### D. Gamma glutamyltransferase

Gamma glutamyltransferase ( $\gamma$ -GT) was measured using commercial kit (Vitro Scient, Egypt) according to the method of Szasz (1969).

#### E. Lactate dehydrogenase

Determination of the total activity of Lactate dehydrogenase (LDH) was by the method of Moss *et al.* (1987).

### Histopathology

Liver tissues were cut into small pieces and fixed in 10% neutral formalin for 24 hours, and were imbedded in paraffin. Cut into five-micron-thick sections using microtom and routinely stained with eosin and counter stain hematoxyline (Junqueira *et al.*, 1995).

### Statistical analyses

The results were analyzed using SPSS 20.0 version. one-way ANOVA was used to analyze the present data,

$p < 0.05$  was considered as statistically significant.

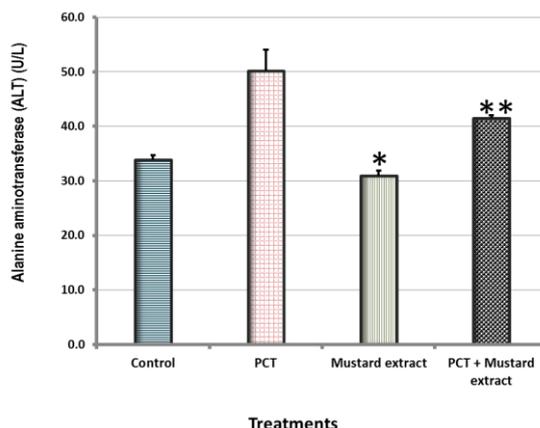
## RESULTS

Figures (1-5) shows the effects of PCT, mustard seed extract and their combination on the indices of serum liver functions, ALT (Fig. 1), AST (Fig. 2), ALP (Fig. 3), GGT (Fig. 4) and LDH (Fig. 5). PCT resulted in a significant increase ( $P < 0.05$ ) in serum ALT, AST, ALP, GGT and LDH, as compared to the control group. Mustard seed extract administration alone for 10 days showed non-significant change compared to the negative control groups. Meanwhile, administration of mustard seed extract in combination with PCT resulted in marked reversal of induced increase in serum ALT, AST, ALP and GGT as well as LDH, as compared with induced liver injury group.

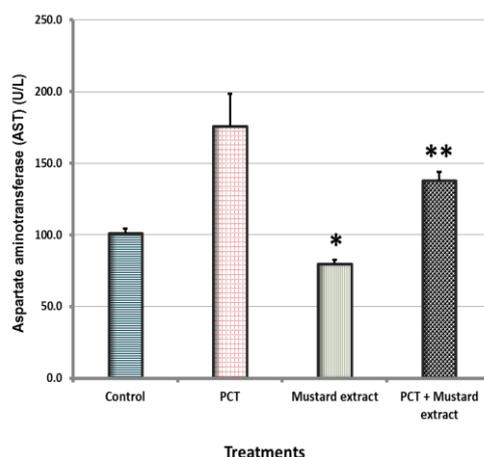
The results obtained from histological sections of livers with hematoxyline and Eosin staining for control and Mustard-treated rats were similar. Liver histologic observations of the control and Mustard-treated rats showed radially arranged hepatic cords around the central vein (Figures 1 and 2). The classic lobule was hexagonal in shape. The livers of the PCT-treated animals showed connective tissue deposition near portal triad resulting in destruction of the lobular architecture (Fig. 4), congestion of central vein and centrilobular hepatocyte swelling (Fig. 4). Section of liver tissue from PCT-treated animals treated with MS-extract showed only mild dilation sinusoids between hepatocytes (Fig. 5) diminished fibrosis, congestion, incidence of inflammatory cells infiltration, centrilobular hepatocyte swelling, as compared with PCT-group.

## DISCUSSION

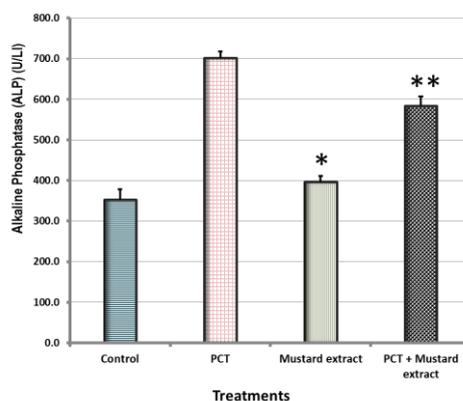
Hepatocytes are the main component that regulates various metabolic activities of liver. Distortion of this organ will result in disorder of body metabolism (Shaker *et al.*, 2010). This study was designed to investigate the ability of mustard seed extract to protect the liver against the oxidative stress and hepatocellular injury that follows a supratherapeutic dose of paracetamol. In agreement with previous studies (Larson *et al.*, 2005; More *et al.*, 2008; Chun *et al.*, 2009), paracetamol administered to rats intraperitoneally at an over dose caused hepatic injury, as evidenced by significant elevation on the serum enzymes levels, namely AST, ALT, ALP, GGT and LDH indicating chemical induced hepatocellular toxicity. It was suggested that the hepatotoxicity of paracetamol was due to formation of a toxic metabolite, N-acetyl-p-benzoquinoneimine (NAP-QI) by cytochrome P450, which induced a dose dependent depletion of intracellular reduced GSH (Parmar *et al.*, 2010; Nimila *et al.*, 2017). Another hypothesis proposed as the mechanism of paracetamol hepatotoxicity is oxidative stress. Lipid peroxidation has been suggested to be closely related to PCT-induced toxicity (Reid *et al.*, 2005).



**Figure (1):** Histogram represents effects of Mustard seed extract daily intake for 10 days, on serum liver function indices, alanine transaminase (ALT) activity in male rats with PCT induced liver injury. Data represents the mean value  $\pm$  SE. from 5 rats / group. (\*) denotes value significantly different from control group using one way ANOVA followed by Duncan ( $p < 0.05$ ). (\*\*) represents a significant difference between experimental groups compared with PCT group using one way ANOVA followed by Duncan. ( $p < 0.05$ ).

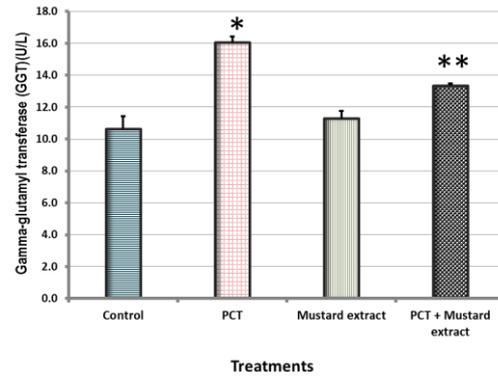


**Figure (2):** Histogram represents effects of Mustard seed extract daily intake for 10 days, on serum liver function indices, aspartate transaminase (AST) activity in male rats with PCT induced liver injury. Data represents the mean value  $\pm$  SE. from 5 rats / group. (\*) denotes value significantly different from control group using one way ANOVA followed by Duncan ( $p < 0.05$ ). (\*\*) represents a significant difference between experimental groups compared with PCT group using one way ANOVA followed by Duncan. ( $p < 0.05$ ).

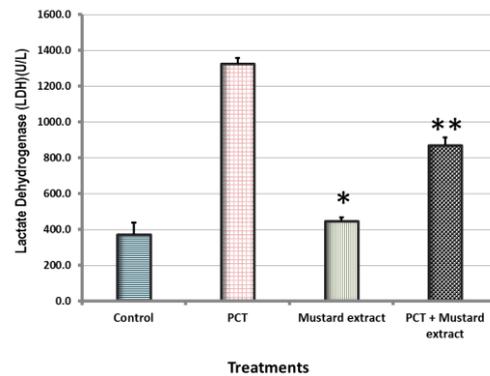


**Figure (3):** Histogram represents effects of Mustard seed extract daily intake for 10 days, on serum liver function indices, Alkaline phosphatase (ALP) activity in male rats with PCT induced liver injury. Data represents the mean value  $\pm$  S.E. from 5 rats / group. (\*) denotes value significantly different from control group using one way ANOVA followed by Duncan ( $p < 0.05$ ). (\*\*) represents a significant difference between experimental groups compared with PCT group using one way ANOVA followed by Duncan. ( $p < 0.05$ ).

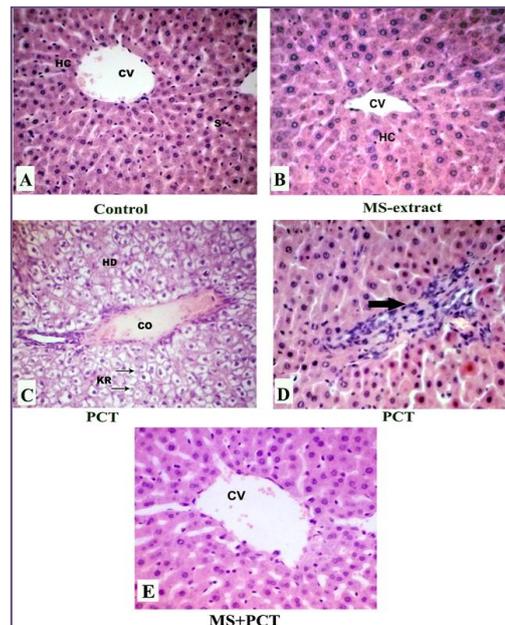
## Hepatoprotective Effects of Mustard Seed Against Liver Injury



**Figure (4):** Histogram represents effects of Mustard seed extract daily intake for 10 days, on serum liver function indices, gamma glutamyl transferase (GGT) activity in male rats with PCT induced liver injury. Data represents the mean value  $\pm$  S.E. from 5 rats / group. (\*) denotes value significantly different from control group using one way ANOVA followed by Duncan ( $p < 0.05$ ). (\*\*) represents a significant difference between experimental groups compared with PCT group using one way ANOVA followed by Duncan. ( $p < 0.05$ ).



**Figure (5):** Histogram represents effects of Mustard seed extract daily intake for 10 days, on serum liver function indices, lactate dehydrogenase (LDH) activity in male rats with PCT induced liver injury. Data represents the mean value  $\pm$  S.E. from 5 rats / group. (\*) denotes value significantly different from control group using one way ANOVA followed by Duncan ( $p < 0.05$ ). (\*\*) represents a significant difference between experimental groups compared with PCT group using one way ANOVA followed by Duncan. ( $p < 0.05$ ).



**Figure (6):** Representative images of liver in (A) control, Mustard-treated (B) PCT-induced liver injury group (C and D), PCT-group treated with 500 mg/kg/day Mustard seed extract (E). In PCT-rats, hydropic degeneration (HD) of hepatocytes around the central vein, Congestion in central vein (CO) were observed. Besides connective tissue deposition near portal triad (arrow). Absence of fibrotic area could be seen following treatment with 500mg/kg/day Mustard seed extract. (Magnification X200)

Regarding histopathological investigation the results of the present study support the findings of the previous studies as in paracetamol treated animals, where the liver showed several alterations including cloudy swelling, infiltration of lymphocytes, sever congestion, necrotic foci and hydropic changes. The degenerated cell has karyolysed nuclei, where others showed pyknosis in which nucleus shrank. In addition, the hepatic portal vein were congested. The histological changes induced by PCT treatment were confirmed biochemically. ALT is a cytosolic enzyme of the hepatocyte and an increase in its activity in serum reflects a leakage in plasma membrane permeability, which in turn, is associated with cell death. ALT is considered to be one of the best indicators of liver necrosis (Rosen and Keeffe, 2000). ALT and AST are the major critical enzymes in the biological processes. They are involved in the breakdown of amino acids into a-ketoacid, which is routed for complete metabolism through the Krebs cycle and electron transport chain (Maiti *et al.*, 2004).

Treatment of rats with Mustard extracts largely prevented paracetamol induced histopathological alterations in the liver as indicated by a reduction in inflammatory cellular infiltration and hepatocytic damages when compared to paracetamol treated group. Mustard was able to attenuate the degeneration action of PTC on the liver tissue. These results strongly supports that Mustard have powerful antioxidant and hepatoprotective properties against PCT induced free radicals damage in the liver. The free radicals scavenging effects of these spices could be attributed to its higher polyphenols and flavonoids contents. Polyphenols are the most significant compounds for the antioxidant properties of plant raw materials (Rice-Evans *et al.*, 1997). The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators (Carocho and Ferreira, 2013). The presence of these antioxidant compounds mustard seed extract could have been the cause for the observed protection of liver from paracetamol induced oxidative damage.

Thus, in conclusion, the present study confirmed that mustard has a powerful antioxidant effects against paracetamol induced hepatotoxicity. Pretreatment with these spices to paracetamol injected rats was able to reduce histopathological changes happened in hepatic tissue. Further studies should be carried out to look into the different antioxidant constituents from these spices that have potent protective effect against induced hepatotoxicity.

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## تأثيرات الممكنة لحماية الكبد بمستخلص بذور الخردل ضد الإصابة الكبدية التي يسببها الباراسيتامول في الذكور فئران البيضاء

هويده السيد خالد

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### الملخص العربي

تم تقييم التأثير الكبدي لمستخلص بذور الخردل (MS) عن طريق تقسيم الحيوانات الي 4 مجموعات، كل مجموعة مكونة من 5 فئران. المجموعة الاولى تعمل كمجموعة ضابطة. المجموعة الثانية معالجة بالمستخلص الايثانولي لبذور الخردل 500 مغ/كغ لمدة 10 ايام. المجموعة الثالثة تمثل الحيوانات التي تم عمل اصابة كبدية لها بواسطة جرعه واحده من ال PCT. المجموعة الرابعة تمثل الفئران ذات الاصابة الكبدية و معالجة بمستخلص بذور الخردل. الانزيمات مثل AST, ALT, ALP, GGT and LDH في المجموعة الثالثة تبين انها مرتفعة مقارنة بالمجموعات الضابطة. وقد لوحظ ان مستوى هذه الانزيمات قد ابدى تحسن ملحوظ في المجموعة التي تم معالجتها بمستخلص بذور الخردل مع PCT; (المجموعة الرابعة). كما لوحظ ان التغيرات الهستوباثولوجيه المحدثه في الفئران المعالجه بال PCT كانتفاخ الخلايا، اتساع الجيوب الكبدية، والتهاب الخلايا الكبدية، وزيادة حجم خلايا كوفر قد اظهرت حدة اقل في المجموعة الي تم معالجتها ببذور الخردل ولكن لم تعود الخلايا الي شكلها الطبيعي. وبالتالي نستنتج ان بذور الخردل لها تأثير في وقاية حماية كبد الفئران من تأثير ال PCT.