

HEPATOPROTECTIVE EFFECT OF MATTHIOLA ARABICA BOISS Against CARBON TETRACHLORIDE INDUCED ACUTE LIVER INJURY

By

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ABSTRACT

Chronic liver diseases one of the most common causes of death in the world due to different etiologies. Carbon tetrachloride (CCl₄) one of the well known toxicant used in most of acute and chronic liver injury. Therefore, several researches have been directed toward natural products to develop a new pharmacologically effective agents in the treatment of liver diseases. *Matthiola arabica* Boiss. one of the brassicaceae's plants which is famous with the presence of pharmacologically active glucsinolates hydrolysis products (GLCsHPs). The present study was performed to investigate the hepatoprotective effect of (GLCsHPs) fraction of *M. arabica* against carbon tetrachloride (CCl₄) induced acute liver injury at a dose of 1ml/kg injected intraperitoneally as a mixture with corn oil in the seventh day. The (GLCsHPs) fraction was administered in different three doses of 7.5,15 and 30mg/kg orally for six consecutive days followed by single dose of CCl₄ at a dose of 1ml/kg injected intraperitoneally as a mixture with corn oil in the seventh day. The hepatoprotective effect was assessed via liver function tests and histopathological liver sections. Significant reduction in the hepatic enzymes levels was found in animals treated with different doses of (GLCsHPs) fraction particularly at a dose of 30mg/kg as well as restoration of hepatocellular architecture. These findings is indicative for the promising hepatoprotective effect of (GLCsHPs) fraction against CCl₄-induced acute liver injury.

Keywords: *Matthiola arabica* ; GLCsHP; Carbon tetrachloride; Hepatoprotective; Rats.

INTRODUCTION

Due to the important vital functions of the liver in the body such as synthesis, storage, metabolism, and excretion (Martini et al., 2006). Besides, the detoxification of xenobiotics and endogenous compounds by converting them to less toxic, easy eliminated compounds by the kidney (Sargent, 2009). Chronic liver diseases have concerned one of the most important health problems in the world caused by many hepatotoxicants such as toxic chemicals, drugs, alcohol, and/or viral infection, which are characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (Loguercio, and Federico, 2003).

Carbon tetrachloride (CCl₄) considered as ordinary hepatotoxic agent which used in the most preclinical animal's studies, due to its ability to induce free radical mediating acute liver injury in rats (**Matkowski, 2008**). This liver injury leads to necrosis, steatosis, and foamy degeneration followed by the progression in to fibrosis (**Recknagel et al., 1989**). The metabolism of CCl₄, catalyzed by liver microsomal cytochrome P450, rapidly overproduces free radicals that deplete hepatic glutathione (GSH) and initiates a chain lipid peroxidation of the hepatocyte membrane and results in oxidative stress and hepatocyte injuries. These pathological changes caused by CCl₄ toxicity resemble the processes of chronic hepatitis and cirrhosis in human (**Guicciardi, and Gores, 2005; Alqasoumi, 2010**).

In the absence of an effective pharmacological therapy for such pathological processes, many researchers have directed toward herbal drugs or plant extracts for the treatment of liver diseases. Brassicaceae is one of the most important plant families due to its richness with glucosinolates, glucose and sulfur containing compounds, which are decomposed by the action of myrosinase enzyme into different pharmacological important products, including substituted isothiocyanates, nitriles, thiocyanates, epithionitriles and oxazolidinethiones. (**Gil and Macleod, 1980; Fenwick et al., 1983**).

Several researches focused in glucosinolates hydrolysis products (GLCsHPs) due to their pharmacological activity such as biocidal (**Vaughn, 1999**) and cancer chemopreventive activities (**Fahey et al., 2001**). These activities encouraged us to investigate the hepatoprotective effect of *Matthiola arabica* Boiss., one of the Brassicaceae's plants which present in Egyptian desert (**Boulos, 1999 and Täckholm, 1974**), in a CCl₄-induced acute hepatotoxicity model.

MATERIALS AND METHODS

Plant material

Matthiola arabica (Boiss.) aerial part was collected from Wadi-Gebal, Saint Catherin protectorate, south Sinai, Egypt in April 2015. It was identified by Dr. Atia Eisa, lecturer of Plant Taxonomy, Faculty of Science, Damanhour University, Egypt. Voucher sample was kept in the botanical Herbarium, Desert Research Center, Egypt.

The fraction rich in glucosinolates hydrolysis products of the aerial part of *M. arabica* (GLCsHPs) was prepared by natural autolysis method according to **Al-Gendy and Lockwood (2003)**.

Animals and experimental protocol

The studies were conducted in accordance with ethical guidelines of Ain Shams University, Egypt. Male albino rats weighing 120-180 g rats were used in this study. They were supplied by Nile Co. for Pharmaceutical and Chemical industries, Egypt and housed in an air-conditioned atmosphere at 22±2°C, under a 12 h light-dark cycle and provided with rodent chow and water ad libitum. Thirty animals were randomly divided into five different groups and treated for one week. Group 1: served as normal control received 1ml/kg of corn oil injected interapretonially on the seventh day. Group 2: received CCl₄ at a dose of 1ml/kg (prepared as 1:1 mixture with corn oil) and injected

interapretonially on the seventh day. Groups 3, 4 and 5: received fraction rich in (GLCsHPs) at three different doses of 7.5,15 and 30 mg/kg (prepared by natural autolysis method and given orally by gastric gavage) for six consecutive days followed by CCl₄ as a single dose of 1ml/kg (prepared as 1:1 mixture with corn oil) and injected interapretonially on the seventh day. At the end of the week, the blood samples were collected from the retro-orbital plexus and allowed to clot. Serum was separated by centrifugation at 1000 g for 10 min and used for the assessment of liver functions. Then, rats were sacrificed and specimens from the three major lobes of each liver were fixed in 10% formalin for histopathological examination.

Assessment of liver function tests

The levels of ALT and AST were measured following the commercial kit's instructions (Spectrum diagnostics, Egypt).

Histopathological examination

For light microscopy, liver specimens were fixed in 10% formalin and embedded in paraffin. Sections of 4µm thickness were cut, stained with hematoxylin and eosin and subjected to photomicroscopic examination.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer as a post hoc test. The 0.05 level of probability was used as the criterion for significance. All statistical analyses were performed using Instat software package (version 3.06). The scores of histopathology were analyzed by non parametric methods using Kruskal – Wallis followed by Dumr as a post hoc test.

RESULTS

Effect of fraction rich in (GLCsHPs) on serum ALT and ASTactivities

Serum ALT and AST levels were significantly increased in the CCl₄-intoxicated group by 3.7 and 3.4 folds, respectively as compared to the control group, while pre-treatment of animals with different doses of the (GLCsHPs) fraction showed a significant decrease in the serum ALT and AST levels as compared to the CCl₄ group, in a dose dependent manner. Indeed, the highest dose (30mg/kg) of the (GLCsHPs) fraction showed the highest hepatoprotection where it decreased ALT and AST serum levels by 60.8 and 51.5%, respectively as compared to the CCl₄intoxicated group .But the low (7.5mg/kg) and moderate (15mg/kg) doses were induced low to moderate significant decrease in ALT and AST levels by 33.2 and 28.6%, respectively of the low dose and 36.6 and 32.3 %, respectively of the moderate dose as compared to the CCl₄ intoxicated group (**Table 1**).

Table 1. Effects of different doses of (GLCsHPs) fraction (7.5, 15 and 30mg/kg given orally for six consecutive days) on serum ALT and AST levels in rats subjected to acute CCl₄ intoxication

Groups	ALT (U/L)	AST (U/L)
Control	29.92 ± 13.06 ^b	87.6 ± 18.43 ^b
CCl ₄	111 ± 16.16 ^a	297 ± 54.29 ^a
CCl ₄ + (GLCsHPs) fraction (7.5mg/kg)	74.2 ± 18.17 ^{a,b}	212.1 ± 36.39 ^{a,b}
CCl ₄ + (GLCsHPs) fraction (15mg/kg)	70.4 ± 23.15 ^{a,b}	201.2 ± 38.01 ^{a,b}
CCl ₄ + (GLCsHPs) fraction (30mg/kg)	43.52 ± 18.72 ^b	144 ± 26.7 ^b

* Data are the mean ± SD (n=5-6). a or b: Significantly different from control or CCl₄ group, respectively at P < 0.05 using ANOVA followed by Tukey-Kramer as a post-hoc test. ALT: alanine aminotransferase ; AST: aspartate aminotransferase

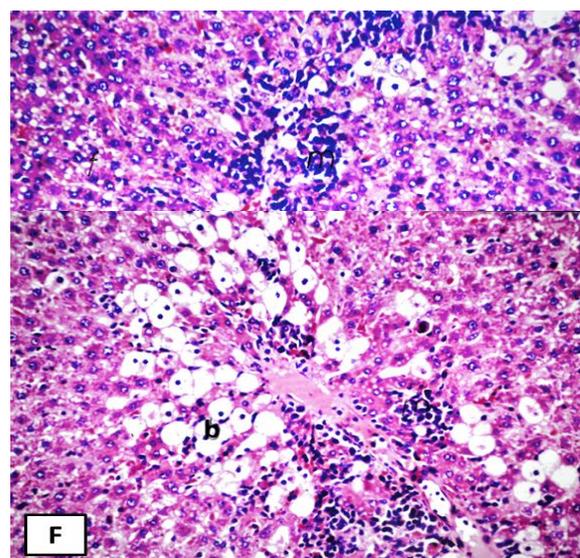
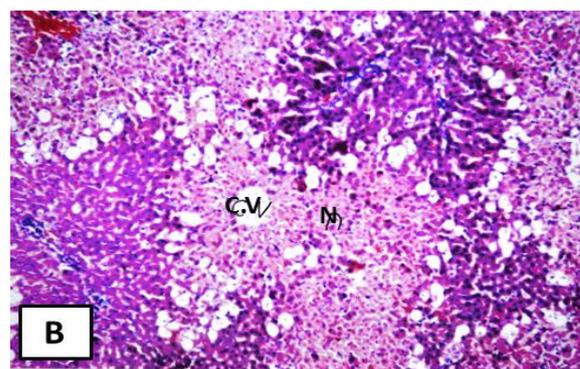
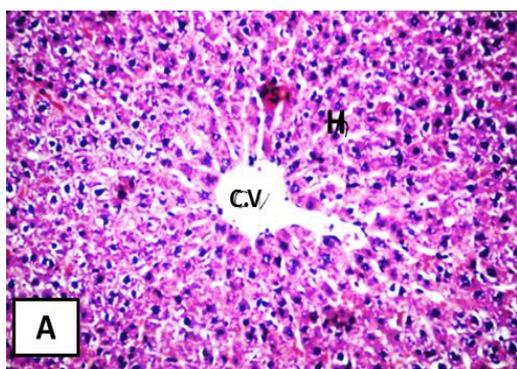
Histopathological findings

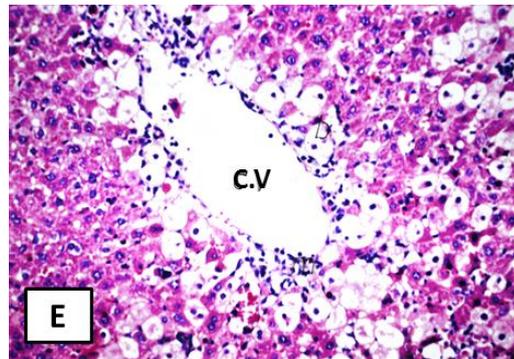
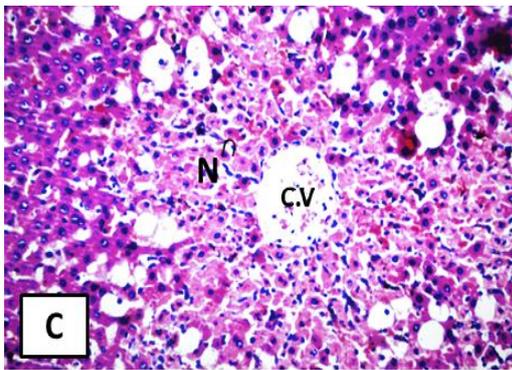
Liver sections taken from the control group and stained with H&E showed normal hepatic architecture where the hepatocytes showed a well-preserved cytoplasm and a well-defined nucleus (**Fig.1A**). However, the CCl₄.intoxicated group showed extensive hepatocellular damage evidenced by the presence of centrilobular necrosis in diffuse manner all over the hepatocytes, ballooning degeneration, inflammatory infiltration of lymphocytes and fatty changes in most hepatic parenchyma (**Fig.1 B& C**). Interestingly, the liver sections taken from the rats pretreated with different doses of the (GLCsHPs) fraction revealed that the fraction was able to reduce or even prevent the development of histopathological damage particularly in the high dose (30mg/kg) (**Fig.1 D, E&F**). These observations supported the results obtained from serum

enzymes assays, where a dose-dependent restoration of cellular integrity was confirmed. A histopathologist graded these histopathological findings (**Table 2**).

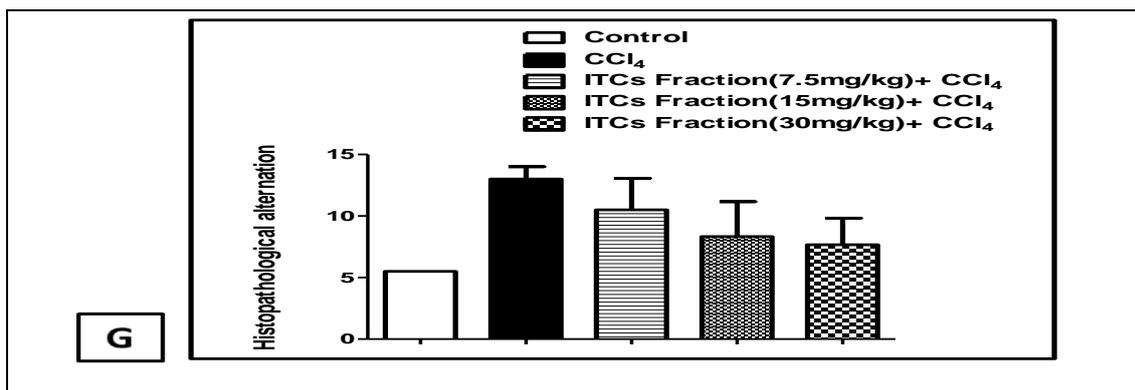
Table 2. Histopathological grading.

Groups	Histopathological Grading		
	Centrilobular necrosis	Ballooning degeneration with inflammatory cells infiltration	Fatty changes in the periphery of lobules
Control	-	-	-
CCl ₄	+++	+	+
CCl ₄ +(GLCsHPs) fraction (7.5mg/kg)	-	++	+
CCl ₄ +(GLCsHPs) fraction (15mg/kg)	-	++	-
CCl ₄ + (GLCsHPs)fraction (30mg/kg)	-	+	-





+++ (Severe), ++ (Moderate), +



(Mild), - (Nil)

Fig. (1): Representative photomicrographs of liver sections stained by H & E (×40) of acute hepatotoxicity. A: section taken from a liver of control rat show normal hepatic architecture, hepatocyte structure and central vein (CV). B and C: Sections taken from liver of rats acute intoxicated with CCl₄ show central vein (CV), sever necrosis (N),

inflammatory infiltrate and ballooning. D, E and F: Sections taken from liver of rats treated with different doses of (GLCsHPs) fraction show ballooning (b) with fatty changes and inflammatory infiltrate in different degrees with absence of necrosis. G: Quantification of histopathological alternation. Values are given as mean \pm SD.

DISCUSSION

The present study was performed to assess the hepatoprotective activity of *Matthiola arabica* Boiss. in rats, against Carbon tetrachloride as hepatotoxin to prove the pharmacological effect of natural products against liver disorder. The changes associated with Carbon tetrachloride induced liver damage presented in form of excessive production of free radicals which cause lipid peroxidation of the hepatocellular membrane and followed by inflammatory cytokines release and, eventually, hepatocellular damage (Weber et al., 2003). The later was evidenced by the significant elevation in the serum levels of the aminotransferases enzymes ALT and AST levels as compared to the control group, because these enzymes release are associated with cellular damage (Recknagel et al., 1989). This biochemical elevation was confirmed with the histopathological sever necrosis, ballooning degeneration and heavy inflammatory infiltration in CCl₄ treated animals.

The pre-treatment of animals with the (GLCsHPs) fraction at different doses (7.5, 15 and 30mg /kg) have significantly reduced serum ALT and AST levels to an accepted extent. It was found that the third dose of 30mg/kg is the most effective dose as it significantly reduced or even prevented the elevated liver enzymes to near control values. This confirmed with the pathohistological examination using H& E staining for routine examination which showed absence of hepatocytes necrosis with the presence of mild ballooning and few inflammatory infiltration in cytoplasm. These findings are confirmed with the previously reported indirect antioxidant and anti-inflammatory effects of glucosinolates hydrolysis products (GLCsHPs) (Kong et al., 2010; Boddupalli et al., 2012).

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التأثير الوقائي لنبات الخمخ ضد الإصابة الحادة للكبد برابع كلوريد الكربون

للسادة الدكتورة

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م

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تعتبر أمراض الكبد المزمنة واحدة من أكثر الأسباب شيوعا المؤدية للموت في العالم لعدة أسباب مختلفة. يعد رابع كلوريد الكربون واحد من أكثر السموم المعروفة والمستخدمه في معظم اصابات الكبد الحادة والمزمنة. لذلك اتجهت كثير من الأبحاث نحو المنتجات الطبيعية لإستخراج علاجات جديدة فعالة في علاج أمراض الكبد. يعد نبات الخمخ واحد من نباتات العائلة الصليبية المشهورة بوجود نواتج التحلل المائي للجلوكوزينولات الفعالة طبيًا. ولقد صممت هذه الدراسة لدراسة التأثير الوقائي للكبد لمستخلص نواتج التحلل المائي للجلوكوزينولات من نبات الخمخ ضد تأثير رابع كلوريد الكربون المسبب لإصابة الكبد بجرعة (١ ميليلتر / كجم عن طريق الحقن في البريتون بنسبة ١:١ بزيت الذرة) في اليوم السابع فقط. وتم حقن مستخلص نواتج التحلل المائي للجلوكوزينولات بجرعات مختلفة (٧.٥ ، ١٥ ، و ٣٠ مليجرام / كجم عن طريق الفم) لمدة ستة أيام متواصلة ثم حقن رابع كلوريد الكربون (١ ميليلتر / كجم عن طريق الحقن في البريتون بنسبة ١:١ بزيت الذرة) في اليوم السابع. ولقد تم تقييم التأثير الوقائي للكبد عن طريق تقييم وظائف الكبد ودارسة النسيج التشريحي لقطاعات الكبد. ولقد وجد انخفاض ملحوظ في إنزيمات الكبد في الحيوانات المعالجة بجرعات مختلفة لمستخلص نواتج التحلل المائي للجلوكوزينولات خاصة في الجرعة ٣٠ (مليجرام / كجم) كما وجد تحسن كبير في أنسجة الكبد للمجموعة ذاتها. وتعد هذه النتيجة واعدة بوجود مستخلص نواتج التحلل المائي للجلوكوزينولات له تأثير وقائي فعال فالكبد ضد تأثير رابع كلوريد الكربون المسبب لإصابة الكبد.