Clinicopathological studies of *Thymus vulgaris* Extract Against Cadmium Induced Hepatotoxicity in Albino Rats

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**Abstract:** Oxidative stress and reactive oxygen species have been implicated in various pollutants including cadmium toxicity. Previous studies suggested that herbs with pharmacological activities may protect against oxidative tissue damage. The aim of this study was to investigate the effect of thyme extract against cadmium chloride induced oxidative stress in albino rats. A single dose of cadmium chloride was used to induce acute cadmium toxicity, where thyme extract was orally given as a protective herbal medicine. Cadmium toxicity induce significant increase in serum aminotransferases, alkaline phosphates, lactate dehydrogenase, gamma glutamyle transferase, bilirubin (total and direct) and malondialdehyde. Moreover, significant decrease in serum total protein, albumin and A/G ratio, catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase. Liver profile was significantly improved in TE treated rats in a dose-dependent manner. Amelioration of serum hepatic enzymes, total protein, albumin, albumin globulin ratio, bilirubin (total and direct) and antioxidant profile has been detected. These biochemical changes were confirmed by liver histopathology. This study indicates that thyme extract could be a potent natural herbal product provide a promising hepatoprotective effect against cadmium chloride induced hepatotoxicity in albino rats.

**Key words:** Thyme Extract • Hepatoprotective • Cadmium Chloride • Hepatotoxicity • Antioxidants

**INTRODUCTION**

Reactive oxygen species and free radicals are implicated in Cd toxicity [1], which reflect tissue damage. Oxidative stress reported to play an important role in acute cadmium induced liver injury [2]. Cadmium (Cd) is one of the most serious environmental and occupational contaminants which represent a serious health hazard to animals and human. Exposure to Cd arises mainly from atmospheric deposition, soil and food contamination. Cadmium toxicity targeting several organs in the body includes lung, liver, kidney and testes. The liver is the detoxifying organ, where cadmium accumulated primarily in the liver, so that it is considered a serious hepatotoxic agent.

Liver damage is associated with oxidative stress which occurs with the activation of mitochondrial pathways and considered an important mechanisms for Cd-induced apoptosis [3]. Acute hepatotoxicity induced by Cd involves, the initial injury where Cd binds to sulfhydryl groups on critical molecules in mitochondria and subsequent injury or inflammation due to the activation of Kupffer cells which release a number of inflammatory mediators such as cytokines and chemokines [4].

Still, there is no effective medication to treat cadmium toxicity [5]. Medicinal plants are considered a new resource for future medication. The safety of these natural products and the harmless alternative conventional medicine to relief the pain and treat different illnesses provide a promising therapeutic applications [6]. Laminaceae family is composed primarily of basil, thyme and rosemary and considered potent natural antioxidants fighting several diseases and even cancers [7, 8]. Recently, it is recommended to use medicinal plants for the treatment of liver diseases in the modern medicine in the absence of reliable hepatoprotective drugs [6].
Thyme (*Thymus vulgaris* L.) is one of the most commercially important species of genus Thymus. It is cultivated worldwide and distributes in different areas of Mediterranean and Asia for medical purposes [9]. It is used in the treatment of different diseases such as, gastroenteric disorders, bronchopulmonary disorders, anthelmintic, antispasmodic, carminative, sedative, plus a numerous biological activities including antifungal and antimicrobial [10].

In this study, we study the antioxidant and hepatoprotective effect of thyme extract (TE) and its correlation with the harmful biochemical effect of cadmium chloride (CdCl₂) on liver. We hypothesized that thyme could help to solve the problem of cadmium intoxication and its future application as a new drug for the prophylaxis and therapy of increased cadmium body burden.

**MATERIAL AND METHODS**

**Collection of Thyme and Preparation of the Extract:** The plant was purchased from SEKEM medicinal company (Cairo, Egypt and prepared at the Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt. The plant material was dried at ambient temperature. The Thyme water extract was prepared according to Patra et al [11] with some modification. Simply, the air-dried plant materials were ground to fine powder in a grinder. About 100 g of ground plant was soaked in 1000 ml of methanol solvent. All the solvents were put in stoppered flasks and agitated for 24-48 h at room temperature with a magnetic stirrer then it was centrifuged at 3000 rpm for 10 min. The residue was re-extracted with 500 ml of distilled water stirring at room temperature for 24 h then centrifuged again at 3000 rpm for 10 min. The methanol was evaporated from the solution at approximately 85°C by using a rotary-evaporator.

**Experimental Animals:** Forty female adult albino rats (120-150 g) were obtained from the Laboratory Animal Colony, Helwan, Egypt. The animals were kept in an environmentally controlled room with clean hygienic metal cages and maintained under a uniform laboratory condition. All animals were under hygienic conditions, given balanced ration and drinking water was allowed *ad libitum* throughout the experimental period. This study complied with the Animal Welfare Act to minimize the animal suffer and pain.

**Acute Cadmium Toxicity:** Cadmium chloride (CdCl₂) (Sigma Aldrich Corporation, St. Louis, Missouri, USA) was used to induce hepatic damage. The calculated dose for induction of acute liver injury was 3.5 mg/kg b.w. dissolved in normal saline (one gm dissolved in 5 ml normal saline) according to Tzirogiannis et al [12]. CdCl₂ was single dose intraperitoneally (IP) injected into rats.

**Experimental Design:** Rats were divided into five groups and acclimatized for 14 days before starting the experiment. Group 1 (n=8), negative control, rats were fed on clean healthy food and water along the experimental period and left under normal condition without any treatment. Group 2 (n=8), positive control (CdCl₂ control group), CdCl₂ was intraperitoneally (IP) injected, 3.5 mg/kg b.w. at the day 31th from the beginning of the experiment. Group 3 (n=8), rats were orally given 100 mg/kg b.w. thyme extract (TE) daily for 30 days then exposed to a single IP injection of CdCl₂ at (day 31th) 24 h after the last TE treatment. Group 4 (n=8), rats were orally given 200 mg/kg b.w. thyme extract (TE) daily for 30 days then exposed to a single IP injection of CdCl₂ at (day 31th) 24 h after the last TE treatment. Group 5 (n=8), rats were orally given 300 mg/kg b.w. thyme extract (TE) daily for 30 days then exposed to a single IP injection of CdCl₂ at (day 31th) 24 h after the last TE treatment. All rats were scarificated at day 32th, the blood samples and liver tissues were collected for further analysis.

**Blood Sampling:** Two-three ml blood was collected from the retro-orbital venous plexus of rats under sterile septic condition at the 32th day. Blood was allowed to flow smoothly into the tubes, left to clot for 2 hours at room temperature then centrifuged at 3000 rpm for 15 min. The clear supernatant serum was collected using sterile Pasteur pipettes. The collected serum was transferred to dry, sterile labeled Eppendorf tubes for chemical analysis.

**Evaluation of Biochemical Parameters:** All parameters were colorimetric measured using commercial kits provided by Biomerieux, Egypt. All analysis was done using spectrophotometer 5010 v5+, Berlin, Germany for biochemical serum analysis.

- *Serum enzymes*, measuring serum alanine aminotransferase (ALT) activity, serum aspartate aminotransferase (AST) activity [13], serum alkaline phosphatase (ALP) activity [14], serum lactate dehydrogenase (LDH) activity [15] and serum gamma glutamyl transferase (GGT) activity [16].

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Serum proteins, serum total protein was colometrically determined according to Krohn [17]. Serum albumin was colometrically determined according to Fernandez, Sobel and Goldenberg [18]. Serum globulin level was determined by subtracting the albumin from the total proteins. Albumin globulin ratio (A/G ratio) for each sample was estimated using albumin and globulin values.

Serum bilirubin, serum total and direct bilirubin was measured, where serum indirect bilirubin was obtained [19] from subtracting the obtained direct bilirubin level from the obtained total bilirubin.

Antioxidants and Lipid Peroxidation Assay: The liver from all treated groups was collected at days 31th. One gram of each liver sample added to 9 ml of normal saline 0.9% and homogenized using electrical tissue homogenizer, centrifuged at 3000 rpm/15 minutes. The supernatant was collected and used for estimation of antioxidants (CAT, SOD, GSH, GPx) and MDA, the marker of lipid peroxidation [20]. Serum hepatic catalase activity (CAT) was performed according to Aebi [21], superoxide dismutase (SOD) was determined according to Weydert and Cullen [22], reduced glutathione (GSH) was estimated according to Beutler, Duron and Kelly [23], Glutathione peroxidase (GPx) was performed according to Weydert and Cullen [22] and malondialdehyde (MDA) was performed according to Valenzuela [24].

Histopathological Examination: A portion of liver tissue from all groups was collected and immediately prepared for hisopatological examination according to Suvarna, Layton and Bancroft [25]. Simply, the specimen labeled and fixed in 10% neutral buffered formalin solution, specimens were processed impeded in paraffin waxes and dehydrated in a series of graded concentrations of ethyl alcohol, cleared in xylene, embedded in melted paraffin at 55-60°C and sectioned at 4-5 µm thickness. These sections were routinely stained with Hematoxylin and Eosin (HandE) and examined with light microscope. Images were taken for each stained specimen.

Statistical Analysis: Statistical Analysis System software package were used to analyze the data by one-way analysis of variance ANOVA [26]. Significant differences between means were determined at a level of (P ≤0.05 or P ≤0.01) by Duncan’s multiple range tests.

RESULTS

Morbidity and Mortality Rate: The positive control group (CdCl₂ treated rats) showed severe signs of illness compared with thyme extract treated rats (100, 200 and300 mg TE/kg b.w.). The clinical signs include anorexia, depression, prostration and harried respiration. Mild signs have been detected in group 3 (100 mg TE /kg b.w.). Meanwhile, groups 4and5 (200 and300 mg TE /kg b.w.), showed no signs of toxicity. The mortality rate in group 2 (positive control) was 37.5% (3 rats died out of 8), meanwhile group 3 showed 12.5 % mortalities (1 rat died out of 8). No mortalities were detected in groups 4and5.

Enzymology Assay: An increase in the serum hepatic marker enzymes (ALT, AST, ALP, LDH and GGT) indicates liver damage. Analysis of these hepatic marker enzymes has been done to evaluate the hepatoprotective effect of TE in cadmium treated rats. Positive control (CdCl₂ treated rats) showed a highly significant (p ≤0.01) increase in serum hepatic enzymes comparing with the negative control. In contrast, the groups pretreated with thyme extract (TE) (100, 200 and300 mg/kg b.w.) showed significantly (P≤0.01) decreased enzyme level in a dose dependent manner with respect to the positive control towards normalization and close to the negative control group (Fig. 1).

Proteinogram Assay: A highly significant (P≤0.01) decrease in serum total protein, albumin and A/G ratio was observed in positive control group (CdCl₂ treated rats), indicate an acute hepatotoxicity, compared with the negative control. Groups (3-5) pretreated with thyme extract (TE) (100, 200 and300 mg/kg b.w.) showed a significant (P≤0.05) increase in the serum total protein, albumin and A/G ratio compared to the positive control (Fig. 2). Meanwhile the serum globulin level showed a non significant change in all treated groups. The proteinogram assay indicates the hepatoprotective effect of TE and the ability to counteract the cadmium induced hepatotoxicity in-vitro in albino rats.

Serum Bilirubin: Intoxication by CdCl₂ is associated with hyperbilirubinemia as an indication for hepatic damage. Analysis of bilirubin (total, direct and indirect) has been done as a hepatoprotective marker to evaluate the effect of TE in cadmium treated rats. A highly significant (P≤0.01) increase in the serum total and direct
Fig 1: Hepatic enzymes titration from rat serum samples. Significant hepatotoxicity was observed after CdCl₂ administration, as indicated by the increases in serum ALT, AST, GGT, ALP and LDH levels. TE exhibited a decrease in the activity of these enzymes. Values are expressed as mean ± SEM. Significant difference was detected \( \text{P} \leq 0.01 \) compared the positive control with negative control group. **\( \text{P} \leq 0.01 \) for the comparison between groups 3-5 with positive control group.

Fig 2: Effect of TE on Proteinogram; values are expressed as mean ± SME. A significance difference was detected \( \text{P} \leq 0.01 \) compared the positive control group with negative control, while *\( \text{P} \leq 0.05 \) for the comparison between groups 3-5 with positive control. While there was no significance difference in all groups for globulin measurement.

Fig 3: Effect of TE on serum bilirubin (total, direct and indirect). Values are expressed as mean ± SME. Significant difference was detected \( \text{P} \leq 0.01 \) compared the positive control with negative control group, while *\( \text{P} \leq 0.05 \) for the comparison between groups 3-5 with positive control.
Fig 4: Effect of TE on the antioxidant profile (CAT, SOD, GSH and GPX) and lipid peroxidation (MDA). Values are expressed as mean ± SEM. Significant difference was detected $P \leq 0.01$ compared the positive control with negative control group. $*P \leq 0.05$, $**P \leq 0.01$ for the comparison between groups 3-5 with positive control.

Fig 5: Histopathology of the liver tissue (x300). (A) Positive control, CdCl$_2$ treated group 2, showed focal necrosis of the hepatic parenchyma and vacuolar degeneration in the hepatocytes. (B) Group 3, showed congestion of the portal vein and portal oedema. (C) Group 4 showed apparently normal hepatic parenchyma and congested blood vessels. (D) Group 5, showed significant improvement in liver tissue represented by the normal hepatic architecture.

bilirubin was observed in the positive control group (CdCl$_2$ treated rats) compared to the negative control. Groups (3-5) pretreated with thyme extract (TE) (100, 200 and300 mg/kg b.w.) showed a significant ($P \leq 0.05$) decrease in the serum total and direct bilirubin compared to the positive control towards the normal level and close to the negative control. Meanwhile, the indirect bilirubin showed a non significant change in groups (3-5) pretreated with thyme extract compared to the negative control (Fig. 3).

**Antioxidant and Lipid Peroxidation Profile:** Cadmium enhances the intracellular formation of reactive oxygen species causing hepatic damage. In the present study...

we analyze the hepatic levels of several antioxidants (CAT, SOD, GSH and GPx) and MDA. Positive control (CdCl₂ treated rats) showed significant (P≤0.01) decrease in the level of CAT, SOD, GSH and GPx compared with negative control group, meanwhile a significant (P≤0.01) increase in MDA level was detected. Groups (3-5) pretreated with thyme extract (TE) (100, 200 and 300 mg/kg b.w.) showed a significant (P≤0.01 or P≤0.05) increase in the level of CAT, SOD, GSH and GPx with significant (P≤0.01 or P≤0.05) decrease in MDA level compared with CdCl₂ treated rats towards the normal level and close to the negative control (Fig. 4).

**Histopathology:** The histopathology of CdCl₂ treated rats group 2 (positive control), showed an alterations in liver histoarchitecture evidenced by focal necrosis of the hepatic parenchyma and vacuolar degeneration in the hepatocytes. Liver sections from rats pretreated with TE showed an improvement in the alteration of liver tissue. Group 3, showed mild congestion of the portal vein and portal oedema, meanwhile group 4 showed apparently normal hepatic parenchyma and slightly congested blood vessels. Group 5, pretreated with TE (300 mg/kg b.w.), showed apparently normal hepatic architecture. The histopathological results indicate the ability of TE to provide a protection against cadmium-induced hepatotoxicity in a dose dependant manner (Fig. 5).

**DISCUSSION**

In the present study, the positive control group (CdCl₂, treated rats) showed anorexia, depression, prostration and harried respiration with mortality rate 37.5%. This may be due to the acute toxic dose of cadmium or it’s highly absorption through the gastrointestinal tract. The lethality associated with acute cadmium exposure resulted from hepatotoxicity [27]. Meanwhile, pretreated rats with thyme extract (100 mg/kg b.w.) (group3) showed mild clinical signs with mortality rate 12.5%. Rats pretreated with thyme extract (200 and 300 mg/kg b.w.), showed no clinical signs with no mortalities. Thyme extract ameliorates the signs of acute cadmium intoxication. This might be due to the neutralizing effect of antioxidants on the free radicals generated due to cadmium intoxication [28].

Lysosomal instability caused by CdCl₂ with leakage of hepatic enzymes (ALT, AST, ALP, LDH and GGT) into the blood stream. A highly significant increase in (ALT, AST and ALP) was detected in the cadmium treated rats (group 2). The increase in the transferases (ALT and AST) may be attributed to hepatic damage. Meanwhile, the alterations in serum ALP damage may be attributed to cholestasis and acute hepatocellular necrosis. Our results indicated that TE has a hepatoprotective activity against CdCl₂-induced hepatotoxicity, where the pretreated groups with TE (100, 200 and 300 mg/kg b.w.), showed an improvement in the ALT, AST and ALP levels. This might be through its direct action on free radicals of cadmium to protects the liver cellular damage by maintaining its membrane integrity [28]. Group 2 (CdCl₂ treated rats) showed a highly significant increase in the serum LDH activity which is an indicative of hepatotoxicity.

Meanwhile, rats pretreated with TE (100, 200 and 300 mg/kg b.w.) showed an improvement in serum LDH level. All members of the Lamiaceae family have hepatoprotective effect via stabilizing the cell membrane viability in a dose-dependent manner, decrease the leakage of the membrane bound enzyme LDH [29]. Gamma glutamyl transferase (GGT) was a highly significant increase in CdCl₂ treated rats (group 2) and associated by a massive focal hepatic necrosis and vacuolar degeneration. Leaking of the enzyme into the blood resulted from attacking of the hepatic cell with free radicals leading to hepatic toxicity and dysfunction [30]. Meanwhile rats pretreated with (100, 200 and 300 mg TE/kg b.w.) showed a significant decrease in the serum GGT level in a dose dependant manner. Thyme extract stabilize the hepatic cellular membrane leading to decrease in the leakage of the enzyme into the blood stream.

A highly significant decrease in the serum total protein and albumin levels with non significant change in globulin was detected in the CdCl₂ treated rats (group 2). This was reflected by a significant decrease in the albumin globulin ratio (A/G ratio). These results may be due to the impaired in liver function or protein synthesis as result of damaged hepatic cells [31]. The decrease in A/G ratio may be due to a great reduction in albumin which cannot be compensated by the serum globulin level. On the other hand, TE treated groups (3-5) showed a significant increase in the proteinogram when compared with the positive control.

Serum bilirubin is a clear marker of hepatic dysfunction. The present work revealed a highly significant increase in the total and direct bilirubin, while there was a non significant increase in the indirect bilirubin in positive control group. These results may be attributed to the hepatocellular damage, as the liver responsible for cleaning the blood from bilirubin by the
conjugation process and so the difficulty to uptake and conjugate the bilirubin [32]. The exposure to CdCl₂ leads to hyperbilirubinemia [32]. Pretreatment of rats with TE (100, 200 and 300 mg/kg b.w.) resulted in a significant decrease of serum total and direct bilirubin compared to the positive control. This may be due to the membrane stabilizing and antioxidant activity of thyme extract [33].

Cadmium depletes glutathione and protein-bound sulphydryl groups, leading to increased lipid peroxidation and enhanced intracellular oxidized states. This includes superoxide radical anion (O₂⁻), hydrogen peroxide and hydroxyl radical (OH). Finally formation of lipid peroxidation associated with necrosis and hepatic damage [34]. Thyme acts as a scavenger for the oxygen-derived free radicals, thus protecting from cellular damage. Catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GPx) are considered great supportive antioxidant enzymes against ROS. Our study showed a decrease in the activity of those hepatic antioxidant enzymes which may be attributed to its consumption in scavenging the free radicals generated by the cadmium [35]. Thyme extract was increased the activity of those hepatic antioxidant enzymes because of its ability to reduce the accumulation of free radicals generated during cadmium-induced lipid peroxidation. MDA was significantly increased in CdCl₂ treated rats compared to the negative control. An increase the level of MDA in rat liver may be due to the ability of Cd to increase lipid peroxidation (LPO) [36], as a consequence of increased free radicals formation [37]. Thyme extract was able to reduce the acute hepatotoxicity caused by cadmium intoxication. The antioxidant protective mechanism decreases the oxidative stress and scavenges the free radical which responsible for the tissue damage and thus inhibit the lipid peroxidation [38].

It could be concluded that, the aqueous thyme extract (TE) has a hepatoprotective effect on cadmium chloride (CdCl₂) induced hepatotoxicity. TE can be a future natural product for counteract the cadmium intoxication. Results showed that TE has a potential hepatoprotective effect in a dose dependant manner. Besides, it has powerful antioxidant activities that minimize or diminish the hepatotoxic effect induced by CdCl₂ intoxication.

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REFERENCES


