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Protective Effect of Hydroalcoholic Extract of Clove on Thioacetamide-Induced Hepatotoxicity Animal Model

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Abstract

Background: The drug-induced liver injury (DILI) has a wide range of clinical presentations, from asymptomatic liver enzyme elevations to cirrhosis. Herbal dietary supplements may be beneficial to reduce the risk of hepatotoxicity. This study aimed to evaluate the effects of different doses of clove extracts on humoral factors in rats with hepatotoxicity induced by thioacetamide. **Materials and Methods:** In this experimental study, rats were divided into nine groups (10 rats per each). The Control group received no treatment. The Sham group was treated with oral administration of distilled water (0.5 ml) for 21 days. The positive control group received thioacetamide (50 mg/kg for three days) intraperitoneally. The clove group was divided into three subgroups and given daily oral administrations of 50, 150, and 300 mg/kg of clove hydroalcoholic extracts (for 21 days). Rats in the experimental group were divided into three subgroups and subjected to 50 mg/kg thioacetamide injection after receiving hydroalcoholic extracts of clove (50, 150, and 300 mg/kg, respectively) for 21 days in the last three days. All rats were sacrificed after 48 hours to measure liver function parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total plasma protein, and albumin). **Results:** The rats that received thioacetamide showed liver damage by increased serum liver biomarkers and decreased levels of total plasma protein and albumin compared to the control group. The different doses of clove extract resulted in a significant improvement of liver damage by reduced serum liver enzymes levels and increased total plasma protein and albumin. **Conclusion:** Oral administration of the different doses of the clove extract (50, 150, and 300 mg/kg) for 21 consecutive days could significantly improve the changes associated with serum biomarkers of hepatotoxicity. **[GMJ.2022;11:e1603]** DOI:10.31661/gmj.v11i.1603

Keywords: Syzygium Aromaticum; Clove; Hepatotoxicity; Chemical and Drug Induced Liver Injury; Rats, Wistar; Plant Extracts

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Introduction

The drug-induced liver injury (DILI) has a wide clinical presentation and ranges from asymptomatic liver enzyme elevations to acute liver failure and cirrhosis [1, 2]. Increased serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are markers of hepatocellular damage [3]. Some medications, such as sulfonamide urea, can cause an asymptomatic elevation in serum aminotransferase level, whereas tacrine may lead to greater than 20 folds of the upper normal limit in ALT [4-6]. Most cases of DILI are benign, and the liver ameliorates after withdrawal of the drug within weeks to months, while, In some cases, fibrosis or cirrhosis occurs, despite discontinuation of the drug [7, 8]. DILI is classified as hepatocellular damage, cholestasis, and combined of both [9, 10]. Acute hepatocellular damage similar to viral hepatitis leads to a marked increase in serum aminotransferase levels. Cholestatic injury can mimic extrahepatic obstructive jaundice and represent raised ALP level. Both can be seen in phenytoin-induced liver injury [4, 11, 12]. Some medications are associated with liver neoplasms, and others lead to hepatic adenoma [13]. Thioacetamide ($C_2H_3N_3$) is a hepatotoxic agent in animal models [14, 15]. It is a colorless, water-soluble crystalline that causes centrilobular necrosis and fibrosis [16]. The P 450 system metabolizes thioacetamide to hepatotoxic thioacetamide S-oxide [17, 18]. It changes cell membrane permeability and runs processes, leading to necrosis of hepatocytes [19]. Thioacetamide is administered as an intraperitoneal or intravenous injection in rats to induce liver damage [19]. Natural products, such as herbal dietary supplements, may be beneficial in reducing the risk of hepatotoxicity. It should be considered that some herbal products can induce hepatotoxic effects; therefore, the selection of an appropriate one is necessary [4]. The clove (*Syzygium aromaticum*) extraction is used for multiple therapeutic purposes, including analgesic, anti-nociceptive, antimicrobial, and anticancer [20-23]. Eugenol and caryophyllene are the main components of clove extract with antioxidant functions [24, 25]. Eugenol and ethanolic clove extract have an anti-inflammatory effect versus *propionibacterium acnes* [26]. Other applications of clove extract in medicines are antifungal effects against *Fusarium oxysporum* and *F. lycopersici*, and protective effects against aflatoxicosis in rats fed an aflatoxin-contaminated diet [27, 28]. One study indicated that single clove garlic had a more protective capability than multi clove garlic against carbon tetrachloride-induced liver injury and might be an effective alternative medicine against acute oxidative hepatotoxicity [29]. Since few studies have compared long-term administration of different doses of hydroalcoholic extract of clove in relation to hepatotoxicity, the aim of this study was to evaluate the effect of three different doses of the clove extracts over 21 days on thiostamide-induced liver injury.

Materials and Methods

Animals

In this experimental animal study, 90 male Wistar rats (10 weeks old, 200±5 g) were provided by the Animal Laboratory of Shiraz University of Medical Sciences. The animals were housed in polycarbonate rat cages (40×25×15 cm^3) in five groups with free access to standard pellet and water. The room temperature was maintained at 25±2 °C and humidity at 55-65% on a 12:12-hours light-dark cycle.

Clove Extracts Preparation

The clove tree was procured from a local market in Shiraz, Iran. The samples were confirmed by a herbalist at the medicinal plants processing research center, at Shiraz university of medical sciences, Iran. The voucher specimen number of the plant is 13954 [30]. They were finely powdered using a clean electric blender. A 50 g of fine powder was extracted with 350 ccs of 70% ethanol for 72 hours using a percolator. The percentage yield of the extract was calculated to be 18.2% (w/w) [30]. The extract was then filtered and made solvent-free with the aid of a rotary evaporator. A semisolid condensed extract was provided after 24 hours using a desiccator [31].

Study Design

All rats were randomly divided into nine groups (10 rats in each) as follows: control group, sham group, positive control, clove group (positive control for extract, three subgroups), and experimental group (three subgroups). The control (native) group received no treatment. The sham group was treated with oral administration of distilled water (0.5 ml) for 21 days. The positive control group was treated intraperitoneally with thioacetamide (50 mg/kg for three days) [32]. The clove group was divided into low (50 mg/kg), moderate (150 mg/kg), and high doses (300 mg/kg) of daily clove extracts for 21 days. [33]. The experimental group was divided into three subgroups and received 50 mg/kg thioacetamide (Merck, Germany) injection after administration of clove extracts (50, 150, and 300 mg/kg for 21 days) in the last three days.

At the end of the study, animals were sacrificed by an overdose of general anesthetic. Blood samples were obtained from the rodent's cardiac puncture for the assessment of hepatic enzymes (AST, ALT, and ALP) activities, serum albumin, and total serum protein.

Hepatotoxicity Evaluations

Hepatotoxicity evaluations include hepatic enzymes activities, serum albumin, and total serum protein determination. AST, ALT, and ALP were measured using a kinetic ultraviolet method defined by the International Federation of Clinical Chemistry [34]. Serum albumin was measured by a bromocresol green, and total serum protein was measured using a Biuret method. An autoanalyzer (Mindray BS-380, Japan) and commercial kits (Pars Azmoon, Iran) were used for all analyses.

Ethical Issues

The Ethics Committee of Fasa University of Medical Sciences approved this study (registration number: IR.FUMS.REC.1400.111). All procedures were performed according to the Helsinki Declaration of 2020 [35].

Statistical Analysis

The results are presented as mean and standard deviation. The one-way ANOVA test was used to compare changes among the groups, and Tukey's post hoc test to identify the differences. The Kolmogorov-Smirnov test was used to test of normality of data. All statistical analyses were performed using SPSS software (Statistical Package for Social Sciences) (version 16.0, Chicago, IL, USA). A P-value≤0.05 was considered statically significant.

Results

1. Serum Levels of ALT, AST, and ALP

1.1. Control, Sham, and Positive Control Groups

The mean AST level in the control group was 183.50±3.26 IU/L (Figure-1). Also, the mean serum level of ALT and ALP in the control group were 62.88±1.33 and 540.13±3.35 IU/L, respectively (Figure-2A and B). The mean AST and ALP serum levels in the sham group were 207.88±2.15 and 572.21±8.91 IU/L, respectively (Figure-2A and B). The post hoc test shows no difference in serum levels of hepatic enzymes between the control and sham groups. However, the mean values of ALT, AST, and ALP (108.4±3.73, 408.23±4.47, and 786.14±10.17 IU/L) in the positive control group were higher than those in the control and sham groups (Figure-1 and -2, P<0.001).

1.2. Clove Subgroups

The mean serum levels of ALT, AST, and ALP in clove extract groups show no significant difference compared with control and sham groups (Figure-1 and -2, P>0.05). However, there was a significant decrease in the hepatic enzymes level of clove subgroups compared with the positive control group (Figure-1 and -2). Also, there was a significant decrease in serum levels of AST and ALP in all clove subgroups compared with the lowest dose (50 mg/kg clove extract +50 mg/kg thioacetamide) of the experimental subgroup (Figure-1 and -2, P<0.001).

1.3. Experimental Subgroups

The mean±SD of serum levels of ALT in 50 (low dose), 150 (moderate dose), and 300

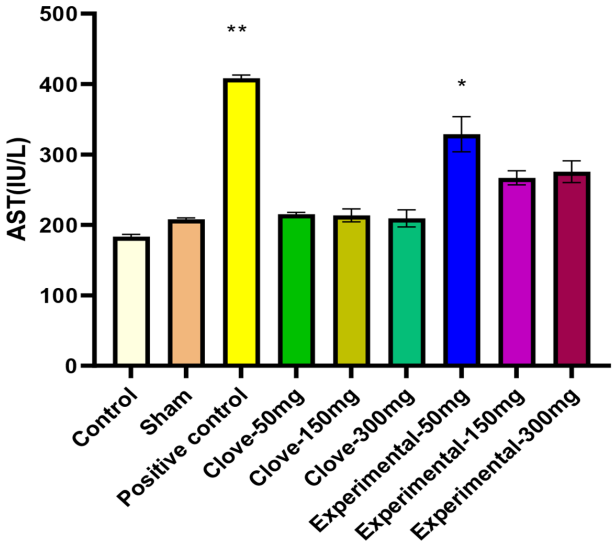

Figure 1. Effects of clove and thioacetamide on hepatotoxicity markers (AST). Results are shown as mean±SD. The one-way ANOVA test analyzed data, and Tukey's post hoc test compared the differences. *P<0.001 vs. all doses of clove and experimental 150 and 300 mg groups,**P<0.001 vs. all groups.

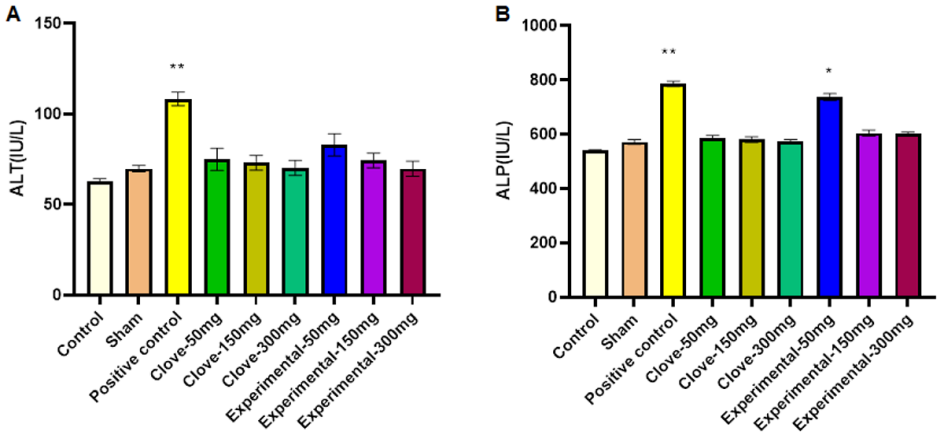

Figure 2. Effects of clove and thioacetamide on ALT (**A**) and ALP (**B**) levels in studied rats. Results are presented as mean±SD. The one-way ANOVA test analyzed data, and Tukey's post hoc test compared the differences. *P<0.001 vs. all doses of clove and experimental 150 and 300 mg groups,**P<0.001 vs. all groups.

(high dose) mg/kg of experimental subgroups were 83.00±6.23, 74.43±4.07, and 69.84±4.15 IU/L, respectively (Figure-2A). Also, the mean serum level of AST in experimental subgroups of low, moderate, and high doses was 328.72±24.98, 266.86±10.04, and 275.62±15.38 IU/L, respectively (Figure-1). In addition, in the high dose experimental subgroup, the ALP level was 737.72±12.53, 605.01±11.39, and 602.63±5.48 IU/L, respectively (Figure-2B). There was a significant decrease in AST and ALP in moderate and high doses of experimental subgroups compared with the low dose of the experimental subgroup (P<0.001). Also, the post hoc Tukey test shows a significant decrease in ALT, AST, and ALP serum levels in all three subgroups compared with the positive control group (P<0.001).

2. Serum Levels of Albumin and Total Protein

2.1. Control, Sham, and Positive Control Groups

The post hoc test shows no difference between the control and sham groups

in the term of serum levels of albumin (4.10±0.23 vs. 4.28±0.19) and total protein (7.68±0.09 vs. 7.81±0.11), respectively (Figure-3, P>0.05). However, the mean value of serum levels of albumin (3.05±0.05) and total protein (7.32±0.12) in the positive control group were higher than those in the control and sham groups (Figure-3, P<0.001).

2.2. Clove Subgroups

The mean±SD of serum levels of albumin and total protein in 50 mg/kg of clove extract was 4.24±0.15 and 7.87±0.12, in the 150 mg/kg group was 4.21±0.07 and 7.83±0.19, and in 300 mg/kg group was 4.19±0.04 and 7.82±0.14, respectively. There was no significant difference between various doses of clove extract and compared with control or sham groups regarding serum levels of albumin and total protein (P>0.05). However, there was a significant increase in serum levels of albumin and total protein of clove subgroups compared with the positive control group (Figure-3, P<0.001).

2.3. Experimental Subgroups

As the showed in Figure-3, serum levels of albumin in 50, 50, and 300 mg/kg of experimental subgroups were 3.64±0.19, 3.84±0.05, and 3.88±0.07, respectively. Also, total protein in those groups was 7.94±0.27, 8.05±0.11, and 7.80±0.16, respectively. The post hoc Tukey test shows a significant increase in serum levels of albumin and total protein in all three subgroups compared with the positive control group (Figure-3, P<0.001).

Discussion

In the present study, we evaluated the ability of various doses of clove extract (50, 150, and 300 mg/kg) to protect the experimental groups from the hepatotoxicity induced by thioacetamide. The rats in the positive control group that received 50 mg/kg of thioacetamide showed liver damage by increasing serum biomarkers (AST, ALT, ALP) and decreased levels of total plasma protein and albumin compared to the control group, which was similar to that of other studies [3, 36]. Salam *et al.* revealed that thioacetamide (300 mg/kg) markedly increased serum ALT, AST, and ALP [3]. Also, Saleh *et al*. found that thioacetamide (200 mg/kg; 3 times/week) induced liver injury and was associated with massive changes in the serum levels of liver biomarkers, oxidative stress markers, and liver inflammatory cytokines [36]. AST, ALT, and ALP are the enzymes naturally present in the cytoplasm and enter the bloodstream through cellular damage [3]. Reports suggest that thioacetamide is not toxic for the liver; however, its metabolite, thioacetamide S-oxide, has hepatotoxic effects associated with producing free radicals and oxidative stress, which affects cell membrane permeability

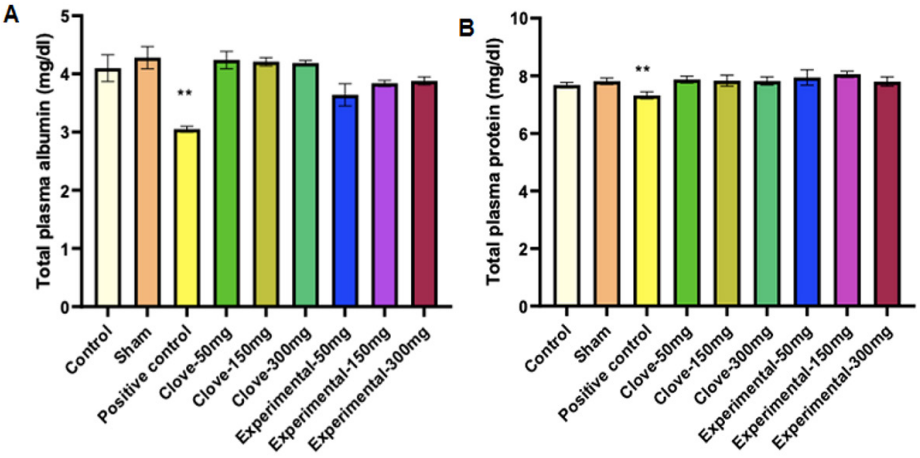

Figure 3. Effects of clove and thioacetamide on total plasma albumin (**A**) and protein (**B**) concentration. Data are presented as mean±SD. **P<0.001 vs. all groups.

[37, 38]. This process increases calcium's intracellular concentration, enlargement of nucleoli, prevents mitochondrial activity and causes necrosis and cell death in zones 1 and 3 [37, 38]. On the other hand, the change in the cell membrane permeability leads to an increase in biochemical parameters such as ALT and ALP [39-42]. Nitric oxide and nuclear factor- κB (Nf-κB) synthesis are also released by thioacetamide, which is another cause of liver necrosis [19].

Regarding the clove subgroups, there were not found any significant differences among subgroups in comparison with control or sham groups. However, the results showed a significant difference in hepatic enzyme activities and serum levels of total protein and albumin compared with the positive control group. The finding of this study presented that clove extracts could prevent thioacetamide-induced hepatotoxicity by decreasing hepatic biomarkers (AST, ALT, and ALP) and increasing levels of total plasma protein and albumin. In agreement with our findings, Prasad *et al*. stated that oral administration of the clove extract (800 mg/kg), containing 50% ethanol, can markedly modify the serum levels of biochemical markers and oxidative stress induced by thioacetamide (400 mg/kg) [43]. Yogalakshmi *et al*. demonstrated that eugenol (derived from the clove tree) reduces CYP2E1 activity, lipid peroxidation, protein oxidation, inflammatory markers, improves antioxidant status, prevents DNA damages, and increases expression of the COX-2 gene in rats [44]. Some other studies reported that the eugenol-rich fraction of clove inhibits hepatic cell proliferation and decreases oxidative stress, which could protect against liver cirrhosis [45]. Another study presented that clove (10 g/kg) improves the growth rate in broiler chickens without hepatic injury [46]. It has been suggested that the potential hepatoprotective role of clove is due to its compounds (i.e., eugenol, eugenol acetate, and polyphenols) that are proton donators leading to inhibit free radical chain reactions and thus protect against thioacetamide S-oxide [47, 48]. One of the limitations of the present study was limited scientific evidence to evaluate various doses of a species extract of *S. aromaticum* on thioacetamide-induced hepatotoxicity. Therefore, the comparison of the present study with similar findings is challenging. However, one animal study administered 25, 50, and 100 mg/kg of hydroalcoholic extract of *Dianthus carryophyllu* (another species of clove tree) for 28 days and found that ALP decreased; however, total plasma protein and albumin decreased compared with the control group [49]. In the above study, it seems that a higher dose of clove extract (100 mg/kg) compared to a lower dose (25 mg/kg) had a greater protective effect. However, this effect was not observed in all measured factors (total plasma protein) [49].

These results may be somewhat consistent with our findings. In our study, all clove extract doses (50, 150, and 300 mg/kg) prevented hepatotoxicity damage. The differences between the results could be due to the use of higher doses in our study (50, 150, and 300 mg/kg v.s 25, 50, 100 mg/kg), the use of different species of cloves (*S. aromaticum vs. D. carryophyllu*), or the type of drug used to cause liver damage (thioacetamide v.s gentamicin). Our findings showed that the moderate and high doses (150 and 300 mg/kg, respectively) had more efficiency than lower doses (50 mg/kg) of clove extract. However, moderate and high doses had the same effects on liver protection. Another limitation of the present study is the lack of pathological evidence to support our results. However, our findings suggest that a moderate dose (150 mg/kg) of hydroalcoholic extract of clove is an optimal dose that can be used in animal models of hepatotoxicity.

Finally, organizing multiple groups, identifying the optimal dose of clove extract to prevent hepatotoxicity induced by thioacetamide, and evaluation of several hepatotoxicity biomarkers are the strong points of this study. For further studies, it is recommended that histological factors be taken into account and use the dose of 150 mg/kg/day instead of various doses.

Conclusion

Treatment with thioacetamide leads to

biochemical and humoral changes, while clove supplement markedly reduces the toxic effects on the liver. Clove extract can improve liver function by itself alone too. Therefore, according to many reports regarding the hepatotoxicity of medications and herbals, clove can be used as a supplement to reduce the hepatotoxicity of medications.

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Conflict of Interest

The authors have no conflict of interest.

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