ARTICLE / INVESTIGACIÓN

# Hepatoprotective effect of *Thyme aqueous* extract in Acetamino-phen induces hepatotoxicity in male rats

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**Abstract:** *Thyme vulgaris* is effective in treating acetaminophen toxicity in clinical trials. The present study investigates Thyme aqueous extract's effect on rats poisoned with Acetaminophen. In this study, the data were obtained from male Wister rats. Animals were divided into three groups: distilled water, acetaminophen (1mg/ kg), and aqueous thyme extract (400 mg/kg). All animals were orally treated for seven days respectively. The animal was sacrificed on the eighth day. ALT, AST, GSH, TAC, and Caspase3 were all measured in plasma obtained from heart derived blood samples centrifuged to determine plasma levels of these enzymes and other antioxidants, malondialdehyde precursors (MDA). Liver enzyme levels were reduced, total antioxidant levels were in creased, and an aqueous extract of thyme compensated for glutathione levels. Caspase3 levels were also reduced. Acetaminophen induced liver tissue damage and inflammatory cell damage were considerably lessened by Aqueous Thyme extract treatment. To protect the liver from Acetaminophen induced hepatotoxicity, aqueous Thyme extract was found to be beneficial.

**Key words:** Acetaminophen, Hepatotoxicity, Thyme aqueous extract, Histopathology.



## Introduction

One of the primary causes for a drug's removal from the market is its hepatotoxicity. Hepatotoxicity caused by drugs accounts for 50% of all acute liver failure cases and 5% of hospitali-zations[1]. It is common to see nonspecific symptoms, including stomach pain, nausea, vomiting, diarrhea, and pruritus, with the nonspecific hepatotoxicity symptoms, such as abdominal pain, jaundice, fever, and rash in patients who have been exposed to hepatotoxicity[2]. Acetaminophen (APAP) is a potent analgesic and antipyretic that has been around for a long time. If you're look-ing for personal medication, you can buy it over the counter or through your doctor's office[3]. Acetaminophen is rapidly absorbed from the gastrointestinal tract after oral administration[4]. Peak plasma concentrations are noticed between thirty minutes to two hours, with protein binding varying from 20 to 50% at traditional therapeutic doses. Acetaminophen is metabolized primarily within the liver into non toxic products[5]. Acetaminophen induced liver damage is charac-terized by the extensive release of cellular contents (liver enzymes), nuclear degradation and inflammatory response. These are typical features of oncotic necrosis. Cell death in vivo and in vitro is caused by oncotic necrosis[6]. The thyme plant belongs to the family Labiatae and is an herbaceous plant spread in the Mediterranean region. The medicinal part of thyme is concentrated in the leaves and the entire plant. It has been widely used as an antiseptic, carminative, anti-spasmodic, rheumatic, dermatological, antifungal, anthelmintic, and analgesic[7]. Thyme is also used in treating cold cases, bronchitis and whooping cough, and it has broad uses in veterinary medicine as an antiseptic for the intestines and an antihookworm. It improves heart rate and lowers blood pressure. It was used as an antibacterial and improves Nutritional efficiency, leads to increased body weight, obesity and appetite in rabbits, and is considered a non toxic plant[8]. Thyme contains many chemicals, including volatile oil, where thyme oil contains 55% of phenols, the most important of which are thymol and carvacrol, and resinous materials such as rais-ing and tannin. It is also a source of thiamine[9]. As for the effects of thyme on reducing sugar, studies have indicated that, injecting thyme extract into rabbits leads to lowering blood glucose, and adding it to the rabbit diet at concentrations of 150-300 mg/kg diet leads to an improvement in food intake, weight gain and food conversion factor[10].

## Materials and methods

### Chemicals

From the GSK firm in the United Kingdom, we acquired a 500 mg acetaminophen tablet, BioMerieux, France, and supplied the reagent kits for the assay of transaminases. Elisa kits for determining total antioxidant capacity were bought from BT LAB, China, for tissue malondialdehyde, glutathione (GSH), caspase 3, and full antioxidant capacity (TAC). Methods for each diagnostic kit's work were followed precisely.

### Thyme Aqueous Extraction

In the Al-Najaf Province, Kufa City, dried leaves of *Thymus vulgaris* were purchased and identified at the Kufa University Herbarium. Using commercially available equipment,

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*T. vulgaris* leaves were pulverized into a fine powder in a grinder. It took 100 grams of fine powder in 200 milliliters of denatured alcohol for 30 minutes of continuous infusion. It was then centrifuged for 10 minutes at 3000 revolutions per minute, then dried in an oven at 60 degrees Celsius. Sterilized bottles were used to hold the dry material[11].

### Experimental Animals

The rats were obtained from a College of Science/ University of Kufa animal house and ranged in age from 10 to 14 weeks and weight from 180 to 200 g. Plastic cages were used to house the animals. Wooden shelves were inserted into the cage, and it was kept at a temperature of (23–25°C) and a humidity level of (60–65%), allowing the animals to drink tap water and consume a standard chow diet. This experiment required using rats that had first spent two weeks acclimated in an animal house to laboratory conditions. This allowed the rats to better cope with the stress of being moved into a new environment. Twentyone male rats were divided into three equal groups and given seven days of treatment in each group.

•Group one: Rats administrated distilled water 1 ml/kg/day orally

•Group two: Rats administrated only distilled water and Acetaminophen at a dose of 1g/kg/day orally for seven days.

•Group three: Rats administrated aqueous thyme extract orally at 400 mg/kg/day for seven days + Acetaminophen at 1 g/kg/day orally on the seventh day. All animals were sacrificed on the nine day.

### Tissue Sampling for Histopathology

The apical portion was preserved and fixed in 10% neutral formalin, then embedded in a paraffin block and cut into sections with a thickness of 5 micrometers for histopathological examinations. Sections stained with hematoxylin and eosin blue were examined under light microscopy[12].

### Statistical Analysis

SPSS version 27 was used for the statistical analysis. (Means SD) was used to represent continuous variables. Three or more groups were compared using an ANOVA test. A p-value of less than 0.05 was deemed significant in this study.

## Results

It is shown in Table 1 that the thyme extract has an effect on liver function tests as well as on biochemical, oxidative, and apoptosis parameters. Aspartate and alanine aminotransferase enzyme activity increased significantly compared to the negative control after an oral dose of 1 mg of Acetaminophen per kilogram of body weight. When used as a treatment, thyme extract significantly reduced serum levels of these enzymes (table 1). Accumulation of lipid peroxidation in liver tissue (MDA level) and depletion of antioxidant defense mechanisms (GSH level) were dramatically ameliorated by treatment with thyme extract (TAC). In the grand scheme of things.

### Effects of Thyme Extract on Liver Histopathology

The liver slices from the positive control group showed extended necrosis, significant hydropic degeneration, and increased Kupffer cell proliferation; thyme extract resulted in only mild degeneration and no necrosis (table 1, figure 1).

The results are presented as mean and standard deviation, with Gp1 representing the control group (no treatment), Gp2 representing the Acetaminophen-treated group, and Gp3 repre-senting the Acetaminophen-treated group (with thymus extract). S. ALT and S. AST represent se-rum alanine aminotransferase and aspartate aminotransferase, respectively. T. GSH represents tissue glutathione, and T. MDA represents tissue.

In this study, the histological evaluation of the liver male rat section of the control group shows normal histological structures. The results are shown in Figure (1).

Histopathological examination of the liver male rat section of group 2 treated with Acetaminophen at a dose (1g/kg, day) showed activation of kupffer cells, irregular and enlarged portal tract, necrosis with the appearance of newly formed bile ductules as shown in Figure (2) compared with the control group. The histopathological score of rat liver group 2 is shown in Table (2).

The results showed that there was damage noted in hydropic degeneration, congestion of the cen-tral vein, no harm in the mitotic figure, and no damage in perisinusoidal fibrosis. Mild toxicity was seen as kupffer cell proliferation. Moderate toxicity was seen as apoptosis and portal In-flammation, and severe toxicity was caught in congestion of the central vein, lobular Inflamma-tion and bile ducts, necrosis, and dilation of the sinusoids. Histopathological examination of the liver male rat section of group 3 treated with thyme aqueous at a dose (of 400 mg/kg per day) shows dilation of the sinusoids with mild mononuclear cells infiltration and an increase in the number of kupffer cells Figure (3) compared with the control group.

The histopathological score of rat liver group 3 is shown in Table (2). The results showed that there was no fibrosis, no congestion of the central vein, no bile duct injury, no necrosis, and no congestion of the central vein. During moderate symptoms of Inflammation and dilation of the sinusoids and mitotic figure, mild symptoms of apoptosis and hydropic degeneration were seen.

| Variables | Gp1 (N=7) | Gp2 (N=7) | Gp3 (N=7) | P value |
|---|---|---|---|---|
| AST (U/L) | 15.963±6.117 | 24.693 ± 7.408 | 17.796 ± 2.215 | <0.698 |
| ALT (U/L) | 62.025 ± 25.351 | 105.342 ± 23.850 | 29.599 ± 14.141 | <0.018 |
| MDA (µmol/g) | 3.123 ± 0.521 | 5.266 ± 1.757 | 3.340 ± 0.227 | <0.159 |
| TAC (mmol/g) | 4.038 ± 0.695 | 5.371 ± 0.347 | 8.214 ± 0.779 | <0.005 |
| GSH (mmol/g) | 69.767 ± 3.889 | 55.695 ± 5.845 | 61.399 ± 7.918 | <0.691 |
| Caspase 3 (ng/ml) | 0.235 ± 0.014 | 0.418 ± 0.123 | 0.248 ± 0.012 | <0.121 |

**Table 1.** Using thyme extract, liver function tests, biochemical and oxidative stress were compared to Acetaminophen and the control group.

| Group 3 | Group 2 | Group 1 | Groups | Histopathological changes |
|---|---|---|---|---|
| 1 | 2 | 0 | Hydropic degeneration | **Hepatocellular changes** |
| 0 | 3 | 0 | necrosis | |
| 1 | 2 | 0 | Apoptosis | |
| 2 | 0 | 0 | Mitotic figure | |
| 0 | 3 | 0 | Congestion of the central vein | |
| 2 | 3 | 0 | Dilation of the sinusoids | |
| 2 | 2 | 0 | Portal | **Inflammation** |
| 2 | 3 | 0 | lobular | |
| 2 | 1 | 1 | Kupfer cells proliferation | |
| 0 | 3 | 0 | bile duct injury | **Bile ducts** |
| 0 | 2 | 0 | bile duct hyperplasia | |
| 0 | 2 | 0 | portal | **Fibrosis** |
| 0 | 1 | 0 | Septal | |
| 0 | 0 | 0 | perisinusoidal | |

**Table 2.** Represents the Histopathological changes that were observed representing (0) no symptoms, (1) mild symptoms, (2) moderate symptoms, (3) severe symptoms (4) acute symptoms.

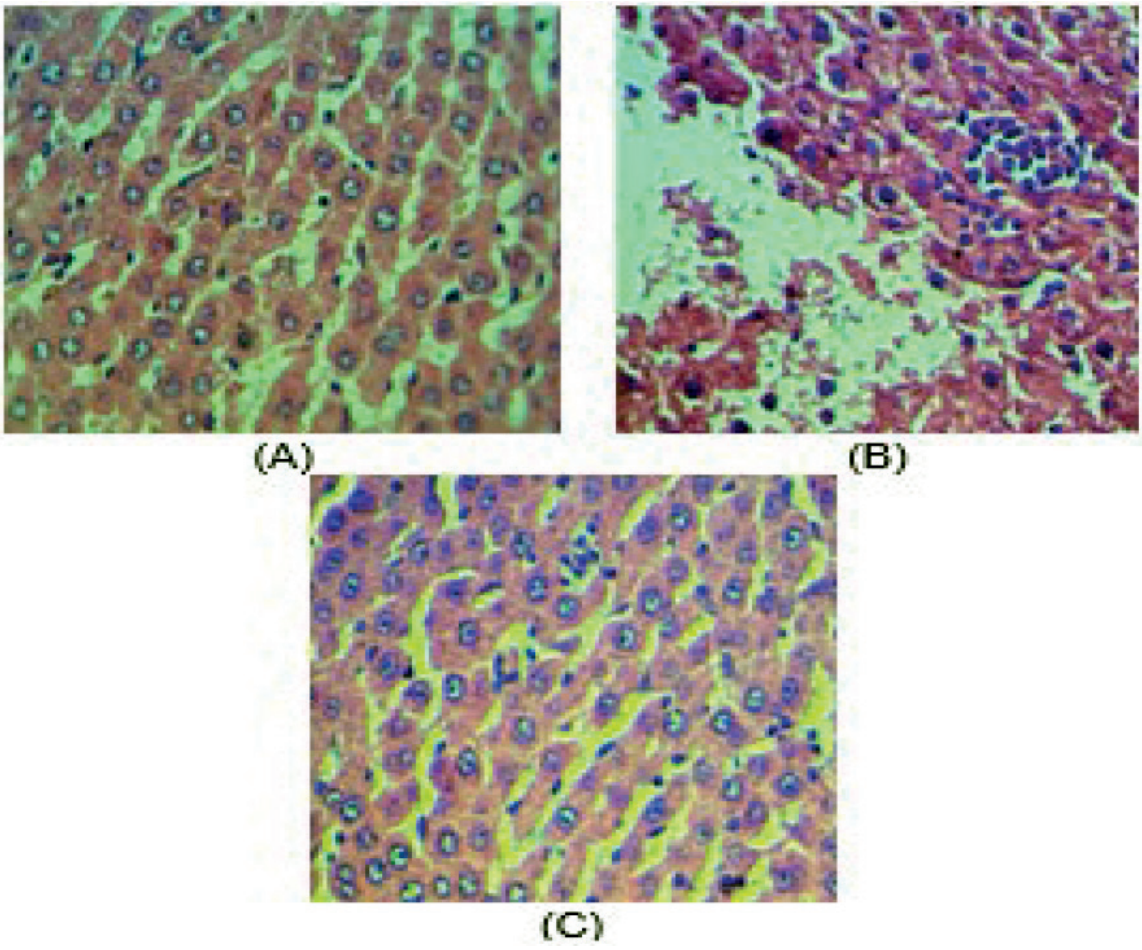


**Figure 1.** Section of liver of albino male rat of study groups (A: Normal group, B: Acetaminophen group, C: Thyme group) on day 8 of the experiment. 400X, H&E.

## Discussion

Even though Acetaminophen has long been considered a generally safe medicine, the general safety of the drug in therapeutic, permitted levels has lately been questioned because multiple studies have shown alanine aminotransferase increases with more than five days of therapeutic dosing. Even though Acetaminophen is widely used and there have been no instances of severe liver injury, the likelihood of developing liver damage is still relatively low[15]. Acetaminophen, a common over-the-counter painkiller and antipyretic. Users include patients suffering from hypertension, migraines or myocardial infarction who frequently and chronically use Acetaminophen to minimize headaches or illdefined pain associated with these conditions[16]. On the other hand, antioxidants can be used in these patients as the central therapeutics or, concomitantly, as a precaution. Acetaminophen can be presented as a drug that can impair liver function as it has a liver-damaging potential[17]. There were no deaths in any of the groups of rats who received APAP or distilled water for 24 hours. Histopathology examination of the control group reported normal morphological features, while Acetaminophen administrated rats' morphological features show hepatotoxicity; activation of kupffer cells and slight congestion of central vein, portal venopathy and the epithelium in the bile duct is irregular, and associated Inflammation is minimal. According to the histopathological severity score, the Acetaminophen group revealed severe toxicity, significantly different from the Control group, which revealed zero histopathological severity score. This histopathological result agrees with a study by Muhammad-Azam *et al.*[18]. Studies by (19). Muhammad-Azam *et al.*[20], agree with the current research, and the study of (21) reported the critical protective role of aqueous thyme extract in hepatocyte morphology and prevention of Acetaminophenmediated apoptosis through the antioxidation and antiinflammatory effects of aqueous thyme extract. The histopathological examination of Acetaminophen revealed severe hepatotoxicity according to histopathological severity score due to Acetaminophen mediated apoptosis, Inflammation and oxidative stress, Histopathological examination of aqueous thyme extract was shown to activate kupffer cells, irregular and enlarged portal tract, scholangitis with the appearance of newly formed bile ductules induced by Aceta-minophen. The effect of aqueous thyme extract on liver enzymes was small and not uniformly consistent.

These findings are consistent with a previously reported conclusion that aqueous thyme extract has cytoprotective effects against oxidative injury caused by acute Acetaminophen toxicity in rat liver and restored the enzyme level[22].

Thyme aqueous extract may exert hepatoprotection through various mechanisms. Free radical scavenging properties and inhibition of lipid peroxidation *in vitro*[23], is one mechanism involved. In the present study, malondialdehyde (MDA) levels, both in serum and liver homoge-nate, which is a marker of oxidative stress, were reduced, and serum glutathione, another charac-teristic of oxidative stress, was marginally increased.

This may suggest that hepatoprotection by Thyme aqueous extract may involve an antiox-idant mechanism.

APAP-induced hepatotoxicity is thought to be caused in part by apoptotic cell death and in-flammatory reactions[24]. According to earlier studies, thyme contains anti-apoptotic and anti-inflammatory properties. In liver slices from lead exposed rats, thyme therapy reduced necrosis, inflammatory cell infiltrations, and hemorrhage. More human clinical investigations are needed to verify the effectiveness of this treatment[25,26].

## Conclusions

Thyme aqueous extract is protective against Acetaminophen-induced hepatotoxicity in rats. The treatment results in a positive result in oxidative stress and apoptosis marker with reten-tion of the average level of liver enzyme and prevents changes in the histopathological section.

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