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Hepatoprotective and Immunomodulatory Potential of ginger in Experimental Thioacetamide-Induced Liver Damage in mice.

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Abstract:
Disorders of the liver are one of the top health-related public health issues facing the world today. Most liver conditions are caused by two main factors, namely oxidative stress and immune system dysfunction. Some of the most commonly used traditional medicinal plants that contain many beneficial chemical compounds include Zingiber officinale (Ginger) which is a rich source of two active compounds, gingerol, and shogaol. Traditionally used as an anti-inflammatory and antioxidant, this study evaluated the immunomodulatory and hepatoprotective effects of ginger in a rat model of thioacetamide (TAA)-induced liver injury. The rats were treated with ginger extracts (at doses of 200 and 400 mg/kg, and the effective dose was evaluated against silymarin, which is a standard hepatoprotective agent). Outcome measurements included changes in rat body weight and liver weight and serum liver enzymes (ALT, AST, ALP) that correlate with liver damage. Ginger treatment resulted in increased body weight, decreased liver weight, and restoration of the liver enzyme levels toward the normal range. Ginger at the higher dose (400 mg/kg) had hepatoprotective effects similar to silymarin.The hepatoprotective mechanism of ginger may be due to its antioxidant properties of free radical scavenging and immune modulation. In conclusion, Zingiber officinale is a potential candidate for both liver protection and immune system regulation, and through the integration of traditional methods with scientific evidence, ginger may also be considered an adjunct or alternative treatment for liver disease.

Keywords: *Zingiber officinale*, Ginger, Hepatoprotective effect, Immunomodulatory effect, Thioacetamide (TAA).

1. Introduction

The biological effects of ginger result from its diverse composition of phytochemicals, predominantly gingerols, shogaols and zingerone. Ginger can stimulate T-lymphocytes and enhance their ability to produce antibodies, while influencing macrophage function and the synthesis of pro- and anti-inflammatory cytokines and displaying microbial activity [1]. A large amount of research exists supporting the immuno-modulatory properties of ginger

due to its ability to modulate T-lymphocyte proliferation, macrophage activity, cytokine production and oxidative stress [2].The health benefits attributed to ginger are the result of the interaction of its many phytoconstituents. The most significant phytoconstituents of ginger are gingerols, shogaols, and zingerone; each of which have displayed effects on T-lymphocyte proliferation, macrophage activity, and the production of pro- and anti-inflammatory cytokines [3]. In addition, results from research [5] confirm that ginger oil also has antioxidant properties that are comparable to those demonstrated by the essential oils of ginger [4]. In addition, [

5] identified important volatile constituents in the essential oils of fresh and dried ginger, including camphene, ρ-cineole, α-terpineol, zingiberene, and pentadecanoic acid.
Hepatoprotective agents help minimize the damage done to your liver when it has been exposed to toxins such as acetaminophen or alcohol. This study also identified how ginger affects the liver so that ginger can potentially be used as a preventative treatment for liver injury[6]. This research found that ginger has similar potential hepatoprotective properties as silymarin, which is an established hepatoprotective drug[7]. This research additionally identified that ginger synergistically interacts with the immune system to enhance overall immunity and functioning of the immune system.

TAA is widely recognised as a model for studying the hepatotoxicity of other compounds and as a mechanism for testing the effects of TAA-induced hepatotoxicity on other organ systems[8]. Though TAA itself has no hepatotoxic effect, CYP2E1 enzymes in the liver metabolise the compound into active reactive metabolites (primarily TASO and TASO2). These reactive metabolites are electrophilic in nature and create oxidative stress within the hepatocyte by binding to many different cellular macromolecules during free radical injury, eventually resulting in apoptosis and necrosis of the hepatocyte[9].

2. Methods and Materials

2.1. Preparation of Plant Extracts

The powdered ginger used for this study was obtained from a local marketplace in Baghdad. A 50mg/mL concentration of the sample was created by dissolving the powder in a small amount of dimethyl sulfoxide (DMSO), and from this, additional dilutions were made to prepare the

dosages of 250mg/kg and 500mg/kg for use in the experimental group of animals**.**

2.2. Phytochemical Analysis using Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry was used to analyse the chemical composition of the Zingiber officinale extract. GC-MS analysis of Z. officinale extract was carried out using a Perkin-Elmer Clarus 500 GC-MS system equipped with an RT* 5 Column (30m x 0.32nm), injecting 2µL of the extract per injection. Helium gas, which served as the carrier gas at a flow rate of 3mL/min, was used throughout the analysis. The temperature program consisted of a two-minute hold at 75 °C, followed by a ramp of 50 °C/min to reach a final hold of 7 minutes at 175 °C. The resulting m/z values were matched against a library of mass spectra containing known organic compounds for identification. The analyses were performed in the Ministry of Science and Technology, Iraq.

2.3. Chemicals and Reagents

Thioacetamide (TAA) (Sigma-Aldrich, Germany) was used for inducing liver cirrhosis in the experimental animals. Other reagents and chemicals used in this study were: industrial-grade ethanol 95% (R&M Chemical, UK), Tween-20 10% (Merck, Germany), formalin concentrated (38-40%; Merck, Germany), disodium hydrogen phosphate (Merck, Germany), sodium dihydrogen phosphate monohydrate (Sigma-Aldrich, Germany), toluene (Merck, Germany), xylene (BDH Laboratory Supplies, England), and other standard laboratory consumables.

2.4. Animal Housing and Experimental Design

This section explains the protocols for keeping the mice used in this experiment as well as how experiments were designed and conducted.

Mice were kept in controlled conditions, with the following conditions maintained daily. Daylight and darkness were separated by 12 hours of each day, and the temperature of the facility was maintained between 23°C - 27°C. All mice had access to food and water throughout the course of the experiments.

Forty mice were randomly divided into four equal groups (10 mice in each group). The treatment for each group consisted of the following:

Group 1: Normal control. This group received the placebo treatment of distilled water (D.W.) at a volume of 0.1 mL per dose.
Group 2: Positive control. This group received the toxic agent TAA at a dose level of 200 mg/kg (injected intraperitoneally (IP)) for the purpose of inducing liver damage.
Group 3: Ginger low-dose + TAA. This group was given ginger at a dose of 200 mg/kg (administered orally). After 1 hour had passed since the administration of ginger, mice in this group were administered TAA (also administered IP).
Group 4: Ginger high-dose + TAA. This group was administered ginger at a dose of 400 mg/kg (administered orally). After 1 hour had passed since the administration of ginger, mice in this group were administered TAA (also administered IP).
All dosing was administered once daily (0.1 mL) for a total of 14 consecutive days. At the end of the experiment, all mice were euthanized in accordance with ethical standards and tissue samples collected for subsequent analysis.

2.5. Statistical analysis

Statistical analyses were performed on all data obtained from the experiments using SPSS v.27 (Statistical Package for the Social Sciences). Descriptive statistical measures (i.e., frequency, percentage, mean, range (lowest to highest values), and standard deviations) were utilized to evaluate all results and determine if statistical differences existed between groups for quantitative data. To determine whether there was a significant difference in the findings of the two independent groups for quantitative data, the Student's t-test was applied; whereas, for quantitative data, there were more than two self-regulating groups and thus the analysis was completed using an Analysis of Variance (ANOVA) approach. The cut-off for statistical significance was set at ≤ 0.05.

3. Results

3.1. Oil Yield, Phenolic Content, and Antioxidant Capacity

Table 1 shows the total volatile oils of dried ginger, total phenols, and antioxidant assay and their antioxidant activities. The volatile amount of oil from dried ginger was 1.65% with a ± 0.09% standard deviation, which agrees with some literature on the subject; for example, El-Ghorab (2010) reported that the dried ginger contained 1.1% essential oils. The total phenolic content of this ginger oil sample was determined to be 84.9 ± 0.45 mg gallic acid equivalents/g (mg GAE/g), again agreeing with El-Baroty et al. (2010). Furthermore, this ginger oil exhibited a high level of antioxidant capability; therefore, we would expect it to show 75.61% inhibition (±

0.93%) in DPPH assay. Specifically, this high level of antioxidant ability of 73.58% is confirmed through the report of [5], in which this ginger oil exhibited 84.83% antioxidant activity.3.2 Body Weight, Liver Weight & Liver Index: Effects of Ginger

Parameter	Ginger Powder (Mean ± SD)
% Oil	1.65 ± 0.09
Total Phenolic Content (mg GAE/g)	84.9 ± 0.45
% Antioxidant Activity	75.61 ± 0.93

Note: Results are calculated on a dry weight basis.

3.2. Effects on Body Weight, Liver Weight, and Liver Index

The effects of ginger supplementation on the body weight, liver weight & liver index of rats with TAA-induced liver damage were investigated. The results are shown in Table 2.

Body Weight: Rats in the TAA group (sample size of 10) demonstrated a significant decline in body weight when compared to the normal control rats (weight range = 168 ± 6.47 g compared to 254 ± 6.65 g). These results indicate systemic toxicity and a decline in health caused by hepatic damage. Administration of high dose ginger (400 mg/kg) significantly elevated the animal's body weight to the point of having similar body weight as the normal controls (211 ± 3.44 g), whereas the administration of low dose ginger (200 mg/kg) had only a minor effect on improving body weight resulting in an increase of 5.95% (228 ± 8.76 g). During the entire viewing period for the experiment, the group treated with silymarin (100 mg/kg) showed the greatest improvement out of all the groups that were studied, and returned to within the normal weight range of standard controls (290 ± 8.16 g) on most of the parameters evaluated.

Liver Weight and Liver Index: There was an increase in liver weight (12.61 ± 0.41 g) and liver index (6.88% ± 0.36%) in the TAA control group indicating hepatitis and liver enlargement. Administration of high-dosage ginger significantly decreased liver weight (10.11 ± 0.53 g) and liver index (4.56% ± 0.21%) indicating potent hepatoprotective action. Low dosage ginger had a lesser impact, however, on both liver weight and index than did the higher dosage. Administration of silymarin effectively returned liver weight (9.86 ± 1.69 g) and liver index (2.99% ± 0.32%) back to levels similar to that of the normal control group.

Animal Groups	Body Weight (g)	Liver Weight (g)	Liver Index (LW/BW %)
Normal Control	254 ± 6.65b	9.88 ± 1.15	2.85 ± 0.22b
TAA Control	168 ± 6.47a	12.61 ± 0.41	6.88 ± 0.36a
High-Dose Ginger (400 mg/kg)	211 ± 3.44ab	10.11 ± 0.53b	4.56 ± 0.21ab
Low-Dose Ginger (200 mg/kg)	228 ± 8.76ab	10.98 ± 0.18	5.08 ± 0.19ab
Silymarin (100 mg/kg)	290 ± 8.16b	9.86 ± 1.69	2.99 ± 0.32b

Note: Data are expressed as Mean ± Standard Error of the Mean (SEM). Means with different superscripts within a column are significantly different ($p < 0.05$). bp < 0.05 compared to TAA control group; ap < 0.05 compared to normal control group.

3.3. Effects on Liver Enzyme Levels

Table 3 illustrates the impact of ginger oil on the levels of key liver enzymes: Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Aspartate Aminotransferase (AST) across the different experimental groups.

Animal Groups	ALT (U/mL)	ALP (U/L)	AST (U/mL)
Negative Control (standard diet)	44.98c	80.99c	24.33c
Positive Control (TAA 200 mg/kg)	158.24a	886.67a	152.67a
Ginger 200 mg/kg + TAA 200 mg/kg	59.14b	99.68b	45.00b
Ginger 400 mg/kg + TAA 200 mg/kg	44.99c	77.98b	30.00bc

Note: Mean values within a column with different letters are significantly different at P>0.05. ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkalin Phosphatase.

To summarize, it is clear that the negative control group has shown, as expected, relatively lower amounts of enzyme activity due to normal functioning of the liver, whereas a much larger amount of liver damage occurred in the positive control group which had been treated with TAA (ALT = 158.24 U/mL; ALP = 886.67 U/L; AST = 152.67 U/mL). The positive effects of ginger appear to increase as the amount of ginger used is increased from 200 mg/kg to 400 mg/kg with the lower dosage of ginger (200 mg/kg) resulting in a reduction in enzyme activity (ALT 59.14 U/mL, ALP 99.68 U/L and AST 45.00 U/mL) compared to the values found in the positive control group that had been treated with TAA. Therefore, the decreased enzyme activities of ALT, AST and ALP reflect on the hepatoprotection provided by

ginger against hepatocellular leakage and the enhanced function for excreting bile fluids.

The higher dosage group of ginger (400 mg/kg) of the present experiment has provided a significant improvement in enzyme activity back toward that of the TAA positive control group (ALT 44.99 U/mL; ALP 77.98 U/L; AST 30.00 U/mL) indicating the hepatoprotective role that ginger provides the liver and is strongly supported by the presence of a positive correlation between the amount of ginger dosage and the reduction of ALT, AST and ALP enzyme levels supporting the positive hepatoprotective property associated with ginger.

4. Discussion

Administration of thioacetamide to the positive control group could induce a significant increase in the levels of ALT, AST, and ALP enzymes with respect to the negative control group. This is clear proof of liver damage as TAA is well known to produce its hepatic injury through the generation of oxidative stress, an inflammatory pathway, and cell death. Meanwhile, the groups that were given ginger together with TAA showed decreased serum activities, with a strong inverse relation with the dosages. At the highest dose of ginger (400 mg/kg), the liver enzyme levels almost normalized, highlighting the strong hepatic tissue healing power of the extract in cases of injury. The marked drop in ALT and AST points to liver cells that remain largely intact, with minimal leakage of enzymes into the blood.A simultaneous decrease of ALP would indicate that biliary flow was enhanced and the injury as a result of cholestasis was reduced. This protective effect is probably due to ginger's millions of active phytochemicals—gingerols, shogaols, and zingerone, among them—compounds that are noteworthy for their strong antioxidant effects, ability to suppress inflammation, and stabilize cell membranes. Through reduction of free radical injury, modulation of inflammatory cascades, and support of cellular membranes, these components attenuate the hepatotoxicity of stress. Similar results have been reported in previous research of chemically induced liver injury, and the response pattern across the doses — the strongest response with 400mg/kg — strongly suggests further studying to better define the optimal treatment range.

5. Conclusion

The present investigation unambiguously attests that Zingiber officinale (ginger) occupies a potent hepatoprotective and immunomodulatory position in a TAA-type liver insult system. Our main results prove that TAA-induced liver injury was reversed upon administration of ginger especially at doses 250 to 500 mg/kg. This was apparent by a marked diminution in the weight of the liver and restoration to almost-normal levels of liver index and enzymatic activities related to liver injury with ALT, AST and ALP, to denote intact hepatocytes and function. Secondly, the high dose of ginger (400 mg/kg) was found to exert quite therapeutic effects as those of Silymarin (100 mg/kg) in most of the parameters, suggesting that ginger can be an effective alternative or adjunct therapy in the management of liver disorders. The immunomodulatory effect of ginger, as shown by its antioxidant and anti-inflammatory properties, suggests a dual mechanism of action in protecting the liver. Future studies should focus on isolating the active compounds and elucidating the precise molecular pathways involved in the hepatoprotective and immunomodulatory effects of Zingiber officinale.

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مستخلص البحث:

يهدف هذا البحث إلى تقييم التأثيرات الوقائية والعلاجية لنبات الزنجبيل (*Zingiber officinale*) في حالات تلف الكبد الناتج عن مادة الثايوأسيتاميد(TAA) ، إضافةً إلى دوره في تنظيم الجهاز المناعي. جرى إعطاء مستخلص الزنجبيل بجرعتين (200 و500 ملغم/كغم) لمجموعات من الجرذان، ومقارنة تأثيره مع عقار السيليمارين المعروف بفعاليته الكبدية. أظهرت النتائج أن الزنجبيل يمتلك تأثيرًا واضحًا في حماية الكبد، حيث ساعد على تحسين وزن الجسم وتقليل وزن الكبد، كما أعاد مستويات إنزيمات الكبد (ALT, AST, ALP) إلى حدودها الطبيعية تقريبًا. وأثبتت الجرعة العالية (400 ملغم/كغم) فعالية مقاربة لدواء السيليمارين. تعزى هذه التأثيرات إلى الخصائص المضادة للأكسدة والالتهاب في مركبات الزنجبيل الفعّالة والتي تعمل على تثبيط الجذور الحرة وتنظيم الاستجابة المناعية. تشير هذه النتائج إلى إمكانية اعتماد الزنجبيل كخيار علاجي طبيعي واعد في حماية الكبد وتعزيز وظائف الجهاز المناعي، مما يجعله حلقة وصل بين الطب التقليدي والحديث.