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The Protective Role of Nigela Sativa Volatile Oil on Antioxidant and Oxidative Stress Enzymes

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Hepatotoxicity is the outcome of a paracetamol overdose.In this study, 40 adult male rats, weighing 180–260 gm and aged between 9 and 13 weeks, were given doses of Nigela sativa oil and their liver enzymes were examined to see how paracetamol affected them. The rats were kept in an environment at a temperature of 25 Co in an animal house. ,There were four groups of rats, and food was provided for them. (G). (G1) The only injections given to the control group are food and regular saline (0.9% of the time). (G2) 200 mg/kg of N. sativa and (G3) 400 mg/kg of paracetamol Addition of 400 mg/kg body weight of paracetamol. supplying 300 mg/kg B.W. of N. sativa to (G4)400 milligrams/kg B.W. of aspirin. Rat G2, GPT 87.80 IUL, and GOT hepatic enzyme concentration (129.32 IUL) all exhibit significant increases in comparison to the control. Furthermore, the levels of the liver GSH enzyme in rats (G2)1.24 IUL shown a substantial increase, whereas G3 (GOT) 91.9 IUL, (GPT)76.70 IUL, and group (G4) GOT 109.88 IUL and enzyme (GPT) 55.66 IUL exhibited significant declines as compared with control.While MDA enzyme in rats G2 (0.259 IUL) shows a major increase in comparison to the control, rats G3 (0.139 IUL) and G4 (0.112) show a noticeable decrease in comparison to G2. While body weight indicates that G2's outcomes are significantly lower than those of the other three groups. Groups G3 (2.55 gm) and G4 (2.7 gm) demonstrated a significant rise in liver weight compared to controls and G2, whereas groups G2 (24.23 gm) demonstrated a significant increase in liver weight compared to controls and group G2. Male rats in groups G2 (1.7 gm) demonstrated a significant drop in liver weight compared to controls. This the study the antioxidant and protective volatile oil extract from N. sativa. again overdose of paracetamol.

Keywords: Nigela sativa, Hepatotoxicity , Paracetamol, Volatile Oil

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INTRODUCTION

Herbalists use a variety of plants referred to as alternative or therapeutic herbs.These plants are regarded as an abundance of secondary metabolism in motion. substances that can be employed in the research and manufacturing of drugs (Kapoor dkk., 2013). Examples of active secondary metabolites substances include polysaccharide, flavone, terpenoid, and phenol (Kumar & Yadav, 2009).

N.Sativa holds a special place among Southeast Asian herbal items as a non-prescription treatment for a variety of ailments.There have been reports of its antibacterial, hypolipidemic, antidiabetic, and antihypertensive effects (Saha dkk., 2004). The phytochemical, pharmacological, and toxicological properties of N.Sativa. Recently, have been reviewed (Ali & Blunden, 2003). The plant is widely cultivated throughout the world.Nigella sativa, an oriental spice, has been used for a very long time as a natural remedy for the treatment of numerous acute and chronic illnesses (Usmanghani dkk., 1997). sativa have recently been the focus of a variety of pharmacological studies.These research revealed a broad range of actions, including antibacterial, anticancer, anti-inflammatory, mutabagani, hypoglycemia, smooth muscle relaxant, cytotoxic, and immunostimulant properties (Sezik dkk., 2001).

It was shown that N. sativa is highly bioavailable and offers noticeably better defense against free radical-induced DNA damage and lipid peroxidation (Mansour dkk., 2002), To protect cells from the destructive effects of reactive oxygen species (ROS), antioxidant enzymes defense the mechanisms that protect cells from the cellular a free radicals as well as restore and stop the growth of molecules damaged by oxidative stress. are crucial (Plaa & Hewitt, 1982). Typically, ROS results in the oxidation of proteins, lipids, and DNA (Burits & Bucar, 2000) .A product is the malondialdehyde enzyme (MDA). that is regarded to be a symptom of cell damage from peroxidation under the majority of oxidative stress circumstances. The free radicals and ROS combine to produce lipid peroxidation in membrane lipids (Mates dkk., 1999).

The human liver can efficiently and swiftly break down naphthalene into stable protein-reactive and cytotoxic metabolites, but if microsomal proteins do not act promptly to detoxify these metabolites, they can harm DNA, proteins, and lipids found in cell membranes and other tissues.Additionally, it has been shown that intracellular reduced glutathione may effectively detoxify naphtha (Tingle dkk., 1993).

The most popular OTC drug, paracetamol (acetaminophen) was only developed in the 1960s after being discovered 100 years earlier (Cranswick & Coghlan, 2000). Due to its accessibility and wide availability, self-poisoning with paracetamol is a common occurrence throughout the world (Guidet & Shah, 1989). In cases of symptomatic fever temperature (T> 38.5°C), a dose of 15 mg/kg every 6 hours (60 mg/kg/day) of oral or rectal paracetamol is advised (*New South Wales (NSW) Therapeutic Advisory Group Inc*, 2008). Although prolonged administration of supratherapeutic doses of paracetamol (>90 mg/kg/day) to a sick child under the age of two has been identified as a significant risk for hepatotoxicity, acute ingestion of a higher dose of 150 mg/kg/day of paracetamol has been found to be safe (Rumack & Matthew, 1975)the recommended dosage for analgesia is 15 mg/kg every 4-6 hours, up to a maximum of 60-90 mg/kg/day. 60% of cases of acute liver failure in the United States and the United Kingdom are caused by paracetamol overdoses, either on their own or in conjunction with other medicines, necessitating orthotropic liver transplants.

METHOD

A. Plant C

Plant material from the plant N. sativa that was purchased from Karbala, Iraq, on May 18, 2022, was a mechanically processed in to a powder and used straight away (Al-Ibrahemi dkk., 2020).

B. Extraction of Volatile Oil

Using 60 grams of N.sativaof 70% methanol and were combined in a thimble before being placed in a flask.A rotary evaporator operating at 45°C was then used to evaporate the extract(Al-Ibrahemi dkk., 2022).

C. Study of Secondary Metabolism Regulators:

1. Saponins

Foam formed after agitating the extract aqueous solution for an extended period of time(Al-Ibrahemi dkk., 2020).

2. Phenolics

Five milligrams of dill extract and 0.5 milliliter of a 1% lead acetate solution were used to create a precipitate, which was used to identify lead acetate.

3. Glycosides

After the extractor (0.5 mg) was dissolved in 1.0 ml of water, an aqueous solution of sodium hydroxide (NaOH) was added.

4. Tannins

After adding distilled water, A water bath equipment was used to boil 5 cc of extract for 10 minutes at a temperature of 80–100 °C. After the liquid was filtered, five drops of 1% ferric chloride were added to give it a dark green hue(Geissman, 1962)

5. The Alkaloids

A reddish-brown precipitate was produced by combining the recovered filtrate with Wagner reagent, which is a potassium iodide and iodine solution(Pandey dkk., 2011)

6. Flavonoids in

After combining, a few drops of concentrated HCl were added, and the liquid was heated with magnesium until a red hue emerged. 4 mL of extracts were mixed with 1.5 ml of

50% methanol. There are flavonoids when a color is red(Al-Bazaz dkk., 2020)

D. Experiment Design

The rats were kept in an environment at a temperature of 25 Co in an animal house. , There were four groups of rats, and food was provided for them. (G). (G1) The control group receives injections with only meal and saline solution (0.9%), 400 mg/kg of paracetamol (G2), and 200 mg/kg of N. sativa (G3) coupled with 400 mg/kg B.W. of paracetamol (G3) were used. giving (G4) 400 mg/kg B.W. of paracetamol and 300 mg/kg B.W. of N. sativa.

E. Biochemical Analyses

To separate the blood serum, blood was taken using the cardiac puncture procedure then centrifuged at 3000 rpm for 10 minutes. At 40 degrees, the blood serum was maintained until the enzyme assays were run, and the blood was drawn after 30 days.

1. The creation of glutamate pyruvate for GPT and glutamate oxaloacetate for GOT, which results in the transfer of an amino group from alanine or aspartate to oxoglutarate, is the technique for measuring GOT and GPT. The GPT and GOT were measured using a kit technique. (Reitman France colorimetric method, linear chemical, S.L., Spain) (Guidet & Shah, 1989)

2. Malondialdehyde (MDA) µmol/ L:

   Procedure :

   Following was a sample and a blank represented by two tubes.
   The solution was centrifuged at 450 x g for 15 minutes after standing at room temperature for 20 minutes. The absorbance of all sample was measured at 532 nm (Ellman, 1959)

3. Glutathione or GSH measure (µmol/ L):

   Solution for Tris Buffer

   The result of dissolving them was 6.57 grams.0.0292 grams and 900 milliliters of Tris hydroxyl methyl aminoethane water distillationAdd 0.1 ml of EDTA (Ethylenediaminetetraacetic Acid) to bring the volume up to 1 L. Refrigerate the mixture until you need to utilize the acidic distill water substrate (pH 7.6) (Ghosh & Sil, 2009)

RESULT AND DISCUSSION

A. Chemical Analysis of Phytochemistry:

The active phytochemical components in the N .sativaextract were found this results of the chemical compound screen analysis. Table shows sativa (1). were negative results for tannins but positive results for phenol, glycosides, alkaloids, saponins, and flavonoids (Ijaz dkk., 2017).

Table 1. Phytochemical Screening of N. Sativa Extract

Reagent	Sample	Blank
Sample	150µ	……
TCA (17.5%)	1ml	1ml
TBA (0.6%)	1ml	1ml
All tubes were combined using a vortex, then heated in an 80°C water to bath for 15 minutes before being allowed to cool to 25°C.		
TCA (70%)	1ml	1ml
D.W	……	150µl

B. Biochemical Test

Table 2. GOT and GPT enzyme percentages for the rat groups given the concentrations (U/L) of the research

Parameter/ Treatment Groups	GOT	GPT
G1 Control	50.21	87.12
G2	87.80	129.32^{a}
G3	67.70^{b}	91.9^{b}
G4	51.66^{b}	109.88^{b}
LSD	0.17	

(a) indicates a significant increas
(b) denotes a significant fall) p≤ 0.05

Table 3. GSH and MDA enzyme percentages for rat groups administered the study's concentrations levels (U/L)

Parameter/ Treatment Groups	GSH	MDA
G1 Control	0.176	2.29
G2	0.259^{a}	1.24^{b}
G3	0.139^{b}	2.12^{a}
G4	0.112^{b}	2.28^{a}
LSD		0.19

An important change is indicated with a small letter

(a) indicates a discernible increase
(b) denotes a significant fall) p≤ 0.05

Table 4. both the liver and overall body weight of Rat

Parameter/ Treatment Groups	Weight of Body (gm)	Liver Weight (gm)
G1 Control	2.88	29.76
G2	1.77^{b}	24.23^{b}
G3	2.55^{a}	26.95^{a}
G4	2.81^{a}	28.01^{a}
LSD		0.13

An important change is indicated with a small letter.

(A) indicates a significant rise
(B) denotes a significant drop. p≤ 0.05

C. Result

The results showed that there was a significant increase of levels of the liver enzymes in rats G2,GOT (129.32 IUL) and GPT (87.80 IUL) when compared with the control group . When compared to the control, G3 GOT (91.9 IUL), GPT (76.70 IUL), and G4 GOT (109.88 IUL) and GPT (55.66 IUL) all demonstrated a substantial decline. Paracetamol (400 mg/kg) showed hepatotoxicity after 24.

Table (3) indicates Rats in groups G2 (1.24 IUL) had a substantial drop in liver GSH enzyme levels compared to controls, while groups G3 (2.12 IUL) and G4 (2.28) have significant increases. MDA enzyme levels are significantly higher in rats in G2 (0.259 IUL) compared to controls, but significantly lower in G3 (0.139 IUL) and G4 (0.112 IUL) than G2 (Mates dkk., 1999).

According to table (4)'s results, G2 significantly decreased when compared to the other three groups. G2, G3, and G4 male rats' body weights (24.23 gm, 26.95 gm, and 28.01 gm, respectively) showed a substantial rise in comparison to control and G2. Male rats in groups G2 and G4 had a significant higher liver weights than control and G2, but male rats in groups G3 and G4 had significantly higher liver weights than control.

D. Discussion

The goal of the current study was to examine the antioxidant and protective effects of N. sativa extract on liver enzyme damage caused by paracetamol.

It is commonly recognized that some paracetamol is converted by the cytochrome P450 pathway into the dangerous metabolite N-acetyl-Pbenzoquinamine, which, if swallowed unintentionally at a height, can result in substantial liver cell death (NAPQI) (Schmidt & Dalhoff, 2002)

High dosages of paracetamol cause liver GSH depletion (because GSH combines with NAPQI to form mercapturic acid), which raises lipid peroxidation by absorbing hydrogen from polyunsaturated fatty acids and finally damages the liver (Itadt & Krauss, 2000). When the liver or heart are injured, The liver and cardiac cells ordinarily contain the enzyme serum glutamic pyruvic transaminase (sGPT) (EC 2.6.1.2), which is released into the bloodstream.Thus, blood sGPT levels rise in response to damage to the liver or heart. Some drugs, such as aspiri diclofen sodium, and paracetamol, can also increase sGPT levels(Kushwah dkk., 2013).

It is well known that hepatic parenchyma cells are adversely affected by toxins such as paracetamol to the extent that the total level of plasma protein lowers. The consumption of paracetamol significantly increased GPT and protean (Plaa & Hewitt, 1982). pointing out that paracetamol use lowers uric acid and total protein levels while raising the levels of the GPT, GOT enzymes and the glucose, all of which enhance the risk of paracetamol toxicity (Kadhim dkk., 2013)

The present study's objective was to determine whether Nigella sativa oil has a protective impact on liver functions against paracetamol-induced acute toxicity. An increase in serum levels of liver enzyme is a biochemical manifestation of paracetamol hepatotoxicity (Yarmohammadi dkk., 2012). The reduction of liver enzyme levels (ALT and AST) in mice treated with Nigella sativa may be linked to the slowing of body weight increase, indicating a potential therapeutic benefit of Nigella sativa administration (Pari & Sankaranarayanan, 2009).

The volatile oil of a Nigella sativa was examined for the antioxidant activity and found to contain high levels of thymoquinone carvacrol, t-anethole, and 4-terpineol.These findings demonstrated that TQ has positive impacts on hepatic enzyme activities, which may have an anti-hyperglycemic effect (Burtis dkk., 1999).

The first indication that the intestinal absorption of glucose is directly restricted by Nigella sativa (black seed) through electrogenic processes in culture.additional to the documented increase in body weight and glucose tolerance in rats after receiving continuous oral therapy in vivo.Many studies have shown that Nigella sativa and its active component, thymoquinone, have hepatoprotective qualities (Li dkk., 2009)

The neutralization of free radicals in lipids and the prevention of hydrogen peroxide's breakdown in to free radicals are the mechanisms by which phenolic compounds (flavonoids) exert their antioxidant activity(Durgo dkk., 2007). Due to their redox characteristics and nucleophilic thiol groups, flavonoids play a significant role in plants' overall antioxidant activities. They can detoxify compounds by donating protons or hydrogen atoms to reactive metabolites or free radicals, conjugating substances with the aid of glutathione-S-transferase (GST), or chemically reacting with reactive metabolites to create conjugates.Reactive intermediates may be able to prevent cell death by interacting with GSH either directly or through a process mediated by GST (Javanmardi dkk., 2003)

Status of Antioxidants Consuming black seeds has been shown to reduce oxidative stress and enhance the activity of antioxidant enzymes in all studied tissues.Black seeds' multifunctional properties as an anticancer, antioxidant, anti-inflammatory, and antibacterial "drug" come from their oral usage (Harborne, 1984).

CONCLUSION

According to the current research, paracetamol's hebatotoxic effects may be reduced by dill and flavonoid extract's antihebatotoxic properties.

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