The protective role of Nigela sativa volatile oil on antioxidant and oxidative stress enzymes

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Abstract :

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 C^{o} in an animal house. ,There were four groups of rats, and food was provided for them. (G). (G1) The control group only receives injections of food and normal saline (0.9% of the time). (G2) (400 mg/kg)paracetamol and (G3) 200 mg//kg of N.sativa 400 mg//kg Body Weight . of paracetamol added. Giving (G4) 300 mg/kg B.W of N.sativa400 mg/kg B.W. of paracetamol. Rat G2, GOT hepatic enzyme concentration (129.32 IUL), and GPT 87.80 IUL show a considerable rise when compared to the control. Additionally, G3 (GOT) 91.9 IUL, (GPT)76.70 IUL, and group (G4) GOT 109.88 IUL and enzyme (GPT) 5[illegible].66 IUL showed significant decreases as compared with control, while the levesl of the liver GSH enzyme in rats (G2)1.24 IUL showed a significant.

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

While male rats in groups G2 (1.7 gm) showed a significant decline in liver weight compared to controls, G3 (2.55 gm) and G4 (2.7 gm) showed a significant increase compared to controls and G2, while male rats in groups G2 (24.23 gm) showed a significant increase compared to controls and group G2. The aim this the study the antioxidant and protective volatile oil extract from N. sativa. again overdose of paracetamol.

Key words: Nigela sativa, Hepatotoxicity , paracetamol, volatile oil

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 [1]. Examples of active secondary metabolites substances include polysaccharide, flavone, terpenoid, and phenol [2].

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 [3]. The phytochemical, pharmacological, and toxicological properties of N.SativaRecently, have been reviewed [4].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 [5].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 [6].

It was shown that N. sativa is highly bioavailable and offers noticeably better defense against free radical-induced DNA damage and lipid peroxidation [7].To protect cells from the destructive effects of reactive oxygen species (ROS), antioxidant enzymes defense mechanisms that protect cells from the cellular free radicals as well as restore and stop the growth of molecules damaged by oxidative stress. are crucial [8]. Typically,

ROS results in the oxidation of proteins, lipids, and DNA[9]. A product is the malondialdehyde enzyme (MDA). that is regarded to be a sign peroxidation damage to cells under the majority of oxidative stress circumstances. free radicals and ROS combine to produce lipid peroxidation in membrane lipids[10].
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[11].

The most popular OTC drug, paracetamol (acetaminophen) was only developed in the 1960s after being discovered 100 years earlier [12]. Due to its accessibility and wide availability, self-poisoning with paracetamol is a common occurrence throughout the world [13]. 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 [14]. The recommended dosage for analgesia is 15 mg/kg every 4-6 hours, up to a maximum of 60-90 mg/kg/day, even though 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, while acute ingestion of a higher dose of 150 mg/kg/day of paracetamol has been found to be safe [15]. In the United States of America and the United Kingdom, paracetamol overdoses, either alone or in combination with other medications, cause 60% of acute liver failure and result in orthotropic liver transplants[16].

Material and method

1:-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 [17, 18].

2:-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[19,20, 21].

3:-Secondary metabolism regeants study :

3:1 Saponins

When the extract aqueous solution was swirled for a long time, foam developed[22].

3:2 Phenols

To detect lead acetate, five milligrams of dill extract were mixed with 0.5 milliliter of a 1% lead acetate solution, resulting in a precipitate.

3:3 Glycosides

Following the dissolution of (0.5 mg) of extractor in (1.0 ml) of water, an aqueous Sodium Hydroxide (NaOH) solution was added.

3:4 Tannins

After adding distilled water, 5 ml of extract was cooked in a water bath apparatus for 10 minutes at a temperature between 80 and 105 °C. Five drops of 1% ferric chloride were added to the liquid after it had been filtered to give it a dark green color[23].

3:5 Alkaloids:

The recovered filtrate was mixed with Wagner reagent, a potassium iodide and iodine solution, to create a reddish-brown precipitate [24].

3:6 Flavonoids:

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[25].

4- Experiment Design:

The rats were kept in an environment at a temperature of 25 C° 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.

5: Biochemical tests

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

5-1: The transfer of the amino group from alanine or aspartate to oxoglutarate, with the synthesis of glutamate pyruvate for GPT and glutamate oxaloacetate for GOT, 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) [26]

5-2 : Malondialdehyde (MDA) μmol/ L:

Procedure :

Following was a sample and a blank represented by two tubes.

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 minutes before being allowed to cool to 25°C.		
TCA (70%)	1ml	1ml
D.W	……	150μl

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 [27].

5-3 : Glutathione (GSH) measure (μmol/ L):

Tris Buffer Solution

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) [28].

Results and Discussion

1: Phytochemical chemical study

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

Table (1) : phytochemical screening of N. sativa extract

ChemicalCompounds	Extract
Phenol	+
Tannins	-
Glycoside	+
Alkaloid	+
Saponin	+
Flavonoid	+

Biochemical tests3

Table (2) GOT and GPT enzymes percentage for rat groups administered the study's concentrations (U/L)

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

An important change is indicated with a small letter
(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	2.29	0.176
G2	1.24[b]	0.259[a]
G3	2.12[a]	0.139[b]
G4	2.28[a]	0.112[b]
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 Rat

Parameter / Treatment	weight of bodyn (gm)	liver weight (gm)
Control	29.76	2.88
G2	24.23[b]	1.77[b]
G3	26.95[a]	2.55[a]
G4	28.01[a]	2.81[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

Result :
The results showed that there was a significant increase of levels of the liver enzymes in rats G2,GOT (129.3 IUL) and GPT (87.80 IUL) when compared with the control group . G3 GOT (91.9 IUL) and GPT (76.70 IUL) and G4 GOT (109.88 IUL) and GPT (55.66 IUL) showte significant decrease as compared with control. 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.
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.

Discussion
The present research was designed to antioxidant and protective role ofN. sativa extract n liver enzyme again paracetamol induced toxicity.
It is well known that the cytochrome P450 pathway transforms certain paracetamol into the hazardous metabolite N-acetyl-Pbenzoquinamine, which, if swallowed unintentionally at a height, can result in substantial liver cell death (NAPQI) [29].
High dosages of paracetamol cause liver GSH depletion (as a result of GSH conjugating with NAPQI to generate mercapturic acid), which in turn causes lipid peroxidation to rise by absorbing hydrogen from of the polyunsaturated fatty acid and, eventually, causes liver damage[30]. When the liver or heart are injured, the enzyme serum glutamic pyruvic transaminase (sGPT) (EC 2.6.1.2), which is normally found in the liver and the heart cells, is released into the bloodstream.Thus, when the liver or the heart are harmed, the blood sGPT levels are raised.Some drugs, such as aspiri diclofen sodium, and paracetamol, can also increase sGPT levels[31].
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 [32,33]. pointing out that paracetamol use lowers uric acid and total protein levels while raising levels of the GPT, GOT enzyme, and glucose, all of which enhance the risk of paracetamol toxicity [34].
The present study's objective was to determine whether Nigella sativa oil has a protective effect on the liver functions against paracetamol-induced acute toxicity. An increase in serum levels of liver enzyme is a biochemical manifestation of paracetamol hepatotoxicity[35]. The slowing of body weight gain in Nigella sativa-treated mice may be connected to the liver enzyme (ALT and AST) level reduction, suggesting a possible therapeutic effect of Nigella sativa administration [36].
The volatile oil of Nigella sativa was examined for 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 [37].

The first proof that Nigella sativa (black seed) directly limits the intestinal absorption of glucose through electrogenic processes in culture.further to the reported rise in glucose tolerance and body weight in rats following prolonged oral treatment in vivo.The hepatoprotective properties of Nigella sativa and its active ingredient, thymoquinone, have been documented in numerous research [38].

The neutralization of lipid free radicals and the prevention of hydrogen peroxide's breakdown into free radicals are the mechanisms by which phenolic compounds (flavonoids) exert their antioxidant activity[39]. Due to their redox characteristics and nucleophilic thiol groups, flavonoids play a significant role in plants' overall antioxidant activities. They can detoxify substances through conjugation with the help of glutathione-S-transferase (GST), chemical reactions with reactive metabolites to form conjugates, or by giving protons or hydrogen atoms to reactive metabolites or free radicals.Reactive intermediates can interact with GSH directly chemically or through a GST-mediated process, potentially avoiding cell death[40].

Antioxidant Status The effects of black seed consumption support the activity of antioxidant enzymes in all examined tissues and lessen oxidative stress.The oral use of black seeds as a multipurpose "drug" is what gives it its anticancer, antioxidant, anti-inflammatory, and antibacterial effects[41].

Conclusions

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

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