



Research Article

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF NIGELLA SATIVA ALCOHOLIC EXTRACT IN A CCL₄ INDUCED RAT

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ABSTRACT

Background: This study investigated the antioxidant, hepatoprotective, and sedative modulatory effects of *Nigella sativa* alcoholic extract (NSAE) in CCl₄-induced hepatotoxicity in rats. **Methods:** Male Wistar rats were divided into six groups (n=6): normal control, CCl₄ control, silymarin (50 mg/kg), and NSAE (100, 200, and 400 mg/kg). Hepatoprotective effects were evaluated through biochemical parameters, oxidative stress markers, and histopathological examination. **Results:** NSAE treatment (400 mg/kg) significantly restored liver function markers, including SGOT (20.95 ± 0.52 IU/L, $p = 0.033$) and SGPT (28.61 ± 0.67 IU/L, $p < 0.001$), compared to CCl₄ control. Total protein and albumin levels were normalized to 5.68 ± 0.54 mg/dL and 3.84 ± 0.48 mg/dL, respectively. Antioxidant parameters showed marked improvement with NSAE (400 mg/kg), increasing GSH (0.26 ± 0.029 μ mol/mg) and CAT (30.19 ± 2.69 μ g/mL) while reducing MDA (0.048 ± 0.008 μ g/mL). Histopathological examination revealed significant protection against CCl₄-induced hepatic and gastric tissue damage, particularly at the 400 mg/kg. **Conclusion:** NSAE exhibited marked hepatoprotective activity comparable to silymarin, predominantly through antioxidant mechanisms and the maintenance of hepatic tissue integrity, indicating its potential as a natural therapeutic agent for managing liver diseases. Because of its hepatoprotective and antioxidant properties, NSAE may be explored in clinical settings as a natural supplement to traditional liver disease therapies or as a prophylactic for people at risk of liver disorders.

INTRODUCTION

Many millions across the world suffer from liver diseases, which create a significant healthcare issue and substantially increase mortality statistics. Statistics reveal that liver disorders are on the rise globally because approximately 2 billion people experience different liver conditions worldwide. Acute hepatitis represents a subset of liver diseases, encompassing both chronic conditions, such as cirrhosis and hepatocellular carcinoma [1].

Hepatic dysfunction develops primarily from environmental toxins mixed with viral infections, together with alcohol consumption and drug-induced liver injury (DILI) [2]. Carbon tetrachloride (CCl₄) has long been one of the most widely used hepatotoxins in hepatotoxicity research. It has contributed enormously to understanding the pathogenesis of liver injury and promising medical treatments. When rat hepatic cytochrome

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P450 2E1 metabolically activates CCl_4 , it liberates trichloromethyl radicals ($\text{CCl}_3^\bullet$) and the subsequent peroxy radicals ($\text{CCl}_3\text{OO}^\bullet$) to cause cell injury [3].

These free radicals cause lipid peroxidation, impair the cell membrane, and elicit oxidative stress, followed by hepatocellular necrosis and inflammation. The CCl_4 -induced liver injury model has been found to emulate human liver pathology concerning oxidative stress, inflammation, and fibrosis. This model has proven helpful in identifying potential hepatoprotective agents, providing consistent experiments, and stereotyping lesions corresponding to human liver disease development [4].

Nigella sativa L., commonly known as black seed, has been widely reported recently as a possible wonder medicinal plant with ethnomedical uses. Apart from TQ at a concentration of 0.1- 20% w/w, other active compounds in the oil include thymohydroquinone, dithymoquinone, and p-cymene. The active constituent that has been reported to have various pharmacological activities includes thymoquinone, where reports have shown that the substance has antioxidant, anti-inflammatory, and hepatoprotective properties [5]. The seed fixed and essential oils contain the unsaturated fatty acids, the linoleic and oleic acids, which are richest in this seed, and several phenolic constituents that give it its therapeutic value. In a pharmacological aspect, recent investigations have shown that *N. sativa* impacts inflammatory agents and antioxidant systems and controls cell death, making it suitable for hepatoprotective disorders [6].

This study intends to examine the abilities of *N. sativa* alcoholic extract to exhibit hepatoprotective and sedative modulatory effects in rat livers injured by CCl_4 . We mainly focus on the extract's impact on some hepatic function markers, indices of oxidative stress, and histological alterations. This research may provide a comprehensive understanding of how antioxidant and hepatoprotective mechanisms synergistically contribute to liver protection.

MATERIALS AND METHODS

MATERIALS

All chemicals and reagents used in this study were of analytical grade. Silymarin ($\geq 98\%$ purity) was procured from Yarrow Chem, India. Carbon tetrachloride (CCl_4 , 99.9% purity), used as

a hepatotoxicity inducer, was purchased from Sciquaint Innovations Private Limited, Pune, India. Additional reagents including hydrochloric acid (HCl, 37%), phosphoric acid (H_3PO_4 , 85%), N-butanol, nitro blue tetrazolium (NBT, $\geq 98\%$), N-N-dimethyl acetamide, hydroxylamine (99%), glacial acetic acid (CH_3COOH , $\geq 99.7\%$), sodium carbonate, Triton-X-100, ethylenediamine tetra-acetic acid (EDTA, $\geq 99\%$), and 10% neutral buffered formalin were obtained from Sigma Aldrich, USA. All solutions were prepared using double-distilled water, and reagents were stored according to the manufacturer's specifications to maintain their integrity throughout the experimental period.

METHODS

Collection and Preparation of Plant Materials

Nigella sativa L. mature seeds (black seed) were collected from the authorized local vendors of herbal stores in Ahmednagar and Pune district of Maharashtra, India. Dr. A. S. Wabale did the identification of seeds from the Department of Botany & Research Centre, PVP College Loni, Ahmednagar, Maharashtra, India, and the specimen was kept in the institutional herbarium with reference number PVPC/Bot./2023-24/231. The seeds were sorted to eliminate chaff and any other contaminants foreseen, such as dust, after which a check was made to separate any loose seeds that may be bad or damaged in any manner whatsoever. The seeds were then washed under running water, and gala samples were selectively removed from the bigger seeds to get pure samples to be further analyzed. After this, the seeds were left to dry for 7 days in the open air at a room temperature of $25 \pm 2^\circ\text{C}$. The dried seeds were milled using a vertical electrical grinder and collected using a 40 mesh sieve to have a uniform size of the powder sample [7].

Preparation of Plant Extract

A standardized Soxhlet extraction method was used to prepare the alcoholic extract of *Nigella sativa*. One hundred grams of finely powdered seeds of the plant materials with a mesh size 40 were placed into a Whatman cellulose thimble (grade 603, size 33×94 mm). At the same time, 500 mL of analytical reagent ethanol (95% v/v, Merck, India) was used to extract the plant materials at $60 \pm 2^\circ\text{C}$. The extraction was done for 8 hours with approximately 10-12 cycles per hour with the solvent to material ratio of 5:1. The obtained extract was then filtered through Whatman No. 1 filter paper and then concentrated using rotavapor (BUCHI R-300, Switzerland) at a temperature of $45 \pm$

2°C. The process was continued until a constant mass was obtained. The final conjugate was dried to get a dark brown viscous mass in 18.6% w/w yield, which was stored in an amber coloured airtight glass container at $4 \pm 1^\circ\text{C}$ before further use. The moisture content of the extracted was kept below 5% throughout the storage period by Karl Fischer titration analysis [8–10].

CCl₄-Induced Hepatotoxicity Study

Healthy male Wistar albino rats (180–220 g, 8–10 weeks old) were randomly divided into six groups (n=6/group) following ARRIVE guidelines and a 7-day acclimatization period (temperature: $22 \pm 2^\circ\text{C}$, humidity: $55 \pm 5\%$, 12-hour light/dark cycle). The experimental design comprised: Group I (Normal Control, NC) receiving normal saline (10 mL/kg, p.o.); Group II (Disease Control, DC) administered CCl₄ (1 mL/kg, i.p., in olive oil 1:1 v/v); Group III (Silymarin Control, SC) treated with silymarin (50 mg/kg, p.o.); and Groups IV–VI receiving *Nigella sativa* alcoholic extract (NSAE) at doses of 100, 200, and 400 mg/kg, p.o., respectively. All treatments were administered daily for seven consecutive days, with CCl₄ being given on days 2, 4, and 6 to induce hepatotoxicity, except in the NC group. Animals were maintained on a standard laboratory diet (Nutrivet Life Sciences, India) and water ad libitum throughout the experimental period. The Institutional Animal Ethics Committee (IAEC) approved the study protocol following CPCSEA guidelines (Approval No. 1942/PO/Re/S/17/CPCSEA/2023/01/03/01) [11].

Blood Sample Collection

After 7 days of treatment, rats were deprived of food for 12 hours, and blood samples were obtained from the jugular vein under mild Diethyl ether anaesthesia (analytical grade Merck Germany). Retro-orbital blood plexus puncture using a sterile disposable glass capillary tube (Sigma-Aldrich Inc., 25 G, 1 inch) was done as per the animal care recommended by ARRIVE guidelines. In all the animals, about 1 mL of blood was drawn aseptically in sterile microcentrifuge tubes (Tarsons, India) with clot activator.

Blood samples collected in the tubes were allowed to clot at room temperature, $25 \pm 2^\circ\text{C}$, for 30 minutes and then centrifuged by a centrifuge model Remi R-8C from India at 3000 rpm for 10 minutes at 4°C . Subsequently, the separated serum was aseptically distributed into pre-labelled Eppendorf tubes before storage at $40 \pm 2^\circ\text{C}$ for biochemical analyses [12,13].

Biochemical Analysis of Hepatic Markers

Blood samples from the experimental animals were collected, and biochemical parameters were assayed using routine diagnostic kits (Erba Mannheim, Germany) on a fully automated biochemical analyser (Erba EM-200, Germany). Serum liver enzymes, aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT), were estimated by using the IFCC method without pyridoxal phosphate activation coefficient variation less than 1.5%. Total Protein assay was determined at 540 nm by the biuret method, whereas Serum albumin was assayed at 630 nm with the help of the Bromocresol green dye-binding method. Total cholesterol was measured using an automatic analyzer by the CHOD-PAP enzymatic method, at a wavelength of 500 nm. Norm was checked using Erba NORM and PATH controls, and the assay was done in duplicates at $37 \pm 0.1^\circ\text{C}$ [14].

Analysis of Oxidative Stress Markers

Liver tissue samples were precisely excised, rinsed with ice-cold isotonic saline (0.9% NaCl), and homogenized (1:10 w/v) in chilled 50 mM Tris-HCl buffer (pH 7.4 ± 0.1) using an ULTRA-TURRAX® T10 homogenizer (IKA, Germany) at 4°C . The homogenate was centrifuged (Eppendorf 5418R) at $10,000 \times g$ for 20 minutes at 4°C , and the supernatant was collected for analysis [15]. Oxidative stress markers were evaluated using standardized methods: SOD activity ($\lambda_{\text{max}} = 560 \text{ nm}$) via nitroblue tetrazolium reduction, CAT activity ($\lambda_{\text{max}} = 240 \text{ nm}$) through H₂O₂ decomposition kinetics, GSH content using Ellman's reagent ($\lambda_{\text{max}} = 412 \text{ nm}$), GPx activity via NADPH oxidation ($\lambda_{\text{max}} = 340 \text{ nm}$), and lipid peroxidation by measuring TBARS ($\lambda_{\text{max}} = 532 \text{ nm}$) using a validated UV-visible spectrophotometer (Shimadzu UV-1800, Japan). All measurements were triplicated with analytical grade reagents (Sigma-Aldrich, USA) [15].

Histopathological Studies

Liver and heart tissues removal was done from euthanized animals and immediately fixed using 10% neutral buffered formalin (pH 7.4 ± 0.2) at room temperature ($25 \pm 2^\circ\text{C}$) for 48 hours [16,17]. All the fixed tissues were processed through an automated tissue processor (LEIC AUTOTOME, TP1020, Germany) and stained through a graded alcohol series (70–100%), xylene, and paraffin (LEIC Tissue-embedding system, EG1150H). Tissue sections of $4 \pm 1 \mu\text{m}$ were made using a rotary microtome; the sections were dried on poly-L-lysine-coated

microscope slides and stained using Harris's haematoxylin and eosin (H&E) according to recommended procedures. Histological evaluation was done at VEDH Laboratory, Pune, and with the help of a digital microscope imaging system (Nikon Eclipse Ci-L, Japan) [18,19].

Microscopic Examination

Histopathological evaluation was performed by a certified veterinary pathologist using a semi-quantitative scoring system on a 4-point scale: 0 (No Abnormality Detected), 1 (Minimal: $\leq 10\%$ involvement), 2 (Mild: 11-20%), 3 (Moderate: 21-40%), and 4 (Severe: $>40\%$). Tissue changes were characterized based on distribution patterns (focal, multifocal, or diffuse), topography, and temporal features. All assessments were blinded, with representative photomicrographs captured at $40\times$ magnification using standardized imaging parameters (Nikon DS-Fi3 camera) [20].

Phenobarbitone induced sleeping time

A total of six adult Wistar rats were randomly assigned to six different groups, with six rats in each group. Animals in the control group (Group I) were administered the vehicle. Oral administration of isotonic saline solution. In group II (DC), rats were administered silymarin orally at 50 mg/kg, while groups III-VI were given *Nigella sativa* extract intraperitoneally at 100, 200, and 400 mg/kg.

The treatments were conducted 30 minutes before administering phenobarbitone sodium (Renaudin, France, 35 mg/kg, i.p.) to all groups. Each rat was monitored to determine the time it took for sleep to begin (latency) and the length of sleep. The criterion for sleep onset was the loss of the righting reflex. In contrast, the duration of sleep or hypnosis was measured by the time the animal lost postural reflexes [45].

Statistical Analysis

All experimental data were expressed as mean $\pm$ standard error of mean (SEM) with $n=6$ animals per group. Statistical analyses were performed using GraphPad Prism software (version 8.0, GraphPad Software Inc., USA). The normality of data distribution was verified using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test. One-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was employed for multiple comparisons between treatment groups and control. The results were considered

statistically significant at three levels: $p < 0.05$ (significant), $p < 0.01$ (highly significant), and $p < 0.001$ (very highly significant). A blinded investigator conducted all statistical analyses, and the data visualization was performed using GraphPad Prism with standardized formatting parameters [21,22].

RESULTS AND DISCUSSION

Effect of NSAE on biochemical parameters

The changes in biochemical parameters of the hepatotoxic rats treated with the alcoholic extract of *Nigella sativa* are presented in Table 1, along with the graphical representation in Figure 1. When compared between the disease control (DC) group and the normal control (NC) group, the disease control group had significantly lower total protein levels, which were 3.45 ± 1.51 mg/dL. Total protein level increased significantly after treatment with NSAE in a dose dependent manner with the highest dose of 400 mg/kg as both 5.68 ± 0.54 mg/dL < 400 mg/kg showing an improvement that was significantly different from the baseline level with $p < 0.001$ and comparable to that observed in silymarin treated group 5.55 ± 0.79 mg/dL. The serum albumin level was significantly lower in the case of the DC group (1.86 ± 0.53 mg/dL) compared to the NC (3.87 ± 0.79 mg/dL). Administration of NSAE at the dose of 400 mg/kg also ameliorated serum albumin concentration (3.84 ± 0.48 mg/dL, $P \leq 0.002$), and this result supported the conclusion about the efficacy of the NSAE treatment, as was the case with silymarin (3.86 ± 0.96 mg/dL).

By the results obtained, it was observed that the cholesterol levels in the NC group (74.91 ± 13.89 mg/dL) were comparatively higher than the cholesterol levels in the healthy control group (40.8 ± 8.02 mg/dL); however, the NSAE treatment significantly reduced the concentration in a dose-dependent manner; especially with the 400 mg/kg dose was recorded to have a cholesterol level of 43.57 ± 2.77 mg/dL ($p < 0.00$).

Liver function indices SGOT and SGPT are up in the Duration of Car Emission group to a higher level (37.39 ± 25.18 IU/L and 34.99 ± 24.88 IU/L, respectively) than in the Normal Car emission (15.15 ± 3.89 IU/L and 25.35 ± 9.73 IU/L, respectively). Federer and colleagues further proved that NSAE treatment at 400 mg/kg appeared to have hepatoprotective effect due to the decrease in both the SGOT level of NSAE treated group (20.95 ± 0.52 IU/L, $p = 0.033$) and the SGPT level (28.61 ± 0.67 IU/L $p < 0.001$) as compared with CCl_4 - induced group and the values were comparable with the Silymarin group (Figure 1D, 1E).

Table 1: Effects of alcoholic extract of *Nigella sativa* (NSAE) on serum biochemical parameters in CCl₄-induced hepatotoxicity in rats

Groups	Treatment	Liver function markers				
		Total protein ^a	Albumin ^a	Cholesterol ^a	SGOT ^b	SGPT ^b
I	Normal Control (NC)	5.53±0.44	3.87±0.79	40.8±8.02	15.15±3.89	25.35±9.73
II	Disease Control (DC)	3.45±1.51	1.86±0.53	74.91±13.89	37.39±25.18	34.99±24.88
III	Silymarin Control (SC)	5.55±0.79***	3.86±0.96**	42.81±3.01***	18.37±2.28*	26.42±2.92***
IV	NS- 100mg/kg	4.79±0.43*	3.04±0.85 ^{ns}	50.03±5.08***	28.49±3.25 ^{ns}	31.39±0.51***
V	NS- 200mg/kg	5.14±0.6***	3.39±1.28*	45.39±2.45***	21.59±0.45 ^{ns}	30.78±0.26***
VI	NS- 400mg/kg	5.68±0.54***	3.84±0.48**	43.57±2.77***	20.95±0.52*	28.61±0.67***

Data are expressed in mean±SD, (n=6)

^a Markers in mg/dL, ^b Markers in IU/U, ^{ns} Values are Not significant (p = 0.12), * Significant (p = .033), ** Very significant (p=0.002), *** Highly significant (p < 0.001) compared to compared to DC group; ns: not significant. SGOT: Serum Glutamic-Oxaloacetic Transaminase; SGPT: Serum Glutamic-Pyruvic Transaminase.

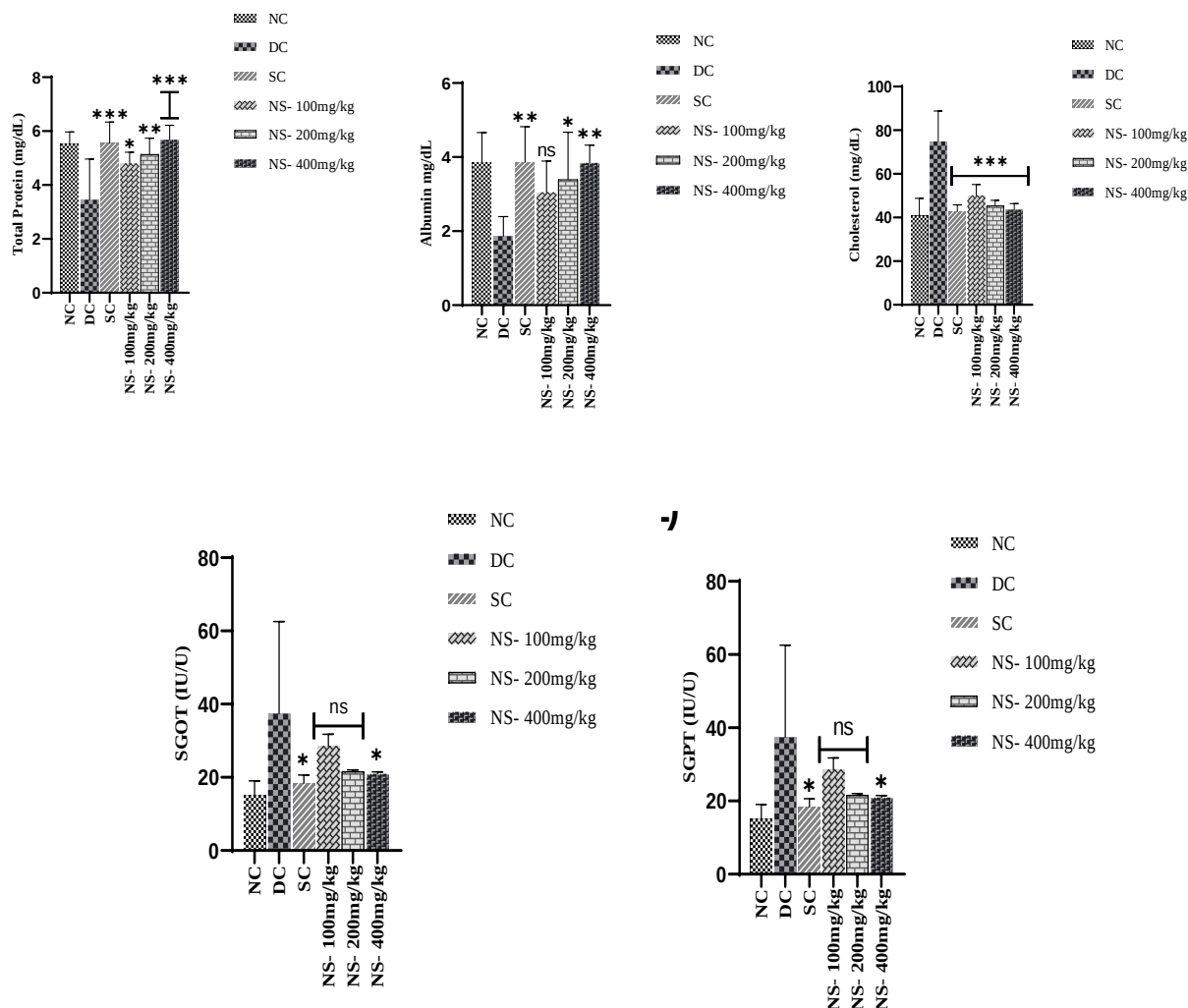


Figure 1: Effects of alcoholic extract of *Nigella sativa* (NSAE) on hepatic biochemical markers in CCl₄-induced hepatotoxicity in rats. The graphs demonstrate: (A) Total protein levels, (B) Albumin levels, (C) Cholesterol levels, (D) SGOT levels, and (E) SGPT levels across different treatment groups. NC: Normal Control, DC: Disease Control, SC: Silymarin Control (50 mg/kg), NS: *Nigella sativa* extract at doses of 100, 200, and 400 mg/kg. Values are expressed as mean ± SEM (n=6). Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001 compared to DC group; ns: not significant. NSAE treatment significantly improved all biochemical parameters compared to the DC group, showing dose-dependent hepatoprotective effects comparable to silymarin.

Effect of *Nigella sativa* (NSAE) on oxidative stress parameter

The role of NSAE on hepatic antioxidant was carried out in the hepatotoxic rats induced by CCl₄ (Table 2, Figure 2). There was a decrease in GSH content in Group 2, the disease control group ($0.17 \pm 0.014 \mu\text{mol/mg}$), as compared with the GSH level in the Normal control ($0.29 \pm 0.01 \mu\text{mol/mg}$) that received the same treatment with CCl₄. NSAE at ever-increasing doses similarly raised the GSH levels to silymarin, and the optimum dose was recorded to be 400 mg/kg ($0.26 \pm 0.029 \mu\text{mol/mg}$) ($p = 0.002$). The results revealed that administration of CCl₄ caused a significant decrease in the activity of the catalase enzyme in the disease control group ($11.53 \pm 3.88 \mu\text{g/mL}$) as compared to the normal control ($43.25 \pm 20.55 \mu\text{g/mL}$).

Cats treated with NSAE at 200 and 400 mg/kg had normal CAT activities: $26.62 \pm 2.9 \mu\text{g/mL}$ ($p = 0.033$) and $30.19 \pm 2.69 \mu\text{g/mL}$ ($p = 0.002$), respectively. These are equally as effective as silymarin, which has a CAT activity of $34.74 \pm 8.61 \mu\text{g/mL}$. The increase of lipid peroxidation in the disease control group was $0.075 \pm 0.019 \mu\text{g/mL}$, whereas it was found to be $0.065 \pm 0.0097 \mu\text{g/mL}$ in the normal control. Chronic NSAE poisoning treatment significantly decreased the level of MDA in a dose-dependent manner; the optimal dose was 400 mg/kg ($0.048 \pm 0.008 \mu\text{g/mL}$, $p < 0.001$), which pointed to the drug's efficacy comparable to silymarin ($0.045 \pm 0.005 \mu\text{g/mL}$). These findings indicate that NSAE has strong antioxidant capacity through hepatic oxidative stress indices alterations.

Table 2: Effects of alcoholic extract of *Nigella sativa* (NSAE) on hepatic antioxidant parameters in CCl₄-induced hepatotoxicity in rats.

Parameter	NC	DG (CCl ₄)	SC	NS- 100mg/kg	NS- 200mg/kg	NS- 400mg/kg
GSH ^a	0.29±0.01	0.17±0.014	0.27±0.028**	0.22±0.073 ^{ns}	0.24±0.06*	0.26±0.029**
CAT ^b	43.25±20.55	11.53±3.88	34.74±8.61**	17.52±4.31 ^{ns}	26.62±2.9*	30.19±2.69**
MDA ^b	0.065±0.0097	0.075±0.019	0.045±0.005***	0.062±0.01 ^{ns}	0.050±0.005**	0.048±0.008***

Data are expressed in mean $\pm$ SD, (n=6)

^a Markers in $\mu\text{mol/mg}$, ^b Markers in $\mu\text{g/mL}$, ^{ns} Values are Not significant ($p = 0.12$), * Significant ($p = .033$), ** Very significant ($p=0.002$), *** Highly significant ($p < 0.001$) compared to DC group; ns: not significant. GSH: Reduced Glutathione; CAT: Catalase; MDA: Malondialdehyde.

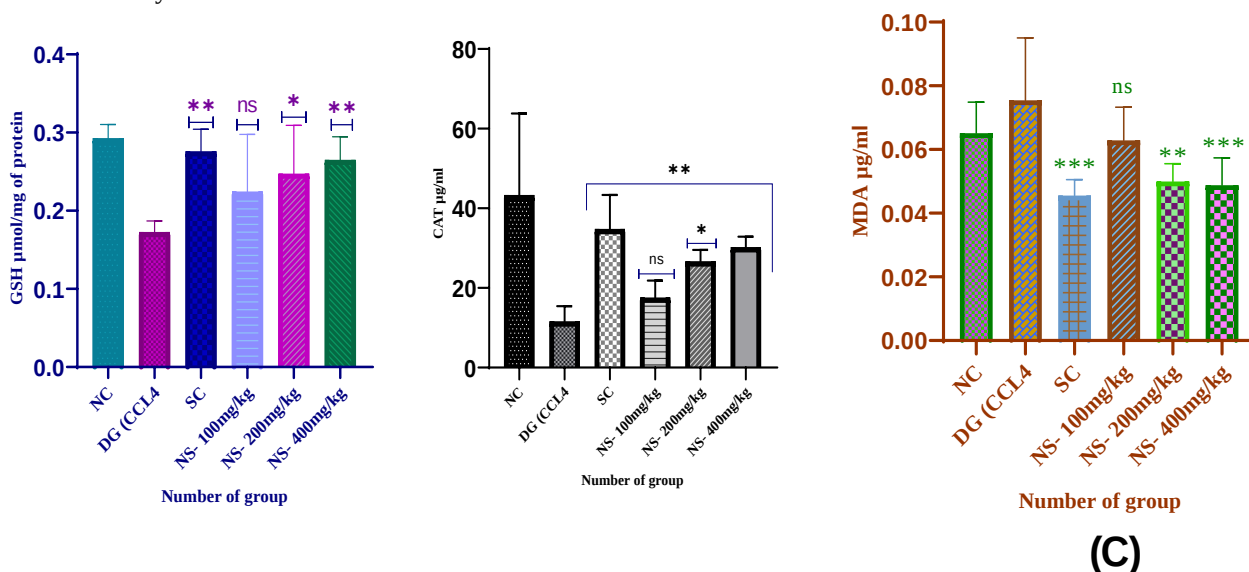


Figure 2: Effects of alcoholic extract of *Nigella sativa* (NSAE) on hepatic oxidative stress markers in CCl₄-induced hepatotoxicity in rats. The graphs demonstrate: (A) Glutathione (GSH) levels, (B) Catalase (CAT) activity, and (C) Malondialdehyde (MDA) levels across different treatment groups. NC: Normal Control, DC: Disease Control, SC: Silymarin Control (50 mg/kg), NS: *Nigella sativa* extract at doses of 100, 200, and 400 mg/kg. Values are expressed as mean $\pm$ SEM (n=6). Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to DC group; ns: not significant. NSAE treatment significantly modulated oxidative stress parameters in a dose-dependent manner, with the 400 mg/kg dose showing maximal antioxidant effects.

Effect of *Nigella sativa* (NSAE) on Organ Weight

The effects of NSAE on organ weights were evaluated in CCl₄-induced hepatotoxic rats (Figure 3). CCl₄ administration slightly

reduced liver weight in the disease control group ($9.27 \pm 2.61 \text{ g}$) compared to normal control ($9.83 \pm 2.57 \text{ g}$). NSAE treatment showed a dose-dependent trend in liver weight modulation, with

the 400 mg/kg dose demonstrating the most notable reduction (8.13 ± 2.68 g), approaching the effect observed with silymarin treatment (7.73 ± 0.19 g).

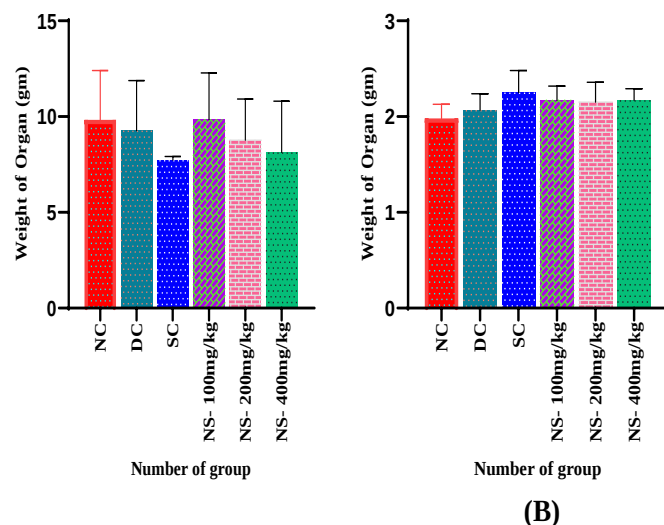


Figure 3: Effects of alcoholic extract of *Nigella sativa* (NSAE) on organ weights in CCl₄-induced hepatotoxicity in rats. The graphs demonstrate: (A) Liver weight and (B) Stomach weight across different treatment groups. NC: Normal Control, DC: Disease Control, SC: Silymarin Control (50 mg/kg), NS: *Nigella sativa* extract at doses of 100, 200, and 400 mg/kg. Values are expressed as mean $\pm$ SEM (n=6). NSAE treatment showed dose-dependent effects on organ weights, particularly evident in liver weight modulation at higher doses

Effect of *Nigella sativa* (NSAE) on Food and Water Intake

The effects of NSAE were also observed in food and water intake in hepatotoxic rats induced by CCl₄ in Figure 4. The water intake was observed in the disease control group, which received CCl₄. It was 19.3 ± 3.4 ml while the water intake of the normal control group was 24.9 ± 3.3 ml. The recorded SAEs suggested that there is an increase in the water intake and the result of the NSAE gave it a dose dependent response, particularly on the 400 mg/kg of the treatment group increasing the amount of water intake (26.9 ± 2.7 mL) to be parallel to with that of silymarin group (27.9 ± 3.9 mL). Therefore, the food consumption rate decreased in the disease control group (17.8 ± 2.8 g) compared to the normal control group (20.2 ± 2.5 g).

The effect of NSAE dose-dependently increased food intake, the maximum being 400 mg/kg of bid, 22.5 ± 3.4 g, which was not very far from the silymarin group, 23.2 ± 3.2 g. The current study's findings imply that NSAE might assist in enhancing the general physiological welfare of hepatotoxic animals through normal feeding and drinking.

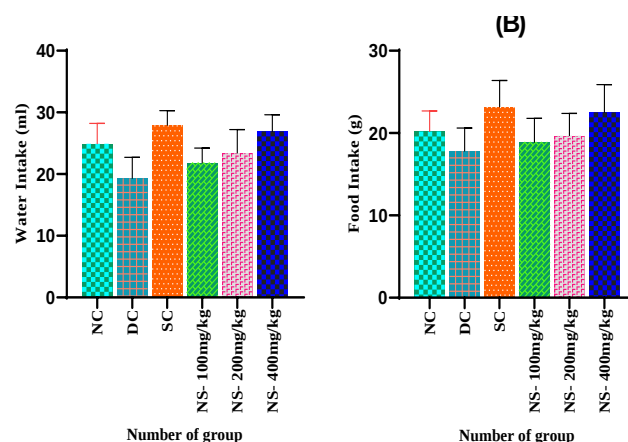


Figure 4: Effects of alcoholic extract of *Nigella sativa* (NSAE) on food and water intake in CCl₄-induced hepatotoxicity in rats. The graphs demonstrate: (A) Water intake and (B) Food intake across different treatment groups. NC: Normal Control, DC: Disease Control, SC: Silymarin Control (50 mg/kg), NS: *Nigella sativa* extract at doses of 100, 200, and 400 mg/kg. Values are expressed as mean $\pm$ SEM (n=6). NSAE treatment showed dose-dependent improvement in both parameters.

Results of Phenobarbital reduced sleeping time

Table 3: Phenobarbital reduced sleeping time

Groups	Treatment	Sleeping Time (hrs)
I	Normal Control (NC)	6 $\pm$ 0.5***
II	Disease Control (DC)	8 $\pm$ 1
III	Silymarin Control (SC)	6.5 $\pm$ 1*
IV	NSAE- 100mg/kg	6 $\pm$ 0.5***
V	NSAE- 200mg/kg	4 $\pm$ 1***
VI	NSAE- 400mg/kg	4.5 $\pm$ 0.5***

Data are expressed in mean $\pm$ SD, (n=6)

ns Values are Not significant (p = 0.12), * Significant (p = .033), ** Very significant (p = 0.002), *** Highly significant (p<0.001) compared to Disease Control (DC) group.

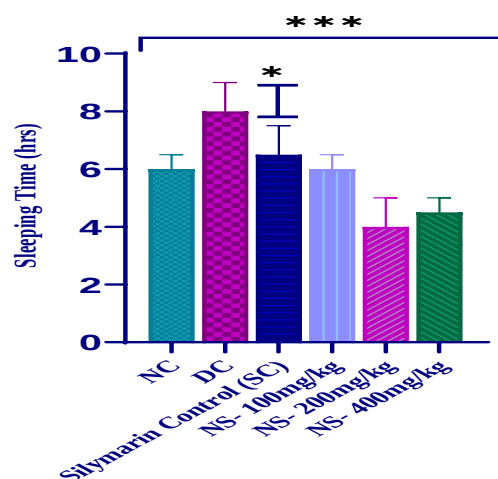


Figure 5: Phenobarbital reduced sleeping time

Histopathology analysis

Histological changes in liver and stomach sections were extensively observed from various tissue alterations as analysed under 40x magnification. In the control group, the marked hepatocellular lesions had features of vacuolisation, infiltration of inflammatory cells, and deranged sinusoidal architecture in liver tissue sections. NSAE-mediated treatment had beneficial effects on hepatic histoarchitecture, depending on the dose used. 400 mg/kg dose possessed a highly hepatoprotective impact as demonstrated by the decrease in the inflammatory infiltration, well-preserved hepatocytes, and minimal cellular degeneration similar to the silymarin group. In the infection control group, stomach sections showed the features of mucosal ulceration and inflammation. In contrast, in the treated group, especially at 400 mg/kg NSAE, there was proper gastric mucosa with normal architecture, lining, and minimal infiltration of inflammatory cells. The histopathological changes support the previous biochemical results, which indicate that NSAE has the potential to shield against CCL₄ elicited hepatic and gastric pathologies.

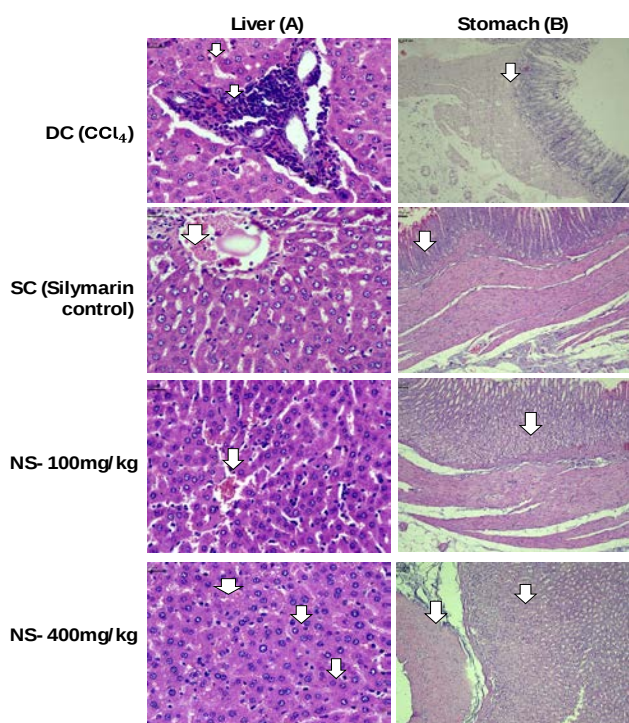


Figure 5: Histopathological analysis of liver and stomach tissues in CCL₄-induced hepatotoxicity model treated with *Nigella sativa* extract. Representative photomicrographs of H&E-stained sections of (A) liver and (B) stomach tissues (magnification 40×). DC: Disease Control showing CCL₄-induced damage; SC: Silymarin Control (50 mg/kg); NS: *Nigella sativa* alcoholic extract at 100 mg/kg and 400 mg/kg. White arrows indicate key histological features.

DISCUSSION

The current investigation demonstrated significant alterations in hepatic biochemical markers following CCL₄-induced liver injury, characterized by decreased total protein and albumin levels, alongside elevated cholesterol, SGOT, and SGPT levels. This biochemical profile indicates severe hepatocellular damage, likely due to CCL₄-mediated free radical generation and subsequent membrane disruption [23]. The reduction in total protein and albumin levels suggests compromised protein synthesis capacity of the liver. At the same time, elevated transaminases (SGOT and SGPT) indicate hepatocyte membrane damage and enzyme leakage into circulation [24]. The observed hypercholesterolemia in CCL₄-treated rats likely results from impaired hepatic lipid metabolism and altered membrane permeability [25].

Treatment with *Nigella sativa* alcoholic extract (NSAE) exhibited remarkable hepatoprotective effects, evidenced by dose-dependent normalization of all biochemical parameters. The highest dose (400 mg/kg) demonstrated efficacy comparable to silymarin, a standard hepatoprotective agent. This therapeutic effect can be attributed to the rich phytochemical composition of *N. sativa*, particularly thymoquinone, which has been reported to possess potent antioxidant and membrane-stabilizing properties. Restoring protein synthesis capacity and normalizing transaminase levels suggests NSAE's ability to preserve hepatocyte integrity and function. These findings align with previous studies reporting the hepatoprotective potential of *N. sativa* extracts [26], though the current research uniquely demonstrates the dose-dependent effects across multiple biochemical parameters in CCL₄-induced hepatotoxicity.

The investigations carried out with the CCL₄-induced hepatic toxicity in rats showed that the antioxidant protection of the liver is reduced, where the GSH level, catalase activity, and lipid peroxidation are completely diminished. CCL₄ hepatotoxicity is mainly exerted through the production of trichloromethyl radicals, which initiate oxidative stress [27]. The decrease in GSH and CAT depicts the decreased antioxidant activity of the cell, while the increase in MDA mainly highlights the level of lipid peroxidation and membrane lipid damage [28]. These changes all depict a severe oxidative stress situation, and this is a characteristic feature of CCL₄-induced hepatotoxicity [29]. By boosting the activity of endogenous antioxidants like SOD, catalase, and glutathione, NSAE seems to improve antioxidant

defence systems. NSAE can lessen liver cell oxidative damage by lowering ROS levels and strengthening the body's natural antioxidant defences. The present study shows that NSAE markedly produces the antioxidant effect at 400mg/kg, almost replenishing all the typical antioxidant parameters. These findings indicate the extract's direct free radical quenching abilities and/or stimulation of the reductive pathways of endogenous antioxidants, such as catalase [30]. Related to this, the significant decrease in MDA levels also suggests that NSAE can protect against lipid peroxidation, which has been attributed to the rich nutrient content, particularly thymoquinone and other phenolic substances present in the plant [31]. The present work also provides evidence that the antioxidant capacity of NSAE is comparable to silymarin, especially at the later concentration, thereby indicating its possible role as a natural hepato-protective agent. These findings align with most conventional assertions concerning *N. sativa* for treatment and establishing its antioxidant action.

The current investigation has found a slight change in magnitude in its variability in the case of organ weights, where it is observed that liver weight has slightly decreased in the disease control group after CCl₄ administration. These results may be due to the direct effect of CCl₄ on the liver, leading to tissue disappearance and changed hepatic structure [32]. The liver is also the one organ whose relative weight was not significantly decreased with the toxic insults, which might imply that the formation of the compensatory mechanisms, inflammatory reactions, and tissue repair may play a role [33]. Small changes in the amount of stomach weight from the treatment group further suggest that CCl₄ has not rendered a systemic effect, such as a sizeable alteration in stomach tissue mass. The present results showed that NSAE exhibited a positive dose-dependent impact on organ weights, which was more conspicuous in the liver organ weights. The decrease in liver weight at higher doses of NSAE, 400 mg/kg, is similar to that of silymarin, which may mean that inflammation processes in the experiment and prevention of injury to liver tissue [34]. These postulates could be attributed to the anti-inflammatory and tissue-protective properties associated with the extract that may have active compounds [35]. Thus, the changes in organ weight should be evaluated considering histopathological changes and biochemical parameters to discern the therapeutic efficacy of NSAE efficiently. The present study additionally reveals that the organ weight changes induced by NSAE are concordant with its

hepatoprotective profile. However, studies are still necessary to establish the patterns that control the changes.

The current outcome of the experiment revealed reduced food and water intake in hepatotoxic animals due to physical deterioration in health, which is consequent on CCl₄. This reduced food consumption has been reported in CCl₄-induced hepatotoxicity due to stress, changes in metabolism, and general well-being of the body [36]. A decrease in food and water intake may also be linked to the ability of CCl₄ to alter appetite and digestive processes since alteration in hepatic capacity to maintain metabolism and feeding drive is fully established [37]. Such behavioural shifts as tails, ears, faeces, and alopecia are valuable regulatory features of the level of hepatic pathology and the state of animals. This study reveals that NSAE at a dosage of 400 mg/kg effectively counteracts the ECS effects induced by stress, leading to a return to regular feeding and drinking habits. The observed improvement in consumption patterns in a dose-dependent manner indicates that NSAE may be advantageous for treating systemic effects caused by CCl₄ and enhancing overall physiological health [38]. The restoration of normal feeding behaviors likely arises from NSAE's hepatoprotective and anti-inflammatory effects, particularly due to its active ingredient, thymoquinone [39]. The balance of food and water intake reflects evidence of improved liver function, showcasing a holistic treatment approach via NSAE. Consequently, this study highlights for the first time that, beyond its hepatoprotective properties, NSAE also enhances vital physiological parameters. However, further investigation is necessary to clarify the relationship between NSAE and these changes. Microscopic examination of liver sections from CCl₄-treated rats revealed severe architectural distortions, characterized by extensive hepatocellular vacuolation, inflammatory infiltration, and disrupted sinusoidal arrangement. These histological alterations are consistent with CCl₄ known mechanism of hepatotoxicity, involving free radical generation and subsequent cellular damage [40]. The observed inflammatory infiltration suggests an active immune response to hepatocellular injury, while the disrupted architecture reflects the extent of tissue damage [41]. The concurrent gastric mucosal changes indicate that CCl₄-induced toxicity extends beyond the liver, affecting multiple organ systems [42].

The exposure to NSAE had been found to possess a remarkably protective impact on all tested tissues, and the significant

protective effects were recorded at 400 mg/kg treatment. Decreased inflammatory infiltration and significant recovery in the hepatic histoarchitecture on reduced doses of NSAE further proved the role of NSAE in preventing the CCl₄ induced cellular damage. The preservation of gastric mucosal integrity in NSAE-treated groups signifies that it has more benefits than merely an anti-ulcer effect; it could be due to the anti-inflammatory and antioxidant effects of the extract [43]. Therefore, the present study provided the first evidence to underscore NSAE's multitissue-protective effect, where improved histology was well reflected in the biochemical alteration. These observations thus offer a tangible morphological basis for NSAE's protective effects on the liver and stomach; however, to understand how, at the cellular level, NSAE can afford the former organ protection, electron microscopy might help enhance these findings [44]. NSAE treatment at 400 mg/kg effectively normalized liver function markers, enhanced antioxidant defence mechanisms, and preserved tissue architecture, comparable to silymarin.

CONCLUSION

The present investigation demonstrates the significant hepatoprotective and gastroprotective potential of *Nigella sativa* alcoholic extract (NSAE) against CCl₄-induced toxicity in rats. NSAE treatment at 400 mg/kg effectively normalized liver function markers, enhanced antioxidant defence mechanisms, and preserved tissue architecture comparable to silymarin. These findings validate the traditional use of *N. sativa* in liver disorders and suggest its potential as a safe, natural therapeutic agent for managing hepatic diseases. The comprehensive protection offered by NSAE could benefit patients with liver disorders, particularly those seeking natural alternatives to conventional treatments. This research allows clinical trials to establish optimal dosing regimens and evaluate long-term safety profiles. At the same time, future studies should focus on isolating active compounds and elucidating their specific molecular mechanisms of action. Furthermore, research targeted at identifying the active ingredients in NSAE and clarifying their unique molecular mechanisms of action will be essential to comprehending its precise therapeutic advantages and enabling its possible incorporation into conventional therapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

AS Devadhe and SS Yadav performed the literature survey, collected the data, and performed the experimental part. SB Dighe and RD Ghogare contributed to the original draft's conceptualization, review, methodology, investigation, and scientific writing. SB Bhawar made corrections in a research paper. All authors checked the final draft.

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