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Research Article

SCREENING OF NEROLI OIL ON PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: The objective of the study was to evaluate the hepatoprotective effect of Neroli oil against Paracetamol induced liver injury in Wistar rats.

Methods: Changes in the serum biomarker enzymes, namely, alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), direct bilirubin, total bilirubin, total cholesterol, and albumin were estimated. Oxidative stress parameter such as MDA, SOD, GSH, and catalase levels was also estimated along with the liver weight and volume.

Results: Neroli oil showed significant reduction in all elevated biochemical parameters (namely, ALT, AST, ALP, direct bilirubin, total bilirubin, and total cholesterol), and significant increase in reduced level of albumin. Oil showed significant increase in GSH and catalase level and decreased in LPO level. Histopathological determinations confirmed biochemical findings.

Conclusion: Neroli oil showed a significant hepatoprotective effect against Paracetamol induced hepatic damage as depicted by its protective activity on functional, physical, biochemical, antioxidant, and histological changes in liver.

Keywords: Neroli oil, Paracetamol, Silymarin, Hepatotoxicity.

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INTRODUCTION

Liver is considered to be one of the most vital organs that functions as a center of metabolism of nutrients such as carbohydrates, proteins, lipids, and excretion of waste metabolites. In addition, it is also handling the metabolism and excretion of drugs and other xenobiotic from the body thereby providing protection against foreign substances by detoxifying and eliminating them. The liver injury is caused by hepatotoxins, microbial infections, and excessive alcohol ingestion or during certain therapy. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. The use of natural remedies for the treatment of various hepatic diseases has a long history and medicinal plants and their derivatives are still used all over the world [1]. Natural remedies from traditional plants are seen as effective and safe alternative treatments for hepatotoxicity. Many bioactive compounds and extracts from plants have thus been investigated for hepatoprotective and antioxidant effects against hepatotoxins-induced liver damage.

Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to extractable glucuronide and sulfate conjugates. However, the hepatotoxicity of Paracetamol has been attributed to the formation of toxic metabolites when a part of Paracetamol is activated by hepatic cytochrome P-45012 to a highly reactive metabolite N-acetyl- P-benzoquinone imine (NAPQI). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH [2].

Silymarin is marketed as one of the standard hepatoprotective herbal formulation. Silymarin is a polyphenolic flavonoid isolated from seeds of the milk thistle *Silybum marianum* (Family Asteraceae). It has been used to treat liver and gallbladder disorders, including hepatitis, cirrhosis, and jaundice, and to protect the liver against poisoning from chemical and environmental toxins, including snake bites, insect stings, and alcohol. Silymarin has also been reported to provide liver protection against CCl4 and Paracetamol-induced liver damage in rat models [3].

Neroli is commonly extracted from aromatic flowers through hydro distillation. In this method, the aromatic crop biomass is loaded into a distillation tank and steam generated either in a boiler or in the distillation tank itself is allowed to pass through the crop biomass. The essential oil present in the biomass vaporizes. Steam and essential oil vapors are passed through a condenser. The condensate (mixture of water and essential oil) is collected in a receiver. The essential oil is decanted, cleaned, made moisture free, and traded. During the process of steam distillation a part of the essential oil becomes dissolved in the condensate or distillation water. Neroli has been well characterized from the chemical, biological, and pharmacological point of view [4]. Vapors of essential oil such as neroli could help reduce anxiety levels. Inhalation of this essential oil may trigger the limbic system of the brain giving the resulting beneficial effects [5]. Since there is no reported activity of neroli oil possessing hepatoprotective activity, this study was planned to evaluate its potential.

METHODS

Reagents

Paracetamol (Montex Bio Pharma) was procured from the pharmacy at Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, India. Ellman's reagent and Silymarin were procured from Sigma-Aldrich, USA. Thiopentone sodium sourced from Neon Laboratories Limited, Mumbai. Tween 80, Gum acacia, Thiobarbituric acid, and Tris-Base were procured from HiMedia Pvt Ltd., Mumbai. Alanine transaminase (ALT) kit and aspartate aminotransferase (AST) kit were obtained from Crest Bio systems, Goa, India. Alkaline phosphatase (ALP) kit, Albumin kit, Total Cholesterol Kit, Total and direct Bilirubin Kit, all were procured from ERBA Diagnostics Manheim GmbH, Germany. All other reagents were of analytical grade.

Oil selection

Based on the traditional usage, neroli oil is used for present study. Neroli oil is obtained as a gift sample from Bansal Aroma, 20-A, Teachers Colony, Samaipur Delhi-42.

Needle selection
Oral dosing needle for rats was of size 18G, 1 and ½ inch, curved ball ended. And for intraperitoneal route the needle size was 26G, ½ inch.

Experimental animals
Adult Wistar rats of either sex weighing 150–200 g were used in the experiment. The animals had free access to laboratory pelleted diet and tap water. They were housed in galvanized iron cages in a thermostatically controlled room and maintained in 12 h dark/light cycle. After 7 days of acclimatization period, they were randomly selected for different experimental groups. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC Reg. No.: 221/CPCSEA) KLEU's College Of Pharmacy, Belgaum, before conducting the experiment.

Selection of dose
LD_{50} cutoff value was determined by neroli oil light (phase). Neroli oil did not showed any significant toxic signs or mortality at a large dose up to 2000 mg/kg, therefore 1 / 5^{th}, 1 / 10^{th}, and 1 / 20^{th} of the maximum dose, that is, 400 mg/kg, 200 mg/kg, and 100mg/kg per o.s. was selected as daily dose and given by making emulsion with tween 80 [6].

Experimental design
Wistar rats weighing between (150 and 200 g) were divided into six groups of six rats in each. Group A served as normal control was given only vehicle orally, per o.s. (p.o.) for 30 days. Group B animals were served as disease control and treated with Paracetamol (1 g/kg p.o.) for 30 days. Group C animals were treated with Silymarin (100 mg/kg p.o.) that served as standard. Animals in Groups D, E, and F were treated with three different doses (low-100 mg/kg, medium-200 mg/kg, and high-400 mg/kg) of neroli oil and serve as treatment group. Groups C, D, E, and F were intoxicated with Paracetamol (1 g/kg p.o.) for 30 days along with co administration of neroli oil in Groups D, E, and F. Animals were anaesthetized with ether; blood was collected through retro orbital puncture and subjected for biochemical studies.

Preparation of blood serum
Blood was allowed to coagulate for 20 min and then centrifuged at 2000× g for 15 min. The serum was separated and used for estimations.

Preparation of liver homogenate
The livers were removed; washed with ice cold normal saline and minced. This was followed by homogenization with an ice cold Tris-HCl buffer saline (0.025 M pH 7.5) in 1:10 ratio, centrifugation at 5000× g for 20 min. The supernatant was used for assays.

Physical parameters
Determination of wet liver weight
Livers isolated from the animals were washed with distilled water and dried on filter paper strips and weighed on an electronic balance and were expressed in gram.

Determination of wet liver volume
After recording the liver weights, the livers were individually dropped into a measuring cylinder containing a fixed volume of distilled water and the volume displaced was recorded and expressed as milliliter [7].

Procedure of parameters evaluated
Assay procedure for estimation of biochemical parameters
AST
Procedure: The samples were prepared using Addition Sequence of AST Kit Reagents. 1.0 ml of working reagent was incubated at the assay temperature (25°C) for 1 min and then 0.2 ml of serum was added. The samples were mixed well and change in absorbance was read at 340nm. The autoanalyzer instrument was set to the specifications mentioned in the protocol supplied with the kit.

Calculation: AST Activity in U/L = ΔAbs/min. × Factor (952). The results were expressed as U/L [8].

ALT
Procedure: The samples were prepared using Addition Sequence of ALT Kit Reagents. 1.0 ml of working reagent was incubated at the assay temperature (25°C) for 1 min and then 0.2 ml of serum was added. The samples were mixed well and change in absorbance was read at 340 nm. The autoanalyzer instrument was set to the specifications mentioned in the protocol supplied with the kit.

Calculation: ALT Activity in U/L = ΔAbs/min. × Factor (952). The results were expressed as U/L [9].

ALP
Procedure: The samples were prepared using Addition Sequence of ALP Kit Reagents. 1000 µl of working reagent was incubated at the assay temperature (25°C) for 1 min and then 20 µl of serum was added. The samples were mixed well and change in absorbance was read at 405 nm. The autoanalyzer instrument was set to the specifications mentioned in the protocol supplied with the kit.

Calculation: Activity of ALP (U/L) = ΔAbs/min × Factor (2713).The results were expressed as U/L [10].

Albumin
Procedure: The samples were prepared using Addition Sequence of Albumin Kit Reagents. The samples were mixed well, incubated at 37°C for 5 min. The autoanalyzer instrument was set to the specifications mentioned in the protocol supplied with the kit and absorbance was measured.

Calculation: Albumin in g/dl = Abs. of Test/Abs. of Standard × Conc. of standard

The results were expressed as g/dl [11].

Serum total cholesterol
Procedure: The samples were prepared using Addition Sequence of Cholesterol Kit Reagents as given in Table 1 [10]. The samples were mixed well and incubated at 37°C for 10 min. The autoanalyzer instrument was set to the specifications mentioned in the protocol supplied with the kit. The absorbance of standard and each test was read at 505 nm against reagent blank.

Calculation: Cholesterol (mg/dl) = Abs. of Test/Abs. of Standard ×200

The results were expressed as mg/dl [12].

Estimation of serum bilirubin
Procedure: The samples were prepared using addition sequence of serum bilirubin kit reagents. All the samples were incubated for 5 min at 37°C, aspirated individually and absorbance was recorded at 546 / 630 nm against reagent blank.

Calculation: Total Bilirubin in mg/dl = Abs. of Test/Abs. of Standard × Concentration of standard

Direct Bilirubin in mg/dl = Abs. of Test/Abs. of Standard × Concentration of standard

The results were expressed as mg/dl [13].

Antioxidant estimation parameters
Lipid peroxidation (LPO)
Procedure: The method involved heating of 0.2 ml of supernatant with 4 ml of Thiobarbituric acid-trichloroacetic acid-HCl reagent for 15 min in a boiling water bath. After cooling, the solution was centrifuged at 3500 rpm for 10 min at room temperature and the precipitate obtained was removed. The absorbance of the clear supernatant was determined at 535 nm against a blank that contained 8ml of reagent with 0.4 ml of distilled water excluding biological sample.

Table 1: Effect of neroli oil on the level of ALT, AST, ALP, total and direct bilirubin biochemical parameters after treatment of paracetamol

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Group A-Normal control	16.87±0.993	15.35±0.994	125.4±0.565	0.618±0.027	0.480±0.071
Group B-Paracetamol toxicant 1 g/kg	53.85±1.619###	60.20±0.417###	330.2±2.221###	1.217±0.033###	1.287±0.025###
Group C-Paracetamol 1 g/kg + Silymarin 100 mg/kg	20.54±1.068***	21.43±1.888***	157.0±1.326***	0.440±0.072***	0.501±0.069***
Group D-Paracetamol 1 g/kg + low dose neroli oil 100 mg/kg	39.22±4.099**	48.14±2.351**	250.6±5.118***	0.823±0.053***	0.998±0.019**
Group E-Paracetamol 1 g/kg + medium dose Neroli oil 200 mg/kg	31.94±3.707***	41.21±2.657***	205±2.792***	0.763±0.071***	0.541±0.090***
Group F-Paracetamol 1 g/kg + high dose neroli oil 400 mg/kg	21.61±0.646***	27.96±1.527***	182.0±1.803***	0.561±0.046***	0.531±0.063***

All the values were expressed as mean±SD using one-way ANOVA followed by dunnett's multiple comparison test, *n*=6. Compared with Normal control: #p<0.01, ##p<0.001, ###p<0.0001. Compared with Disease control: *P<0.01, **P<0.001, ***p<0.0001. ALT: Alanine transferase, AST: Aspartate transferase, ALP: Alkaline phosphatase

Calculation: The malondialdehyde (MDA) equivalents of the sample were calculated using an extinction coefficient (a) of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as moles of TBARS/g of tissue for liver homogenate

A = abc (Beer-Lambert's law)
Where, A = absorbance of the solution
a = molar extinction coefficient, b = 1 cm, c = concentration of MDA equivalents in moles
In this case, except "c" remaining values we know hence we calculated "c" from the above formula [14].

Antioxidant enzyme catalase
Procedure: The reaction was initiated by adding 0.2 ml of enzyme to 0.8 ml of 0.2 M hydrogen peroxide (H_2O_2) and 2.0 ml of 0.01 M phosphate buffer (pH 7.4). The mixture was incubated at 37°C accurately for 1.0 min. The reaction was stopped with 4.0 ml of dichromate-acetic acid reagent. Further, the samples were heated in boiling water bath for 15 min. Tubes were centrifuged at 5000 rpm for 15 min and supernatant was used to quantify the amount of catalase activity at 570 nm against a blank that contained 2.4 ml of 0.2 M H_2O_2 and 6 ml of 0.01M phosphate buffer with 0.6 ml of distilled water excluding enzyme and represented as moles of H_2O_2 consumed/min/g of tissue for liver homogenate.

Stock solution of 1M H_2O_2 was prepared from the marketed H_2O_2. From this Stock solution (1 M), serial dilutions ranging from 0.005 M to 0.1 M were prepared as represented in Table 2 [15].

To each 1 ml of dilution 4.0 ml of dichromate-acetic acid reagent was added. Further, the samples were heated in boiling water bath for 15 min. The mixture was allowed to attain room temperature and the absorbance was read at 570 nm against blank (excluding H_2O_2), a standard graph was plotted between absorbance versus moles of H_2O_2 remaining in the solution as depicted in Graph 1.

Detoxifying enzyme GSH assay
Procedure: 1 ml of enzyme supernatant was pipetted out and precipitated with 2 ml of 5% trichloroacetate (TCA), then centrifuged at 3200 rpm for 20 min. Supernatant was collected and 1 ml was treated with 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The mixture was thoroughly mixed and the absorbance was read at 412 nm against blank containing 1 ml of reagent and 6 ml of phosphate buffer with 2 ml of distilled water except enzyme, expressed as µM of TNB/g of tissue for liver homogenate.

10 mg of reduced GSH was dissolved in 100 ml of distilled water. From this Stock solution (100 µg/ml), serial dilutions ranging from 1 µg/ml to 50 µg/ml were prepared as represented in Table 3 [16].

To each 1 ml of dilution, 0.5 ml of Ellman's reagent, 3.0 ml of phosphate buffer (0.2 M, pH 8.0) and 0.4 ml of distilled water was added. The mixture was thoroughly mixed and the absorbance was read at 412 nm against blank (excluding GSH), a standard graph was plotted between absorbance versus µM of TNB/g of liver tissue. From this graph, the concentrations of TNB were determined by interpolation (Graph 2).

Table 2: Addition sequences of catalase standard graph reagents

Concentration (M)	Volume of stock solution (ml)	Volume of distilled water (ml)	Total volume (ml)
0.005	0.05	9.95	10
0.01	0.1	9.9	10
0.02	0.2	9.8	10
0.04	0.4	9.6	10
0.08	0.8	9.2	10
0.1	1	9	10

Table 3: Addition sequence of glutathione standard graph reagents

Concentration (µg/ml)	Volume of stock solution (ml)	Volume of distilled water (ml)	Total volume (ml)
1	0.1	9.9	10
5	0.5	9.5	10
10	1	9	10
20	2	8	10
30	3	7	10
40	4	6	10
50	5	5	10

Estimation of superoxide dismutase (SOD)
Test solution containing 2.9 ml of tris buffer mixture and 10 µl supernatant was added to 0.1 ml of pyrogallol reagent against blank solution containing 2.9 ml of tris buffer mixture. Control solution contained 0.9 ml of Tris buffer mixture and 0.1 ml of pyrogallol. Absorbance was taken at 420 nm and readings were taken at 90 s and 210 s.

$$\text{Calculation: SOD}\left(\text{unit / ml}\right)=\frac{\left(C-T\right)\times 100}{\left(C\times 50\right)\times h}$$

Where h = volume of homogenate taken.
C (control) = (absorbance at 210 s – at 60 s) ÷2
T (test) = (absorbance at 210 s – at 60 s) ÷2
Note: One unit of SOD = amount of enzyme required for 50% inhibition of pyrogallol auto-oxidation [17].

Statistical analysis
Results were expressed as Mean±S.D., where n = 6. Differences among data were determined using one-way ANOVA followed by Dunnett's

multiple comparison tests. Data were computed for statistical analysis using GraphPad Prism software, INC.

Histopathological studies

All animals were sacrificed, liver were dissected out and post fixed overnight in 10% formalin. Sections of the tissues fixed in paraffin were prepared and stained with hematoxylin and eosin and observed for pathological changes.

RESULTS

Acute toxicity studies

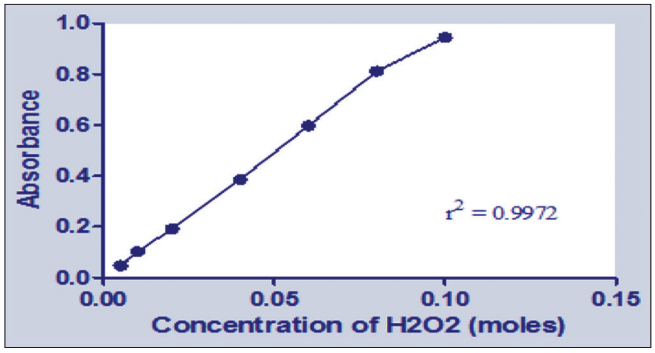
Acute toxicity studies revealed that neroli oil was found to be safe and showed no mortality up to 2000 mg/kg. Therefore, 1 / 5th, 1 / 10th, and 1 / 20th of the maximum dose, that is, 400 mg/kg, 200 mg/kg, and 100 mg/kg p.o. were selected for the study.

Effect of neroli oil on liver weight and liver volume after paracetamoltreatment

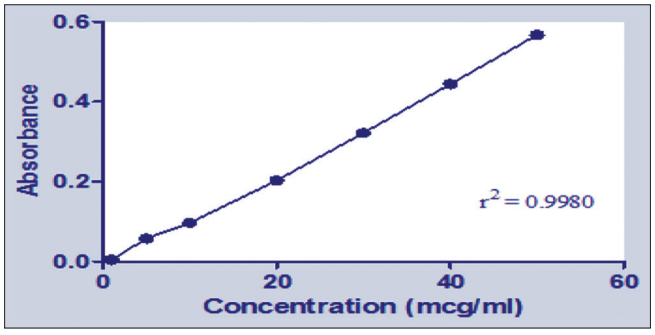
Effect on liver weight and liver volume of the animals in the control and experimental groups after Paracetamol treatment is shown in Table 4. There was a significant ($p<0.001$) increase in liver weight and liver volume in the Paracetamol toxicant group compared to that of normal control group. Standard drug Silymarin (100 mg/kg p.o.) showed significant ($p<0.001$) reduction in liver weight and liver volume when compared with normal. Treatment with neroli oil (100 mg/kg, 200 mg/kg, and 400 mg/kg) showed ($p<0.001$) a significant reduction in liver weight and liver volume as shown in Table 4.

Effect of neroli oil on the level of ALT, AST, ALP, total, and direct bilirubin biochemical parameters after treatment of paracetamol

A significant ($p<0.001$) increase in ALT, AST, and ALP levels was observed in Paracetamol (1 g/kg) treated group when compared to normal control. Increased levels of these biochemical parameter were decreased significantly ($p<0.001$) by treatment with Silymarin (100 mg/kg) and neroli oil (100 mg/kg, 200 mg/kg, and 400 mg/kg), respectively. Total bilirubin and direct bilirubin levels were also reduced significantly ($p<0.001$) with neroli oil treatment (Table 1, Graphs 3 and 4).



Graph 1: Standard graph for Catalase



Graph 2: Standard graph for GSH

Effect of neroli oil on serum cholesterol and albumin biochemical parameters after treatment of paracetamol

The level of lipid profile parameter total cholesterol in serum was significantly ($p<0.001$) increased in Paracetamol (1 g/kg) treated group when compared with normal control group. Treatment with neroli oil (100 mg/kg, 200 mg/kg, and 400 mg/kg) in Paracetamol induced hepatotoxic group significantly ($p<0.001$) decreased the level of this parameter in serum. Neroli oil treatment did not show any dose dependency.

The levels of serum marker albumin were significantly ($p<0.001$) decreased in Paracetamol (1 g/kg) induced hepatotoxic group when compared with normal control group. Treatment with neroli oil (100 mg/kg, 200 mg/kg, and 400 mg/kg) in Paracetamol induced hepatotoxic group significantly ($p<0.001$) increased the level of these enzymes in serum as shown in Table 5 and depicted in Graphs 5 and 6.

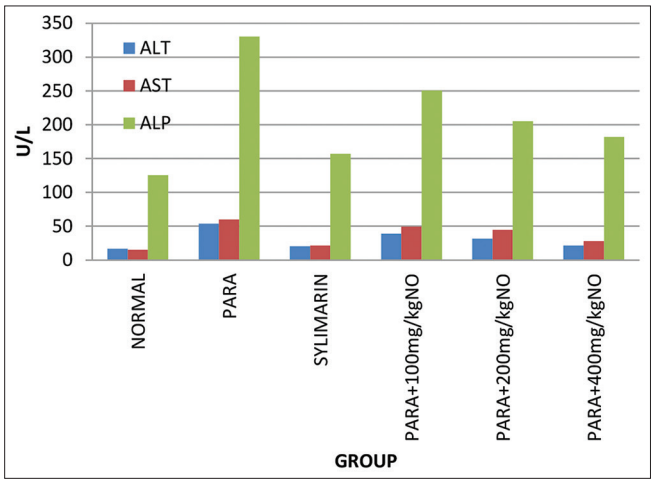
Effect of neroli oil on antioxidant estimation of catalase activity, GSH, MDA, and SOD after treatment of paracetamol

The level of TBARS in liver homogenate was significantly increased in Paracetamol induced hepatotoxic group when compared with normal control group. Treatment with neroli oil (100 mg/kg, 200 mg/kg, and 400 mg/kg) in Paracetamol induced hepatotoxic group significantly

Table 4: Effect of neroli oil on liver weight and volume after paracetamol treatment

Group	Liver weight (g)	Liver volume (ml)
Group A-Normal control	5.720±0.1911	55.00±0.8367
Group B-Paracetamol Toxicant 1 g/kg	7.590±0.5664$^{###}$	64.80±0.8602$^{###}$
Group C-Paracetamol 1 g/kg+silymarin 100 mg/kg	5.815±0.2242***	56.00±0.7071***
Group D-Paracetamol 1 g/kg+low dose Neroli oil 100 mg/kg	6.656±0.4798***	57.60±1.030***
Group E-Paracetamol 1 g/kg+medium dose neroli oil 200 mg/kg	6.056±0.4550***	56.00±1.225***
Group F-Paracetamol 1 g/kg+high dose Neroli oil 400 mg/kg	5.761±0.2378***	55.00±0.7071***

All the values were expressed as mean±SD using one-way ANOVA followed by Dunnett's multiple. Comparison test, n=6. Compared with Normal control: $^{#}p<0.01$, $^{##}p<0.001$, $^{###}p<0.0001$. Compared with Disease control: *p<0.01, **p<0.001, ***p<0.0001. SD: Standard deviation



Graph 3: Effect of neroli oil on the level of ALT, AST, and ALP biochemical parameters after treatment of Paracetamol

(p<0.001) decreased the level of TBARS in liver homogenate. Standard drug Silymarin (100 mg/kg) reduced the levels of TBARS significantly (p<0.001).

The level of catalase and GSH in liver homogenate was significantly (p<0.001) decreased in Paracetamol (1 g/kg) treated group when compared with normal control group. Treatment with Silymarin and neroli oil significantly (p<0.001) increased the level of catalase and GSH level in liver homogenate.

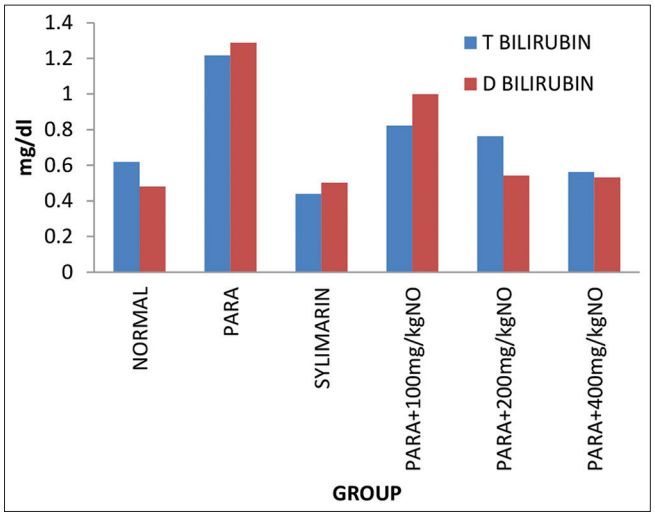
The level of MDA and SOD in liver homogenate was significantly (p<0.001) decreased in Paracetamol (1g/kg) treated group when compared with normal control group. Treatment with Silymarin and neroli oil significantly (p<0.001) increased the level of catalase and GSH level in liver homogenate as shown in Table 6, Graphs 7 and 8.

Effect of neroli oil on histopathological analysis of liver in control and experimental animals after treatment of paracetamol

Histology of liver from normal control group shows normal lobular architecture. There is no sign of kupffer cell hyperplasia. Hepatocytes, sinusoids, and central vein are all normal. There is no sign of any form of necrosis and no sign of nuclear changes. Liver histopathathogy from Paracetamol treated group reveals central vein congestion and sinusoidal congestion. Mild focal hemorrhage is observed. Degeneration and mark ballooning of hepatocytes are observed. Moderate necrosis and fibrosis are also presents. Moderate inflammation and kupffer cell hyperplasia is observed. Liver section from Neroli oil treated groups showed recovery from hepatic injury but some of the feature shown in Paracetamol toxicant group are present to a lesser extent. Silymarin treated group illustrated better recovery than the hyssop treated group. Only a mild sinusoidal and central vein congestion is present. Liver has normal lobular architecture. Liver shows no kupffer cell hyperplasia (Plate 1).

DISCUSSION

In the present study, hepatotoxicity was induced chemically using Paracetamol, as to assess the dose-dependent modulatory effect of neroli oil. The extent of toxicity was estimated by biochemical parameters (viz. AST, ALT, ALP, total cholesterol, direct bilirubin, total bilirubin, and albumin), antioxidant estimation (viz. catalase activity, MDA, GSH, and SOD), and histopathological studies. Neroli oil did not cause any mortality and found to be safe up to 2000 mg/kg, p.o. Hepatoprotective offered by this oil in Paracetamol induced liver damage was significant

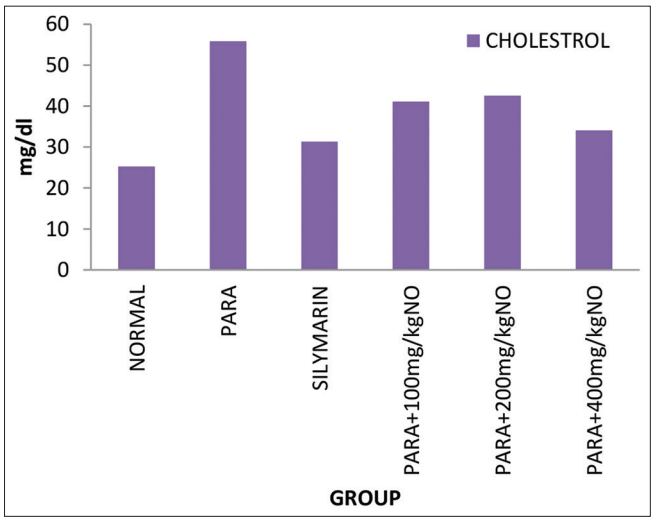


Graph 4: Effect of neroli oil on the level of total bilirubin and direct bilirubin biochemical parameters after treatment of Paracetamol

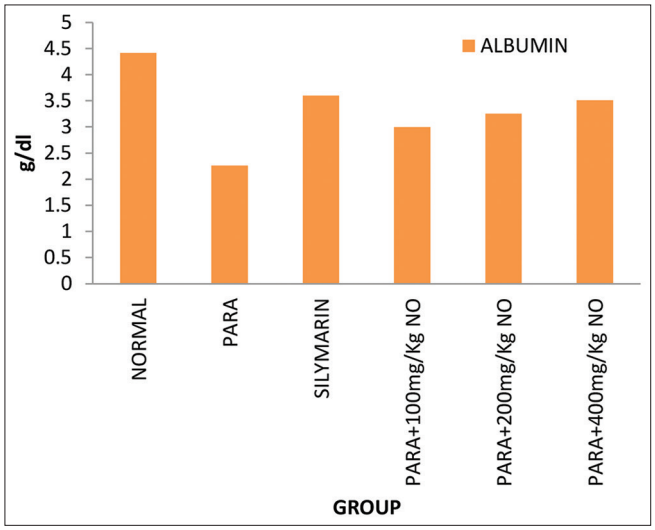
Table 5: Effect of neroli oil on serum cholesterol and albumin biochemical parameters after treatment of paracetamol

Group	Total cholesterol (mg/dl)	Albumin (g/dl)
Group A-Normal control	25.25±4.232	4.415±0.2303
Group B-Paracetamol toxicant 1 g/kg	55.5±6.650###	2.263±0.1953###
Group C-Paracetamol 1 g/kg+silymarin 100 mg/kg	31.30±1.058***	3.597±0.1687***
Group D-Paracetamol 1 g/kg+low dose neroli oil 100 mg/kg	41.07±2.751*	2.995±0.09054**
Group E-Paracetamol 1 g/kg+medium dose Neroli oil 200 mg/kg	42.53±1.297*	3.255±0.05993***
Group F-Paracetamol 1 g/kg+high dose neroli oil 400 mg/kg	34.07±1.364***	3.513±0.1775***

All the values were expressed as mean±SD using one-way ANOVA followed by Dunnett's multiple comparison test, n=6. Compared with Normal control: #p<0.01, ##p<0.001, ###p<0.0001. Compared with Disease control: *p<0.01, **p<0.001, ***p<0.0001



Graph 5: Effect of neroli oil on serum cholesterol after treatment of Paracetamol



Graph 6: Effect of neroli oil on albumin after treatment of Paracetamol

Table 6: Effect of neroli oil on antioxidant estimation of catalase activity, glutathione, malondialdehyde, and superoxide dismutase after treatment of paracetamol

Group	Catalase (Mol of H_2O_2u tilized/min/g of liver tissue)	GSH	MDA	SOD
Group A-Normal control	0.0716±0.002	913.4±21.17	158.4±4.825	88.35±2.107
Group B-Paracetamol toxicant 1 g/kg	0.0262±0.002###	669.4±29.58###	272.0±6.025###	54.64±2.661###
Group C-Paracetamol 1 g/kg+Silymarin 100 mg/kg	0.0672±0.003***	894.4±30.39***	191.2±5.791***	81.68±2.203***
Group D-Paracetamol 1 g/kg+low dose Neroli oil 100 mg/kg	0.0486±0.004**	780.8±15.05**	247.2±2.223**	63.81±1.895*
Group E-Paracetamol 1 g/kg+Medium dose Neroli oil mg/kg	0.0480±0.004**	817.1±20.61**	219.8±2.53***	66.33±0.925**
Group F-Paracetamol 1 g/kg+High dose neroli oil 400 mg/kg	0.0586±0.004748***	877.9±21.86***	198.8±7.940***	72.95±1.502***

All the values were expressed as mean±SD using one way ANOVA followed by Dunnett's. Multiple comparison test, n=6. Compared with Normal control: #p<0.01, ##p<0.001, ###p<0.0001. Compared with Disease control: *p<0.01, **p<0.001, ***p<0.0001. GSH: Glutathione, MDA: Malondialdehyde, SOD: Superoxide Dismutase

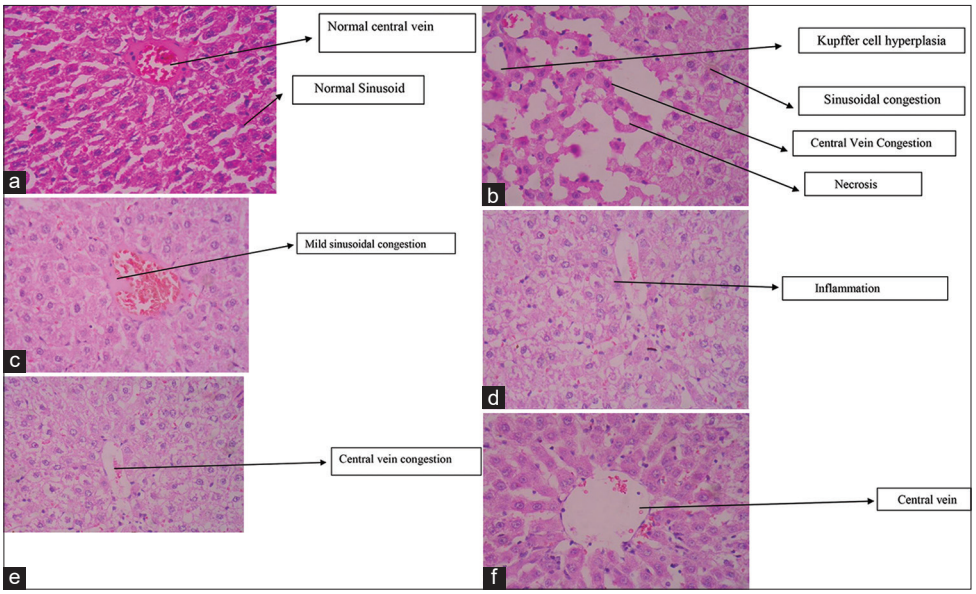
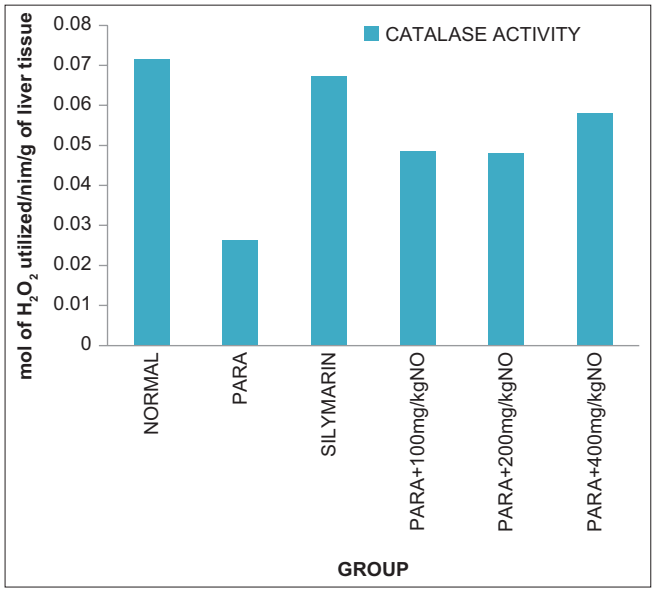
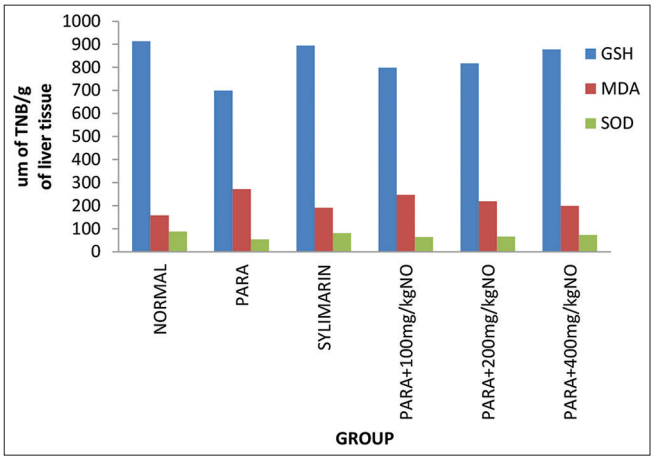


Plate 1: (a) Normal control group, (b) PCM Toxicant, (c) PCM + Silymarin, (d) PCM + Neroli low dose, (e) PCM +Neroli medium dose, (f) PCM + Neroli high dose



Graph 7: Effect of neroli oil on antioxidant estimation of catalase activity after treatment of Paracetamol



Graph 8: Effect of neroli oil on GSH, MDA, and SOD after treatment of Paracetamol

and also delayed the development of liver damage. Standard drug Silymarin showed significant hepatoprotective activity in both the model, neroli oil also showed significant hepatoprotective activity which was nearly comparable to Silymarin.

Hepatic toxicity has been induced by Paracetamol which is clear from the presence of marked central vein and sinusoidal congestion, focal hemorrhage, inflammation, necrosis, hepatocyte degeneration, nuclear changes, and kupffer cell hyperplasia. Significantly elevated biochemical

parameters (*viz*. AST, ALT, ALP, total cholesterol, total bilirubin, direct bilirubin,) and significantly decreased albumin level showed extended hepatotoxicity. Reduced level of catalase, GSH, SOD indicates that there was significant hepatic damage when compared with normal. When there is LPO of hepatic membrane by paracetamol which results in elevation of transaminases of plasma or serum. These serum activities presumably increase as a result of cellular membrane damage and leakage. Elevated levels of bilirubin often indicate an obstruction of bile flow or a defect in the processing of bile by the liver.

The data obtained during the research work suggested that high dosage of N-acetyl-para-aminophenol (APAP) in the liver could lead to decreased levels of antioxidant enzymes (catalase) and present a significant level of hepatotoxicity in the course of the treatment. However, neroli oil raised the levels of catalase, and GSH against the APAP-induced oxidative stress mediated by ROS and RNS. MDA was one of the main LPO products; its elevated levels could reflect the degrees of LPO injury in hepatocytes. The MDA equivalent of the sample was expressed as moles of TBARS/g of tissue for liver homogenate. Treatment with neroli oil prevented significantly LPO either directly or through GSH by scavenging the free radicals. Neroli oil showed significant reduction in the elevated enzyme level induced by Paracetamol treatment. There is subsequent recovery towards normalization of the enzymes suggesting the capability of the oil to recover the hepatocytes injury (as observed in histopathological observation) to accelerate parenchymal regeneration, thus protecting against membrane fragility and subsequently decreasing leakage of marker enzymes into the circulation. Histological observations of the liver, treated with neroli oil, are found to have normal architecture with mild central vein and sinusoidal congestion when compared with Paracetamol treated liver which signifies hepatoprotective activity of neroli oil.

CONCLUSION

From the studies, it can be concluded that neroli oil showed a significant hepatoprotective effect against Paracetamol induced hepatic damage as depicted by its protective activity on functional, physical, biochemical, antioxidant, and histological changes in the liver.

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AUTHORS CONTRIBUTIONS

Design of research work, data collection, and drafting of manuscript was done by Annasaheb Patil. Review and final editing of manuscript was done by Neha Shivathaya.

CONFLICT OF INTEREST

The authors hereby declare that there are no conflicts of interest.

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