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Original article

Nepeta paulsenii Briq. inhibits hepatic toxicity in albino rats: Phytochemical analysis and chemical profiling

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ABSTRACT

Nepeta paulsenii is a less explored plant found in the South-Eastern hilly region of Pakistan. Local people consider it a medicinal plant. The protective effect of methanol extract of N. paulsenii leaves on carbon-tetrachloride (CCl_4) tempted liver damage in male rats was assessed. Forty-eight rats were equally divided into 8 groups and various concentrations of extract + CCl_4 were induced for 30 days. Crude methanolic extract of N. paulsenii plant was examined through phytochemical analyses and gas chromatograph coupled with mass spectrometre (GC–MS) before biological testing on albino rats. Phytochemical analysis of extract showed coumarins, flavonoids, terpenoids, saponins and betacyanins, while GC–MS results revealed the existence of several compounds belonging to diverse classes, responsible for the hepatoprotective attribute of N.paulsenii. CCl_4 exposure substantially decreased the antioxidant enzymes activity while increased the thiobarbituric acid reactive substances and reactive oxygen species levels. A remarkable increase in the aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase levels was observed in CCl_4 treated rats. Comet-parameters were observed after CCl_4 administration. N. paulsenii extract administration substantially improved the antioxidant enzyme activity, hepatic markers as well as comet parameters and reversed the CCl_4 induced histopathological damages. The results revealed that N. paulsenii has therapeutic effects against CCl_4-induced liver damages.

1. Introduction

The liver is a vital and second-largest organ in the body, representing 1.4–2.4 % of lean body mass (Dai et al., 2021) that plays an essential role in eradicating toxic materials from the body and aids in the digestion of food stuff (Vancells Lujan et al., 2021). The liver involves excretory, vascular, immunological, secretory and metabolic functions and plays a significant role in fat, carbohydrate and protein metabolism (Panzitt and Wagner 2021). Liver is main organ which is attacked by reactive oxygen species because hepatic parenchymal cells are prone to oxidative stress (Shi et al., 2021). Liver diseases can be caused by drugs, virus infiltration from infection or ingestion of toxic chemicals (Cheemerla and Balakrishnan 2021). Toxins are converted into intermediate free radicals that are very reactive and unstable. These radicals produce hepatotoxic effects (Lu et al., 2021). Presently, drugs that are used against liver diseases are often inadequate and have some severe side effects so there is need to explore the alternative medicines to replace with

Abbreviations: OS, Oxidative stress; ROS, Reactive oxygen species; CCl_4, Carbon tetrachloride; LP, Lipid peroxidation; PUFA, Polyunsaturated fatty acid; NaOH, Sodium Hydroxide; $CHCl_3$, Chloroform or trichloromethane; H_2SO_4, Sulfuric acid; HCl, Hydrochloric acid; DNA, Deoxyribonucleic acid; UAF, University of Agriculture Faisalabad; CAT, Catalase; POD, Peroxidase; GSR, Glutathione reductase; TBARS, Thiobarbituric acid reactive substance; NMA, Normal melting agarose; $OOCCl_3$, Proxy trichloromethyl; CCl_3, Trichloromethyl free radical; ALP, Alanine-transaminase; ALT, Alkaline-phosphatase; AST, Aspartate-transaminase; GST, Glutathione-*S*-transferase; GSH, Reduced glutathione; SOD, Superoxide dismutase.

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existing medicines of uncertain efficiency and security (Rubiano et al., 2021).

The models of experiment for hepatotoxicity can be designed by using CCl_4, paracetamol or alcohol. CCl_4 is a strong hepatotoxic agent, generally used as a standard toxicant to provoke chemical injury (Samad et al., 2020). CCl_4 causes accumulation of fat and induces centrilobular necrosis in liver. CCl_4 disrupts the triglycerides and total protein levels (Amir et al., 2021). CCl_4 induced hepatic injury is because of the free radical production and lipid peroxidation (Sajid et al., 2016). CCl_4 forms CCl_3 and $OOCCl_3$ radicals and these radicals bind with PUFA and form peroxy and alkoxy radicals. These radicals induce damages in tissue and alter the activity of different enzymes (Cheng et al., 2022).

Medicinal plants are used to cure many diseases due to their therapeutic potential (Almoshari 2022, Ayidh AlThobaiti 2023). Plants are a source of natural biologically active antioxidant components like alkaloids, flavonoids (15-C compounds), saponins, tannins and these components are present in the different parts of plants which are extracted by proper extraction technique (Pandey et al., 2021). Oxidative stress induces hepatic damage due to many factors such as environmental pollutants, drugs, alcohol, and irradiation which are responsible to cause some liver diseases. Reactive oxygen species (ROS) break the strands of DNA, initiates lipid peroxidation and oxidize all molecules that are present in biological tissues and membranes and cause injury. Due to the high production of ROS and low production of antioxidants, oxidative stress is produced. Antioxidants signify a rational therapeutic strategy to cure and prevent hepatic diseases. A variety of antioxidants present in medicinal and edible plants frequently displays a strong antioxidant abilities (Raeeszadeh et al., 2021).

Genus *Nepeta* belongs to Lamiaceae family having more than 250 species (Talebi 2021). Due to their anti-asthma, diuretic, anti-spasmodic, diaphoretic, antitussive characteristics various species of *Nepeta* genus have been used in preparation of traditional medicines (Alkahtani et al., 2022). Plants of *Nepeta* genus are traditionally used for curative-purposes due to the extensive diversity and high contents of flavonoid, phenolic and terpenoid compounds in *Nepeta* species. Several species of *Nepeta* genus contain antioxidant characteristics and also show anti-fungal, anti-viral and anti-bacterial activities (Azizian et al., 2021). Phytochemicals existing in *Nepeta* plants are beneficial for both animals and humans and most of these phytochemical substances are biologically active compounds which are used for the manufacturing of new drugs (Samad et al., 2020). Many experiments have been carried out on many plants of the *Nepeta* genus, but the *N. paulsenii* Briq. plant is hardly ever used in this respect. To explore the phytochemicals and to investigate the hepato-protective and antioxidant functions of *N. Paulsenii* Briq. Crude methanolic extract against CCl_4 intoxicated rats, the present study was planned.

2. Materials and methods

2.1. Collection of sample and extract-preparation

Nepeta paulsenii Briq, was gathered from Skardu (Gilgit-Baltistan), Pakistan. The plant was identified by Prof. Dr. Mansoor Hameed, and voucher specimen number 1002 was banked at the science herbarium of Quaid-e-Azam University, Department of Botany, Islamabad, Pakistan. The plant sample was collected at mature stage and the identification was carried out on the basis of morphological characteristics of sample. Leaves of *N. paulsenii* plant were dehydrated under shadow at RT and grinded to a very fine powder. Leaves powder was placed in Soxhlet apparatus for methanolic extraction. Extracted material was dried under vacuum with rotary-evaporator.

2.2. Phytochemical-analysis

Various qualitative trials were performed to recognize the classes of phytochemicals that exists in crude methanolic extract of leaves.

2.2.1. Flavonoids assessment

Concisely, 1 mg of specimen was permitted to react with 2 N NaOH (1 mL). Development of light yellow color was the symbol of existence of flavonoids in sample (Harborne 1998).

2.2.2. Coumarins assessment

Sample (1 mg) was mixed with 1 mL of NaOH (10 %). The confirmation signal of the existence of coumarins in sample was the appearance of yellow color (Harborne 1998).

2.2.3. Saponins assessment

A 2 mg solution was applied to distilled water (2 mL) of and mixed robustly. The development of approximately 1–2 cm soapy layer was a proof of the presence of saponins (Harborne 1998).

2.2.4. Terpenoids assessment

Development of reddish brown layer at the interface of 2 reacting layers verified the presence of terpenoids on mixing ½ mg of sample with 3 mL of $CHCl_3$ and 3 mL of conc. H_2SO_4 (Harborne 1998).

2.2.5. Assessment of betacyanins and anthocyanins

One mg sample was boiled for 10 min. in 2 mL of 1 N NaOH. A yellow color formation indicate the betacyanin, and formation of bluish green color indicates the anthocyanins (Trease and Evans 1989).

2.2.6. Assessment of anthraquinones

One mg sample was mixed with 2 mL of diluted 2 % HCl and change in color towards red indicated the presence of anthraquinones in the extracted material (Harborne 1998).

2.3. Gas chromatograph coupled with mass spectrometre (GC–MS) analysis

N. paulsenii was scrutinized for the existence of active compounds for mass determination on the gas chromatograph“ Thermo GC-Trace Ultra Ver; 5.0 ” supporting with a“ Thermo MSSDSQ II. The elements were parted on a 60 m long ’ZB 5-MS Capillary Standard Non-polar Pole’ with a film thickness of 0.25-µm.- The temp. was increased from 70 – 260 °C at a rate of 6 °C per min. throughout the experiment. The rate of flow of carrier gas was 1 mL per min. of helium, while the sample injection vol. was 1 µL. The-characterization of chemical components was based on analogy with those obtained from genuine sample and Wiley library spectra.

2.4. Experimental design

In the UAF animal house, male albino rats were separated for this trial. Tap water and food chaw ad libitum were given to rats. The rats of different groups were kept in separate cages. Eight-groups (n = 6/ group) of male albino rats were established to scrutinize the hepatoprotective role of *N. paulsenii* extract. Group-I was referred as control. Group-II was treated with olive oil and dimethyl-sulfoxide (1:1, v/v) at a dose of 1 mL per kg bw orally. Rats in group III were treated with Olive oil + CCl_4 (8:2, 1 mL per kg bw). CCl_4 + silymarin (50 mg per kg bw) in dimethyl sulfoxide was administered to group IV rats. Doses of 200 and 400 mg/kg bw of *N. paulsenii* extract were orally administered to group V

and VI rats respectively with CCl_4. Dose of 200 and 400 mg/kg bw in *N. paulsenii* extract only were given to VII and VIII group rats respectively.

Trial was carried out for thirty days and after completion; all of the rats were examined then blood sample was stored in sterilized tubes and liver of every single rat was removed out. For removal of plasma, centrifugation of blood carried out for 15 min at 3000 revolution per minutes and kept at −20 °C for further analysis. Liver was washed with ice cold saline. A segment of each liver was packaged in zip lock bag and kept at −20 °C for evaluation of anti-oxidant enzymes and DNA damage studies. And other part of liver was used for histopathological studies. This protocol was certified by the ethical board of Agriculture University, Faisalabad-Pakistan.

2.5. Anti-oxidant enzymes assessment

The assessments of anti-oxidant enzymes were carried out according to the reported procedures (Ijaz et al., 2021, Rehman et al., 2022). Finally, the standard curve was plotted (Hayashi et al., 2007).

2.6. Biochemical studies of serum

Liver marker enzymes, i.e., ALT, ALP and AST were analyzed utilizing relevant commercially available diagnostic kits (Wiesbaden-Germany).

2.7. Comet assay

For the estimation of DNA-injury, process of (Dhawan et al., 2009) was followed. According to this method, a reasonable section of liver tissue was placed and crushed in a number of fragments on autoclaved slides and then immersed in 0.5 % of low melting agarose and 0.6 % of NMA (Normal melting agarose) and let it set at normal room temp. After solidification, these slides were bathed in cold lysis solution for 1 hr. at 4 ℃. For the DNA un-winding, these slides were kept in electrophoretic buffer for 20 min. and then processed for electrophoresis for 30 min. After that, Tris-hydrochloric acid buffer at 25 °C was used to bring pH 7.0 of each slide. Ethidium bromide (1 %) was used for staining of slides before studying under fluorescence microscope. For DNA assessment, injury level image software (TRITEK) was used. Parameters i.e., comet number, olive moment, tail moment, head length, tail length, comet length % DNA contents in head and tail were considered for DNA integrity.

2.8. Histopathological examination

Histopathological analysis was performed to observe hepatic injuries. Samples were fixed in fixative solution (glacial acetic acid10 %, absolute alcohol 70 %, formaldehyde 20 %). For the gradual dehydration process, the tissues were passed through various grades of ethanol (70 %, 90 % and 100 %). For embedding Paraffin wax was used. Thick slices of tissues were cut to the 4–5 μm using spencer rotatory microtome (model 820) was used for this purpose. Lastly stained using the stain (hematoxylin-eosin) which was dissolved in 70 % of alcohol. Light microscope (Nikon model 187–842, Japan) was used to observe these slides at × 40 and microphotography was done using Leica LB microscope was adapted to utilize the photographs.

2.9. Statistical analysis

Results are displayed as Mean ± SEM. The difference between the results of different groups was evaluated as reported earlier (Samad et al., 2020).

3. Results

3.1. Qualitative phytochemical analysis

The phytochemical examination of crude extract is shown in Table 1. The existence of flavonoids, coumarins, saponins, terpenoids and betacyanins in methanol extract of *N. paulsenii* was confirmed by a qualitative study.

3.2. GC–MS analysis

3.2.1. Identification of components

N. paulsenii crude extract was designated for GC–MS study to reveal the compounds accountable for hepatoprotective activity. GC–MS exploration specified that *N. paulsenii* contains 10 chemical-components eluted between 2.0 and 10.0 min. (Fig. 1). The documentation of chemical components was based on a comparison of their mass spectra and relative-rt with those attained from reliable samples and Wiley libraries spectra (Table 2). In *N. paulsenii*, out of these 10 chemical constituents, there was 2-glycerin (4.37 % as per peak area and 2.32 min. R/T), geranic acid (4.87 % and 4.08 min.), 6-octadien-1-ol (2.32 % and 4.22 min.), 2-pentadecanone (3.74 % and 6.53 min.), benzenepropanoic acid (2.20 % and 6.95 min.), ascorbic acid (22.1 % and 7.05 min.), phytol (10.8 % and 7.67 min.), 9, 12, 15-octadecatrienoic acid (36.1 % and 7.80 min.), octadecanoic acid (7.78 % and 7.87 min.) and 1,2-benzenedicarboxylic acid (4.43 % and 9.71 min.) (Fig. 2).

3.3. Effect of crude N. Paulsenii extract on antioxidant enzymes activity

Antioxidant enzymes protect the cell against toxic radical reactions and show defensive effect against cellular damage. Variation in activity of antioxidant enzymes was assessed after CCl_4 treatment to illustrate the protecting effect of *N. paulsenii* extract. Action of CAT, SOD, GST, POD, GSH & GSR were substantially ($p < 0.05$) reduced in comparison with control after CCl_4 administration. *N. paulsenii* extract reinstated the antioxidant enzymes activities in co-treated groups and it was statistically analogous to the group treated with silymarin at its higher dose. Oral administration of *N.paulsenii* extract alone demonstrated a marginal difference in the activities compared to control (Table 3).

3.4. Effect of N. Paulsenii extract on ROS and TBARS levels

Compared to control group, the rats treated with CCl_4 reported a significant ($p < 0.05$) eleviation in ROS and TBARS levels. The levels of TBARS and ROS reduced remarkably ($p < 0.05$) when co-treated with CCl_4 + silymarin as opposed to the CCl_4 group. As matched with CCl_4-intoxicated group, the *N. paulsenii* + CCl_4 groups showed significant decreases in ROS and TBARS levels (dose-dependent). Rats administered with *N. paulsenii* extract alone showed ROS and TBARS levels within standard range (Table 4).

Table 1
Phytochemical analysis of *N. paulsenii* leaves methanolic extract.

Compound class	*N. paulsenii* extract
Flavonoids	+
Coumarins	+
Saponins	+
Terpenoids	+
Anthraquinones	_
Betacyanins	+

(+) present (-) absent.

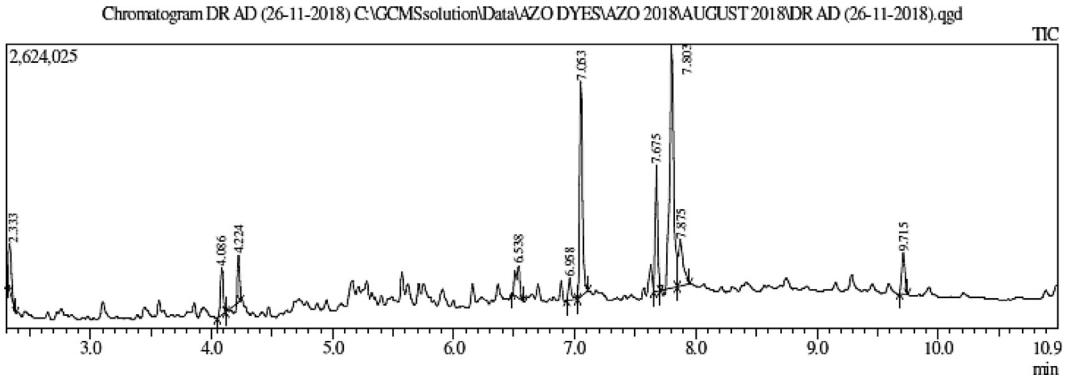


Fig. 1. GC–MS chromatogram of leaves methanol extract of *N. paulsenii* plant.

Table 2
GC–MS analysis of *N. paulsenii* leaves extracts (methanol).

Sr#	Name of compound	Peak area %	Class	R/T (mins.)
1	glycerin	4.37	Alcohol	2.33
2	geranic acid	4.87	Polyunsaturated fatty acid	4.08
3	2,6-octadien-1-ol	2.34	Alcohol	4.22
4	2-pentadecanone	3.74	Ketone	6.53
5	benzenepropanoic acid	2.20	Carboxylic acid	6.95
6	1-(+)-ascorbic acid	22.1	Vitamin C	7.05
7	phytol	10.8	Diterpene alcohol	7.67
8	9,12,15-octadecatrienoic acid	36.1	Linolenic acid	7.80
9	octadecanoic acid	7.78	Stearic acid ester	7.87
10	1,2-benzenedicarboxylic acid	4.43	Plasticizer	9.71

3.5. Effect of N. Paulsenii extract on serum markers

Levels of ALP, AST and ALT are directly linked to oxidative stress in liver tissues (Table 5). Treatment of CCl_4 significantly ($p < 0.05$) enhanced the level of hepatic serum markers (ALP, AST and ALT) which was diminished significantly ($p < 0.05$) by co-treatment with *N. paulsenii* extract. However, in 200 and 400 mg/kg dose of *N. paulsenii* alone treated groups, the serum markers levels were almost comparable to control group values.

3.6. Effect of N. Paulsenii extract on DNA damage

CCl_4 intoxication remained significant ($p < 0.05$) in comet length, comet-numbers, tail length, tail and olive moment, whereas a major decrease was reported in head length and percentage DNA in head as compared with control rats. CCl_4 + silymarin treatment exhibited a notable ($p < 0.05$) reduction in comet number, comet and tail length, % DNA in tail, tail and olive moment and a substantial ($p < 0.05$) rise in percentage DNA in head and head length was observed as compared to CCl_4 intoxicated rats. In identical way, significant reductions in tail length, comet number, comet length, percent DNA in tail, tail moment and olive moment, and a significant increase in percent DNA in head and head length relative to CCl_4 administered rats were observed in *N. paulsenii* + CCl_4 administered groups dose dependently. Rats administered with *N. paulsenii* extract alone showed normal parameters as indicated in the control group (Table 6).

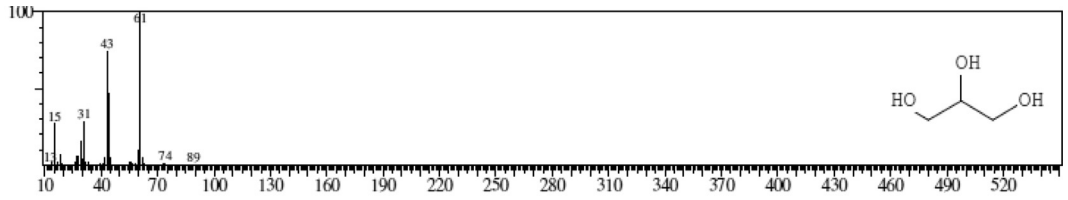
3.7. Effect of N. paulsenii extract on histomorphology of liver

Effect of *N. paulsenii* extract in various groups with different doses and CCl_4 treatment on histology of liver is presented in Fig. 3 (40X). Vehicle control and Control group indicated normal histomorphology and did not show any disruption in central venule and hepatocytes. CCl_4 administration resulted in severe variations that occur in histomorphology of liver tissues. The substantial variations noticed in CCl_4 intoxicated group were deterioration of lobule, fatty change, congested blood vessels and severe steatosis. Co-treatment of *N. paulsenii* extract with CCl_4 exhibited tissue recovery and abolition of chronic damages at different doses. *N. paulsenii* extract (200 mg/kg b.w.) treatment showed a visible level of tissue recovery.

3.8. Discussion

Liver performs metabolic functions in the body. Hepatic diseases are a major problem even in advanced countries (Adewusi and Afolayan 2010). Hepatic injury is related with distortion of these metabolic functions. There is a lack of effective medicines which stimulate the functions of liver and protect the liver from damage and regenerate the hepatic cells so there is need to replace the current medicines with new effective medicines (Adewusi and Afolayan 2010). Mostly new drugs are being synthesized from natural products (secondary metabolites) and from those compounds that are obtained from natural products Genus *Nepeta* have medicinal properties because many species of genus *Nepeta* are used in folk medicines and components found in species of *Nepeta* genus have biological activities (Karaman 2007). Herbal medicines that are synthesized from plants have been used against hepatic diseases for a long time and some preparations are accessible in market. Many active components are present in plants from where herbal medicines are synthesized. Therefore, the present investigation emphasizes the hepatoprotective potential of *N. paulsenii* and its active ingredients, which can be further studied as a pharmacore for reducing the toxic effects of carbon tetrachloride.

Many phytocomponents identified in preliminary phytochemical analysis of extract like coumarins, terpenoids, flavonoids, saponins and betacyanins. Among these phytochemicals, flavonoids, coumarins, saponins and betacyanins have increased a certain importance because of their wide range antioxidant-activities



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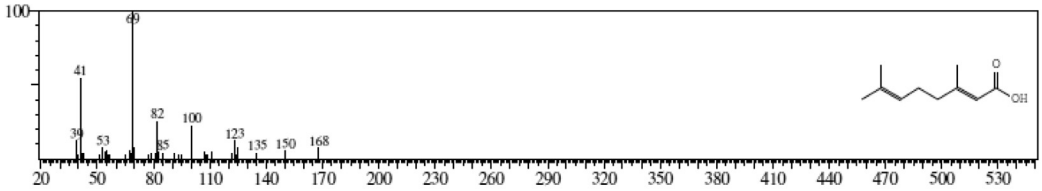

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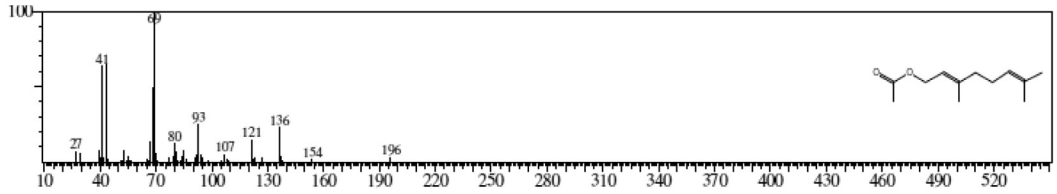

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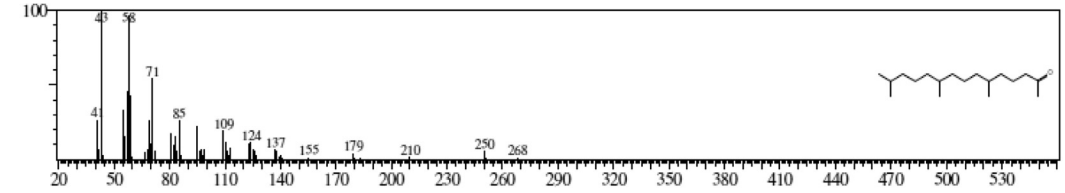

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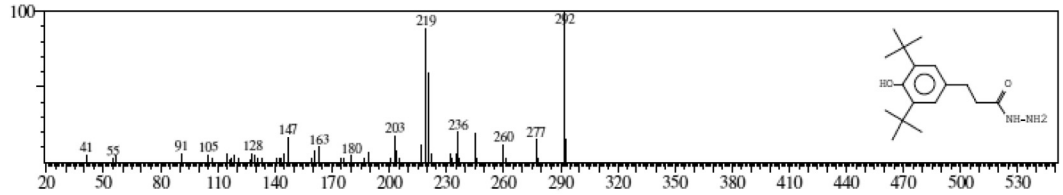
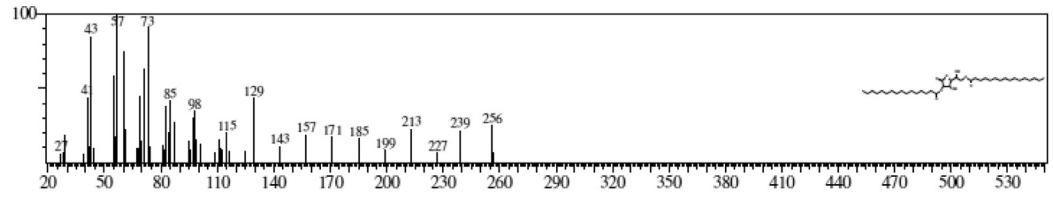


(e)

Fig. 2. **(a)** MS of glycerin (RT: 2.33) **(b)** MS of geranic acid (RT: 4.08) **(c)** MS of 2, 6-Octadien-1-ol (RT: 4.22) **(d)** MS of 2-Pentadecanone (RT: 6.53) **(e)** MS of Benzenepropanoic acid (RT: 6.95) **(f)** MS of 1-(+)-ascorbic acid (RT: 7.05) **(g)** MS of phytol (RT: 7.67) **(h)** MS of 9, 12, 15-octadecatrienoic acid (RT: 7.80) **(i)** MS of octadecanoic acid (RT: 7.87) **(j)** MS of 1, 2-benzenedicarboxylic acid (RT: 9.71).

(Sajid et al., 2016). Ten components identified in further analysis (GC–MS) which have medicinal properties these components are glycerin, geranic acid, phytol, octadecanoic acid, 2,6-octadien-1-ol, 2-pentadecanone, benzene propanoic acid, 1-(+)-pscorbic acid, 1,2-benzene-dicarboxylic acid and 9,12,15-octa-decatrienoic acid. benzenepropanoic and 1-(+)-ascorbic acids have anti-oxidant activity (Akpuaka et al., 2013). So benzenepropanoic acid and 1-(+)-ascorbic acid are foremost components providing to the antioxidant activity of the plant. Benzenepropanoic acid is used to extend the shelf life of foods as an antioxidant, and it is also used to protect the shelved foods from deterioration by microorganisms. Moreover, 9,12,15-octadecatrienoic acid has hepatoprotective effect and it is the one of major constituents of the plant (Akpuaka et al., 2013).



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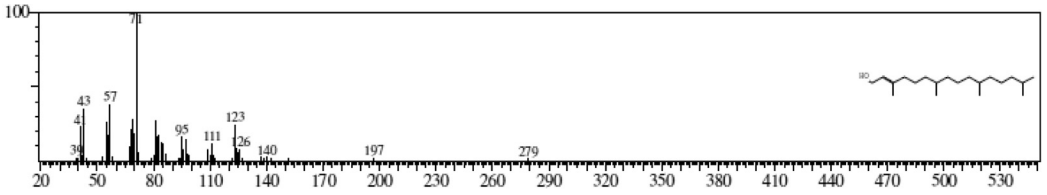

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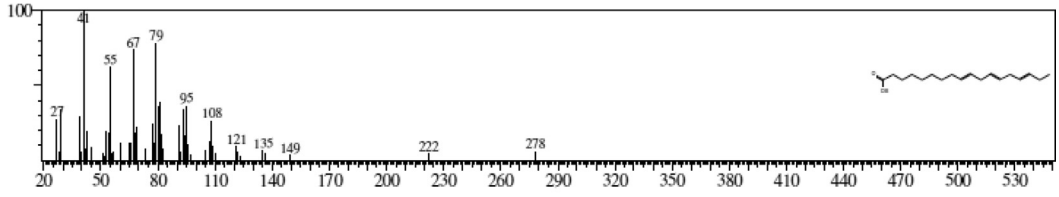

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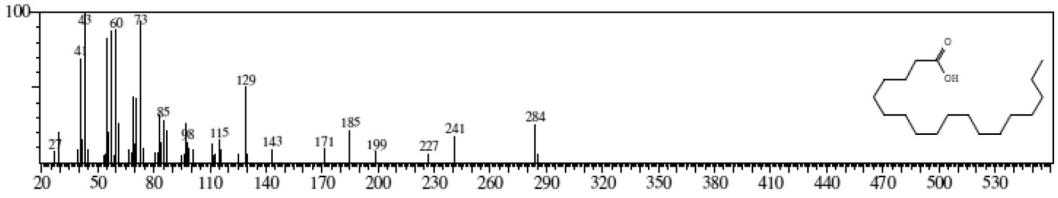
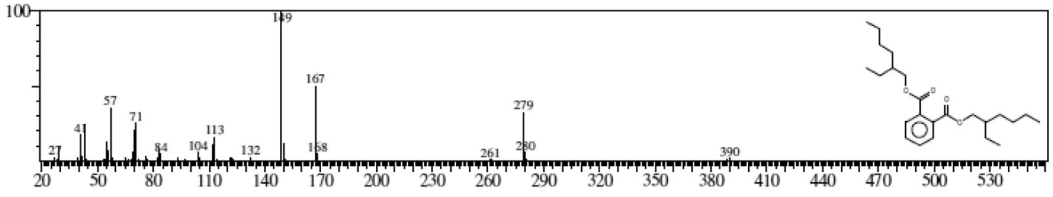


(i)



(j)

Fig. 2 (*continued*)

Antioxidant enzymes are considered to be the foremost species to defend the biological molecules. The metabolic function of the liver is to detoxify the effect of xenobiotics on ROS development, where CAT, POD, SOD, GSH, GSR and GST play a crucial role in oxidative stress deterrence in liver (Khan et al., 2013). CAT reacts with hydrogen peroxide to form molecular oxygen and water. Administration of CCl_4 influenced the activity of POD, CAT, SOD, GSH, GST and GSR while the levels of ROS and TBARS were elevated in CCl_4 administered group. Lipid peroxidation of hepatocytes caused when CCl_4 is metabolized into highly reactive metabolites by liver. The observed decline in the level of CAT, SOD, POD due to CCl_4 exposure is in consensus with former observation of (Sajid et al., 2016), who reported that treatment with CCl_4 reduced the level of these antioxidant enzymes. Reduction in the level of these antioxidant enzymes indicated the oxidative damage in liver (Cheng et al., 2013). Administration of plant extract instigated a noteworthy recovery in activities of enzymatic antioxidants CAT, SOD, POD, GSH, GST and GSR, however, ROS and TBARS levels were

Table 3
Protective effects of methanolic leaf extracts of *N. paulsenii* on anti-oxidant enzymes in rats livers.

Group	CAT (U per min.)	POD (U per min.)	SOD (U per mg)	GSH (nMmin./mg/protein)	GSR (nm NADPH oxidized/min/mg tissue)	GST (mg per dl)
Control	5.69 ± 0.23^{a}	8.45 ± 0.12^{a}	6.28 ± 0.06^{a}	11.8 ± 0.34^{a}	4.37 ± 0.083^{a}	24.63 ± 0.93^{a}
Vehicle control	5.58 ± 0.14^{a}	8.27 ± 0.07^{a}	6.35 ± 0.04^{a}	11.7 ± 0.43^{a}	4.33 ± 0.06^{a}	24.72 ± 0.77^{a}
CCl_4 (1 mL/kg)	2.18 ± 0.10^{b}	3.29 ± 0.06^{b}	2.51 ± 0.06^{b}	5.11 ± 0.13^{b}	0.91 ± 0.04^{b}	10.37 ± 0.37^{b}
Silymarin + CCl_4	5.38 ± 0.20^{a}	6.51 ± 1.00^{c}	5.30 ± 0.05^{c}	9.65 ± 0.24^{c}	3.82 ± 0.04^{c}	21.33 ± 0.41^{c}
N.P (200 mg/kg) + CCl_4	4.64 ± 0.14^{c}	6.56 ± 0.02^{c}	4.72 ± 0.09^{d}	9.47 ± 0.19^{c}	3.41 ± 0.04^{c}	18.56 ± 0.74^{ac}
N.P (400 mg/kg) + CCl_4	4.88 ± 0.04^{c}	6.80 ± 1.70^{c}	5.13 ± 0.03^{c}	9.87 ± 0.13^{c}	3.61 ± 0.07^{c}	21.71 ± 0.65^{c}
N.P (200 mg/kg)	5.68 ± 0.02^{ac}	8.43 ± 0.04^{a}	6.29 ± 0.05^{ac}	11.75 ± 0.22^{ac}	4.35 ± 0.11^{c}	24.67 ± 0.61^{c}
N.P (400 mg/kg)	5.74 ± 0.03^{a}	8.48 ± 0.07^{a}	6.32 ± 0.07^{a}	11.89 ± 0.55^{a}	4.44 ± 0.19^{a}	24.81 ± 1.09^{a}

Superscripts with different alphabets in a same column means significantly different.

Table 4
Protective effects of methanolic leaf extracts of *N. paulsenii* on TBARS and ROS in rats livers.

Group	TBARS (nM/min./mg protein)	ROS (U per mg protein)
Control	14.31 ± 0.47 ^{a}	0.90 ± 0.10 ^{a}
Vehicle control	14.62 ± 1.03 ^{a}	0.97 ± 0.92 ^{a}
CCl_4 (1 mL/kg)	27.59 ± 1.03^{b}	8.41 ± 0.58^{b}
Silymarin + CCl_4	17.14 ± 0.39^{c}	1.81 ± 0.08^{c}
N.P (200 mg/kg) + CCl_4	19.06 ± 0.16 ^{d}	2.09 ± 0.13^{c}
N.P (400 mg/kg) + CCl_4	18.28 ± 0.61 ^{cd}	1.66 ± 0.11^{c}
N.P (200 mg/kg)	14.37 ± 0.25^{c}	0.92 ± 0.11 ^{a}
N.P (400 mg/kg)	14.11 ± 1.46 ^{a}	0.85 ± 0.11 ^{a}

Superscripts with different alphabets in a same column means significantly different.

Table 5
Protective effects of methanolic leaf extracts of *N. paulsenii* on hepatic serum markers in rats liver.

Group	ALT (U per I)	AST(U per I)	ALP(U per I)
Control	41.0 ± 0.40 ^{a}	49.5 ± 1.04 ^{a}	60.3 ± 1.25 ^{a}
Vehicle control	39.5 ± 0.64 ^{a}	46.8 ± 2.39 ^{a}	58.5 ± 1.19 ^{a}
CCl_4 (1 mL/kg)	395.8 ± 3.06^{b}	347 ± 4.02^{b}	230.5 ± 4.40^{b}
Silymarin + CCl_4	81.75 ± 0.85^{c}	87.8 ± 1.10^{c}	93.0 ± 1.82^{c}
N.P (200 mg/kg) + CCl_4	140.3 ± 1.70 ^{d}	144 ± 2.67 ^{d}	170.5 ± 1.55 ^{bd}
N.P (400 mg/kg) + CCl_4	113.5 ± 2.53 ^{cd}	134.8 ± 2.21 ^{d}	141.0 ± 2.48 ^{d}
N.P (200 mg/kg)	41.11 ± 1.93^{c}	47.25 ± 1.10 ^{a}	60.13 ± 0.91^{c}
N.P (400 mg/kg)	37.33 ± 1.10 ^{a}	44.76.0 ± 1.08 ^{a}	58.21 ± 1.25 ^{a}

Superscripts with different alphabets in a same column means significantly different.

significantly reduced. This demonstrates that plant extract can scavenge free radicals that might cause reduction in oxidative damage to liver tissue and restore the antioxidant enzymes activities. The existence of bioactive phytochemicals in the methanolic extract of the *N. paulsenii* may be a major cause of its curative potential.

CCl_4-administration significantly increased the AST, ALP and ALT levels in treated rats. The biochemical damage and cellular function defects can be detected by the levels of these enzymes (Khan et al., 2011). Previous studies have shown that high levels of serum ALT, AST, and ALP have a strong linkage with oxidative liver damage (Sharifi-Rigi et al., 2019). Aminotransferases including ALT, ALP and AST are enzymes ked with liver parenchyma cells. These enzymes are normally present in the cytosol, but due to the damage in plasma membrane of hepatic cells, these markers are released into the bloodstream, which indicates liver damage. So, the level of these enzymes is considered as an important marker that is used in the diagnosis of liver diseases (Carobene et al., 2013). Deleterious effects of CCl_4 may be linked with its reactive metabolites which are formed intermediately during its metabolism in the body. Previous investigations showed that over production of ROS impairs the structural integrity of hepatic cells, as indicated by the unusual increase in hepatic serum markers. CCl_4 treatment caused noteworthy hepatic injury as evident by increased levels of ALP, ALT and AST that are indicator of disturbance in functional-integrity of liver. Treatment with *N. paulsenii* extract reduced the hepatic markers level which is the sign of stabilization in hepatic functions. It has been reported earlier, phytochemicals (flavonoids, coumarins, saponins and betacyanins) act as an antioxidant which acts as both free radical scavengers and antioxidant (Engwa 2018). This reduction in the level of hepatic serum markers can be accredited to antioxidant potential of *N. paulsenii* extract.

DNA can be damaged by several exogenous and endogenous factors. Common factors include ROS produced during the normal metabolic functions, spontaneous or enzymatic conversions and errors during DNA replication (Tiwari and Mishra 2017). It was previously reported that several cytotoxic agents pose genotoxic impacts through the generation of ROS, which results in single/double-stranded breakdowns and disrupt the DNA integrity (Srinivas et al., 2019). In the current investigation, the outcomes of the comet parameters show significant variations in comet parameters. CCl_4 administration induced a remarkable reduction % DNA in the head and in head length compared to the standards,

Table 6
Protective effects of methanolic leaf extracts of *N. paulsenii* on comet parameters in rats liver cells.

Group	Number of comets	Comet length	Tail length	Head length	% DNA in head	% DNA in tail	Tail Moment	Olive tail moment
Control	22.2 ± 0.51 ^{a}	25.61 ± 0.87 ^{a}	4.35 ± 0.11 ^{a}	40.89 ± 0.86 ^{a}	97.17 ± 0.16 ^{a}	2.82 ± 0.16 ^{a}	1.13 ± 0.13 ^{a}	2.41 ± 0.09 ^{a}
Vehicle control	23.7 ± 0.71 ^{a}	25.78 ± 1.22 ^{a}	4.62 ± 0.08 ^{a}	40.83 ± 1.02 ^{a}	96.74 ± 0.16 ^{a}	3.25 ± 0.16 ^{a}	1.17 ± 0.08 ^{a}	2.43 ± 0.09 ^{a}
CCl_4 (1 mL/kg)	48.4 ± 1.61^{b}	58.6 ± 3.34^{b}	17.49 ± 0.51^{b}	24.1 ± 0.55^{b}	86.33 ± 0.21^{b}	13.66 ± 0.21^{b}	2.48 ± 0.14^{b}	4.31 ± 0.18^{b}
Silymarin + CCl_4	30.9 ± 1.21^{c}	33.94 ± 0.67^{c}	6.61 ± 0.18 ^{ac}	35.34 ± 1.54^{c}	95.26 ± 0.14^{c}	4.74 ± 0.14 ^{ac}	1.85 ± 0.04 ^{ac}	2.91 ± 0.04 ^{ac}
N.P (200 mg/kg) + CCl_4	32.9 ± 1.14^{c}	36.22 ± 0.59^{c}	7.94 ± 0.19^{c}	33.33 ± 0.73^{c}	94.56 ± 0.23^{c}	5.43 ± 0.23^{c}	1.96 ± 0.03^{c}	3.08 ± 0.07^{c}
N.P (400 mg/kg) + CCl_4	32.6 ± 1.06^{c}	35.02 ± 0.22^{c}	6.66 ± 0.11 ^{ac}	36.82 ± 0.79^{c}	95.25 ± 0.26^{c}	4.74 ± 0.26 ^{ac}	1.90 ± 0.04^{c}	2.94 ± 0.07 ^{ac}
N.P (200 mg/kg)	23.4 ± 0.87 ^{ac}	25.47 ± 0.51^{c}	4.52 ± 0.12 ^{ac}	40.92 ± 1.03 ^{a}	96.82 ± 0.08^{c}	2.99 ± 0.08 ^{ac}	1.13 ± 0.06 ^{a}	2.42 ± 0.04 ^{a}
N.P (400 mg/kg)	22.1 ± 1.12 ^{a}	24.77 ± 1.46 ^{a}	4.12 ± 0.15 ^{a}	40.08 ± 0.91 ^{a}	95.49 ± 0.27 ^{a}	2.56 ± 0.27 ^{a}	1.01 ± 0.19 ^{a}	2.31 ± 0.09 ^{a}

Superscripts with different alphabets in a same column means significantly different.

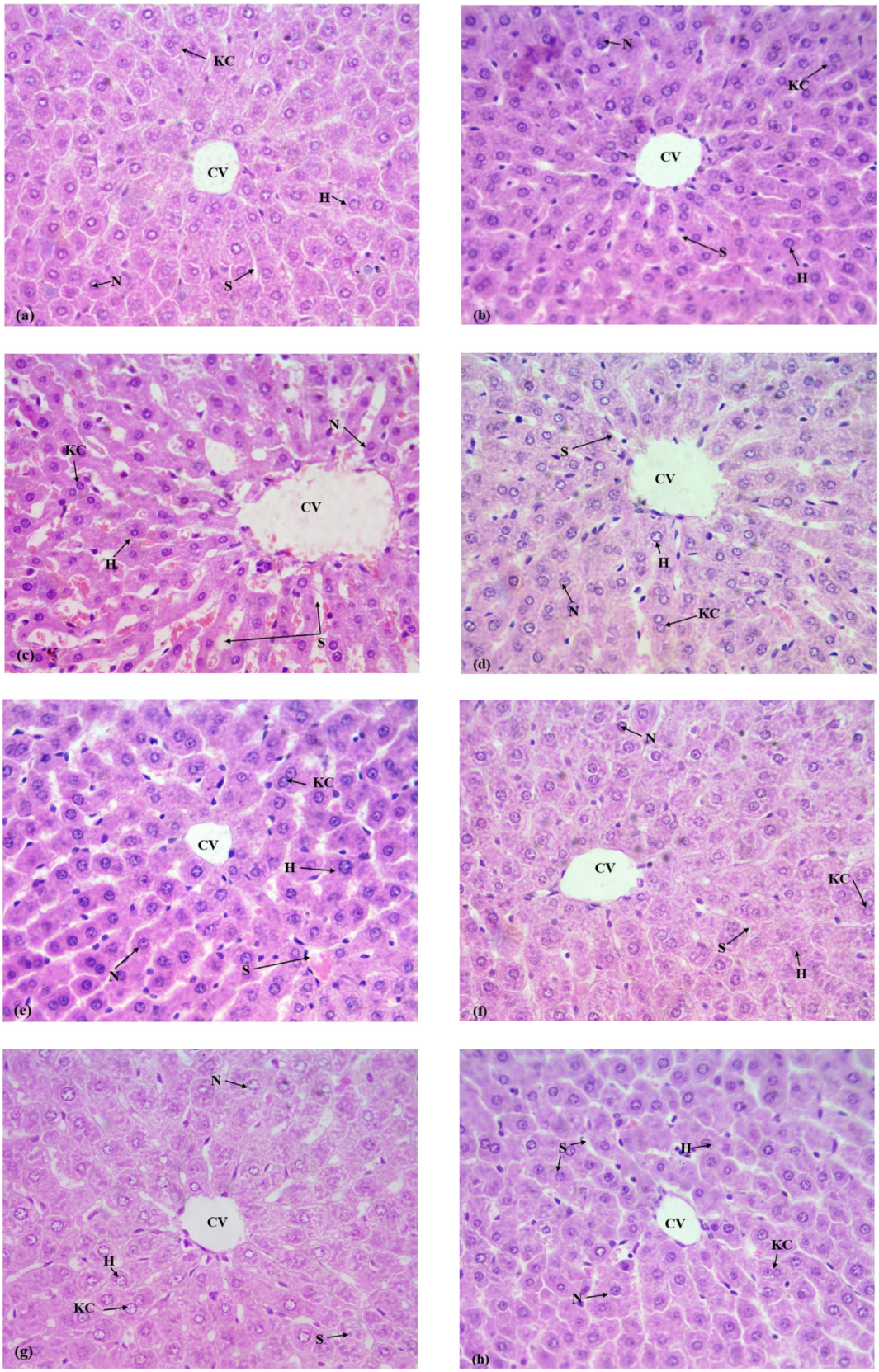


Fig. 3. Microscopic images of liver tissues were obtained from different groups (H&E 40X). **(a)** Normal histoarchitecture (control) **(b)** Vehicle control (normal histoarchitecture) **(c)** CCl_4 (1 mL/kg) Tissues show extensive and marked necrosis **(d)** CCl_4 + Silymarin (decrease in necrosis throughout the liver tissues and recovery of damaged tissues **(e)** N.P (200 mg/kg) + CCl_4 (decreased necrosis) **(f)** N.P (400 mg/kg) + CCl_4 (shows recovery in damaged tissues) **(g)** N.P (200 mg/kg) shows normal histoarchitecture **(h)** N.P (400 mg/kg)shows normal histomorphology: H; Hepatocytes, CV; Central venule, S; Sinusoids, KC; Kupffer cells, N; Nucleus.

on the other hand, increased the comet length, comet numbers, tail length, olive-tail moment, and % DNA in tail. It has been reported that CCl_4 potentially generates ROS, which leads toward DNA fragmentation (Alkreathy et al., 2014). However, *N. paulsenii* plant extract recovered the DNA injury provoked by CCl_4. Our study evaluated that *N. paulsenii* plant extract is a potent bioactive agent to avert CCl_4-induced DNA damage by decreasing OS in the liver tissues which is further supported by (Samad et al., 2020), who observed that *N. paulsenii* plant extract decreased oxidative stress produced due to CCl_4 administration in the testicular tissues of rats.

Histopathological study of liver showed the severe damages like hepatic damages, inflammatory cell infiltration, fatty changes, degeneration of lobular structure and degeneration of nuclei in CCl_4 treated group. Level of fatty degeneration and necrosis were obvious after treatment of CCl_4. CCl_4 induced hepatic tissue injury is due to the production of free radicals and lipid peroxidation that induces cellular damage. CCl_4 forms a CCl_3 and $OOCCl_3$ radicals then these radicals bind with polyunsaturated fatty acids (PUFA) and form peroxy and alkoxy radicals. These radicals produce injury in cell membrane, hepatic damage and altered the activity of different enzymes. These radicals bind with macromolecules and become source of oxidative degradation of cellular membrane leading to necrosis of hepatocytes (Ranawat et al., 2010). Administration of high and medium dose of *N. paulsenii* plant extract reduced the injury and fatty degeneration. This may be due to the presence of phytochemical components like coumarins, terpenoids, flavonoids, saponins and betacyanins. These components exhibited hepatoprotective effect and work individually or combined action against hepatotoxicity. Histological study exhibited a defensive effect of *N. paulsenii* plant extract on CCl_4 induced hepatoxicity which may be attributed to the active components of the plant extract.

3.9. Conclusion

The current study revealed that the hepatoprotective nature of *N. paulsenii* leaves extract is possibly due to the endogenous antioxidant-molecules and scavenging of free radicals. Natural antioxidants tend to promote this effect in *N. paulsenii* plant, which greatly decreased oxidative stress and contributed to normal liver functions. Further research is needed to expose the pathways concerning the hepatoprotective function of *N. paulsenii* at molecular level. Moreover, ethanolic extract of *N. paulsenii* needs to be studied in future as for the intake of oral drugs ethanolic extracts may be relatively safer compared to methanolic extracts. Also, LC-MS based studies needs to be carried out for *N. paulsenii*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102542.

References

Adewusi, E., Afolayan, A.J., 2010. A review of natural products with hepatoprotective activity. J. Med. Plant Res. 4 (13), 1318–1334.
Akpuaka, A., Ekwenchi, M., Dashak, D., et al., 2013. Biological activities of characterized isolates of n-hexane extract of Azadirachta indica A. Juss (Neem) leaves. Nature and science. 11 (5), 141–147.
Alkahtani, J., A. Asma, M. Adil, et al., 2022. Phytochemical Investigation and Antimicrobial Potential of Medicinal Plant Nepeta distans Royle ex Benth. Journal of Food Quality. 2022.
Alkreathy, H.M., Khan, R.A., Khan, M.R., et al., 2014. CCl4 induced genotoxicity and DNA oxidative damages in rats: hepatoprotective effect of Sonchus arvensis. BMC Complement Altern. Med. 14 (1), 1–7.
Almoshari, Y., 2022. Medicinal plants used for dermatological disorders among the people of the kingdom of Saudi Arabia: A narrative review. Saudi Journal of Biological Sciences. 29, (6). https://doi.org/10.1016/j.sjbs.2022.103303 103303.
Amir, M., Ahmad, W., Sarafroz, M., et al., 2021. Hepatoprotective effect of a polyherbal formulation (Aab-e-Murawaqain) against CCl4 induced liver toxicity in Wistar albino rat model by suppressing proinflammatory cytokines. South African Journal of Botany.
Ayidh AlThobaiti, S., 2023. Protective effect Spirulina against Monosodium glutamate-induced hepatic dysfunction: A biochemical, molecular, and histopathological study. Journal of King Saud University - Science. 35, (2). https://doi.org/10.1016/j.jksus.2022.102464 102464.
Azizian, T., Alirezalu, A., Hassani, A., et al., 2021. Phytochemical analysis of selected Nepeta species by HPLC-ESI-MS/MS and GC–MS methods and exploring their antioxidant and antifungal potentials. Journal of Food Measurement and Characterization. 15 (3), 2417–2429.
Carobene, A., Braga, F., Roraas, T., et al., 2013. A systematic review of data on biological variation for alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transferase. Clin. Chem. Lab. Med. 51 (10), 1997–2007.
Cheemerla, S., Balakrishnan, M., 2021. Global epidemiology of chronic liver disease. Clinical Liver Disease. 17 (5), 365.
Cheng, X., Jia, J., Zhang, T., et al., 2022. Heparanase Expression Propagates Liver Damage in CCL4-Induced Mouse Model. Cells. 11 (13), 2035.
Cheng, N., Ren, N., Gao, H., et al., 2013. Antioxidant and hepatoprotective effects of Schisandra chinensis pollen extract on CCl4-induced acute liver damage in mice. Food Chem. Toxicol. 55, 234–240.
Dai, H., Xiang, J., Hou, Y., et al., 2021. Fat mass to fat-free mass ratio and the risk of non-alcoholic fatty liver disease and fibrosis in non-obese and obese individuals. Nutrition & metabolism. 18 (1), 1–12.
Dhawan, A., Bajpayee, M., Parmar, D., 2009. Comet assay: a reliable tool for the assessment of DNA damage in different models. Cell Biol. Toxicol. 25 (1), 5–32.
Engwa, G. A., 2018. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. Phytochemicals: Source of Antioxidants and Role in Disease Prevention. BoD–Books on Demand. 7 49-74.
Harborne, A., 1998. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media.
Hayashi, I., Morishita, Y., Imai, K., et al., 2007. High-throughput spectrophotometric assay of reactive oxygen species in serum. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 631 (1), 55–61.
Ijaz, M.U., Ahmed, H., Ashraf, A., et al., 2021. Vitexin attenuates cisplatin-induced renal toxicity by reducing oxidative stress and inflammation. Journal of King Saud University - Science. 33, (8). https://doi.org/10.1016/j.jksus.2021.101657 101657.
Karaman, S., 2007. Essential Oil Composition of Nepeta cilicia Boiss. Apud Bentham and Phlomis viscosa Poiret from Turke. Int. J, Botany.
Khan, M.R., Khan, G.N., Ahmed, D., 2011. Evaluation of antioxidant and fertility effects of Digera muricata in male rats. Afr. J. Pharmacy Pharmacol. 5 (6), 688–699.
Khan, R.A., Khan, M.R., Sahreen, S., 2013. Attenuation of CCl4-induced hepatic oxidative stress in rat by Launaea procumbens. Exp. Toxicol. Pathol. 65 (3), 319–326.
Lu, S., Zhang, J., Lin, S., et al., 2021. Recent advances in the development of in vitro liver models for hepatotoxicity testing. Bio-Design and Manufacturing. 4 (4), 717–734.
Pandey, S.K., Bhandari, S., Sarma, N., et al., 2021. Essential oil compositions, pharmacological importance and agro technological practices of Patchouli (Pogostemon cablin Benth.): A review. Journal of Essential Oil Bearing Plants. 24 (6), 1212–1226. https://doi.org/10.1080/0972060X.2021.1995511.
Panzitt, K., Wagner, M., 2021. FXR in liver physiology: Multiple faces to regulate liver metabolism. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 1867, (7) 166133.
Raeeszadeh, M., M. Moradi, P. Ayar, et al., 2021. The antioxidant effect of Medicago sativa L.(alfalfa) ethanolic extract against mercury chloride (HgCl2) toxicity in rat liver and kidney: an in vitro and in vivo study. Evidence-Based Complementary and Alternative Medicine. 2021.
Ranawat, L., Bhatt, J., Patel, J., 2010. Hepatoprotective activity of ethanolic extracts of bark of Zanthoxylum armatum DC in CCl4 induced hepatic damage in rats. J. Ethnopharmacol. 127 (3), 777–780.
Rehman, A., Tahir, A., Ehsan, N., et al., 2022. Protective effects of aucubin against nonylphenol-induced liver toxicity by improving biochemical, inflammatory and histopathological indices. Journal of King Saud University - Science. 34, (4). https://doi.org/10.1016/j.jksus.2022.102033 102033.

Rubiano, A., Indapurkar, A., Yokosawa, R., et al., 2021. Characterizing the reproducibility in using a liver microphysiological system for assaying drug toxicity, metabolism, and accumulation. Clinical and translational science. 14 (3), 1049–1061.

Sajid, M., Khan, M.R., Shah, N.A., et al., 2016. Phytochemical, antioxidant and hepatoprotective effects of Alnus nitida bark in carbon tetrachloride challenged Sprague Dawley rats. BMC Complement Altern. Med. 16 (1), 1–17.

Samad, A., Ijaz, M.U., Ashraf, A., et al., 2020. Methanolic extract of Nepeta paulsenii as an ameliorative agent against CCl4 induced testicular damage in male albino rats. Journal of King Saud University-Science. 32 (1), 1168–1174.

Sharifi-Rigi, A., Heidarian, E., Amini, S.A., 2019. Protective and anti-inflammatory effects of hydroalcoholic leaf extract of Origanum vulgare on oxidative stress, TNF-α gene expression and liver histological changes in paraquat-induced hepatotoxicity in rats. Arch. Physiol. Biochem. 125 (1), 56–63. https://doi.org/10.1080/13813455.2018.1437186.

Shi, Y., Hu, Y., Wang, Z., et al., 2021. The protective effect of taurine on oxidized fish-oil-induced liver oxidative stress and intestinal barrier-function impairment in juvenile Ictalurus punctatus. Antioxidants. 10 (11), 1690.

Srinivas, U.S., Tan, B.W., Vellayappan, B.A., et al., 2019. ROS and the DNA damage response in cancer. Redox biol. 25, 101084.

Talebi, S.M., 2021. Leaf Anatomical Evaluation of Some Nepeta L., Taxa in Iran. Iranian Journal of Science and Technology, Transactions A. Science. 45 (4), 1211–1222.

Tiwari, P. and Mishra, 2017. Role of Flavonoids in DNA Damage and Carcinogenesis Prevention'. J. Carcinog. Mutagen. 8 (4) 1000297.

Trease, G., Evans, W., 1989. Pharmacognsy. Macmillian publishers, Brailliar Tiridel Can.

Vancells Lujan, P., Viñas Esmel, E., Sacanella Meseguer, E., 2021. Overview of non-alcoholic fatty liver disease (NAFLD) and the role of sugary food consumption and other dietary components in its development. Nutrients. 13 (5), 1442.