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HEPATO PROTECTIVE EFFECT OF *MYRTUS COMMUNIS* IN CARBON TETRACHLORIDE-INDUCED HEPATIC INJURY IN ANIMAL MODEL

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Abstract

The present study was designed to assess the hepatoprotective potential of Myrtus communis extract against CCl_4 induced liver injury in rats. Liver diseases which are attributed to radical reactions, inflammation and toxins products are among the major ailments affecting human beings all over the world. Myrtus communis is known to contain various bioactive components including flavonoids, terpenes and phenolic acids. This plant has been used in traditional medicine and most notably for the purpose of liver health. For the purpose of evaluating its hepatoprotective efficacy, plant leaves of Myrtus communis were collected, prepared, and subjected to several bio-chemical and phyto-chemical studies. A total of six groups of rats were used in the study and these groups include the control group and the CCl_4-treated groups expected to have liver problem. One group received varying concentrations of Myrtus communis extract together with CCl_4 while the other received only CCl_4. Serum samples were also analysed to determine the levels of liver enzymes (ALT, AST, ALP) as well as antioxidant enzymes (SOD), glutathione (GSH), and malondialdehyde (MDA) and the histological analysis was on the liver tissues. This research findings revealed the lowering of liver enzyme levels in rats which were treated with Myrtus communis, probably resulting in decreased hepatocellular injury. It also raised the levels of SOD and GSH and lowered the MDA level, a fact pointing towards the extract's use in moderation of oxidative stress. Histopathologically it is also evident that liver injury was ameliorated by Myrtus communis treatment as there was a good return of liver tissue architecture, little inflammation and less necrotic areas. Thus, based on the results of this study, Myrtus communis has a high potential for efficacy in the treatment of liver disorders and can be attributed to the group of hepatoprotective agents; the mechanisms of this plant's action are connected to its antioxidant and anti-inflammatory properties. The descriptive study reaffirms it as having natural cure claims on liver diseases and opens up the real possibilities of its therapeutic uses.

Keywords: *Antioxidant, CCl_4-induced injury, Hepatoprotective, Liver damage, Myrtus communis*

INTRODUCTION

Therapeutic plants are as essential part of the human life as health itself and the more science discovers new uses of them, the more vital they become. Such plant is *Myrtus communis* well known for its anti-bacterial, anti-septic, and hypoglycemic properties since a long time ago (1). It is preferred because it is safer, easily accessible, and has few negative effects as compared to synthetic drugs in traditional medicine. As the cases of hepatic diseases increase across the globe, more attention is being paid to natural remedies for the disease with less side effects as those of standard treatments (2). As is witnessed with hepatic injuries induced by toxic agents such as carbon tetrachloride (CCl4), infections can generate health threats of great magnitude (3). It was found that liver injury is normally associated with inflammation and oxidative stress. It is effective, but often, it has side effects, by which the health of the patient is at risk. However, much

research is still lacking in the literature though more evidence has shown that *Myrtus communis* possess hepatoprotective activity. Most of the published studies has focused on the generic health-enhancing properties of the plant with little emphasis on their specificity in chemically induced liver injuries (4). To the best of our knowledge, the current studies fail to elucidate much about the details through which *Myrtus communis* contributes to the liver protection. This work has research relevance since it fills the current research gap by evaluating the hepatoprotective potential of *Myrtus communis* comprehensively. The scope of the study is to provide significant information on the possibility of the traditional medicinal plant to cure liver diseases by the chemical profiling of the plant extract together with the investigation of the possible ameliorative impacts on CCl4 instigated liver damage (5). Besides, the current study focuses on *Myrtus communis* , and increased concentration on this plant may lead to the discovery of new work bioactive compounds with therapeutic impact, whereby new medication for the treatment of hepatic disorders can be developed (6). Finally, the presented work focuses on the need for dose and extracts standardization using *Myrtus communis* as an example. The rationale for the trail is to obtain more accurate estimate of the effectiveness of the plant and reduce variability in the outcomes through establishing procedures for administering the plant and extracting it. This is a significant development towards *Myrtus communis* to be considered as part of the innovative healthcare that is characterized by reliability and consistency.

MATERIALS AND METHODS

PREPARATION OF AQUEOUS ETHANOLIC EXTRACT

Originally, *Myrtus communis* leaves were washed with tap water so that any dust or any unwanted object that may have attached to it can be washed away. Sukim's part of the plant material was sliced into very narrow strips. A mechanical comminution, shedding dried and stored in close vessels were performed on the material. Fresh whole plant was dried, ground into powder then 4 grams of the powdered dry plant material was soaked in a solvent mixture (1:4 deionised water:ethanol) of 400 ml water and 1600 ml ethanol for 7 days. It was implicate that filter paper was supposed to filter the mixture but after that the filtrate was concentrated using rotary evaporator at a temperature of 40ºC under reduced pressure. For later use, the aqueous ethanolic extract (M. C) was dried at 40ºC under heat lamp in the dry heat oven. The whole procedure was made in Swedish college of Rahim Yar Khan.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

MC underwent the general vernalisation for the phytochemical components including but not limited to alkaloids, glycoside, flavonoids, saponin, protein and carbohydrates which were tested for (7). Each of the phytochemical analysis mentioned below was performed in the pharmacology lab of the Swedish college Rahim Yar Khan.

QUANTITATIVE ANALYSIS

ANALYSIS OF FLAVONOIDS CONTENT

The amount of total flavonoids was determined by using aluminium chloride colorimetric method. In trial one 3 ml of methanol was added with 1 ml of solution i. e. 1 mg/ml. Next, 5.6 ml of the filtered water and, 0.96 µl of 1 M K Acetate solution, 0.2 ml of 10% aluminium chloride ($AlCl_3$) were added. After the 30 minutes of dark incubation, the mixture that was formed was read for absorbance value at 420nM with reagent blank which did not contain extract. As a blank, distilled water was used in the study. The standard concentrations of quercetin employed include 1, 0.50, 0.25, 0.10, 0.05, 0.02, 0.01 and 0, mg/ml used to establish a quercetin equivalent (QE) mg/g of total extract curve. Quercetin dihydrate with purity greater than 98% was used in the study and ten milligrammes were dissolved in ten millilitres of 80% ethanol and made up to a volume of fifty millilitres to give the quercetin stock solution at a concentration of 1 mg/ml. After that, 80% Ethanol was used to dilute the stock solution to the concentrations used for making the standard curve. This analysis was done in Swedish College RYK to define the financial management of a company specifically within the scope of the organization.

ANTIOXIDANT TESTING

DPPH RADICAL SCAVENGING ACTIVITY

These findings were further supported with DPPH free radical scavenging assay Confirming the antioxidant potential of extracts from plants. To determine the ability of Extracts in the removal of free radicals and possible antioxidant activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (C18H13N5O6) as the free radical containing, a 1% TWEEN solution was prepared. Namely, standard DPPH solution containing 0.004g of DPPH per/ml of HPLC grade methanol was prepared. Secondly, an entity that has just been produced in the manufacturing process of the third order is a newly created 3.5 ml DPPH solution was combined with 0.25 ml of an extract solution of compound prepared in methanol. Both the materials were then blended together effectively and then the mixture was incubated at 28°C in a dark area for a period of 30 minutes. After that, for determination of absorbance at 517 nm, Spectra-Max 190 coming from Meigu Molecular International Co. Ltd. Shanghai, China, was used. Methanol was used as the negative control while DPPH and methanol were used as the experimental controls. A theoretical equation develop from the decrease of the absorbance was used to approximate the inhibition percentage of the obtained DPPH solution. With reduced absorption, higher free radical scavenging activity was suggested for both the plant extracts tested. This study was done in BZU pharmaceutical lab. The formula is as follows: A Sample is the sample absorbance of the solution and Ablank is control on the absorbance of the solution (8).

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Structural studies of the extracts were conducted using functional group analysis using Fourier Transform Infrared spectroscopy (FTIR). The spectra recorded had a resolution of 4 cm^{-1} and the range covered was from 400-4000 cm^{-1}. Background spectrum was also recorded and was subtracted from the sample spectrum to arrive at the final FTIR spectrum (9).

LABORATORY ANIMALS

Male Wistar albino rats were bought from Multan, Pakistan, weighing 200-250g were used in this experiment and maintained at 25°C, 60% relative humidity and 12:12 light/dark cycle. They were also provided with basics such as food throughout the day as seen to have consumed according to research. There was water followed by grumbling for all people. This study was carried out in Pharmacology lab, BZU using Berna Biotech rabbit anti human selenoprotein P, conjugated to horseradish peroxidase.

INDUCTION OF HEPATIC DAMAGE

More than 2 ml/kg is considered as the lethal dose in 50 percent of patients. In case of human, for 14 days, toxic effects will be seen at doses between 0. Five and two milliliters per kilogram. The rats were accordingly given CCl4 1ml/kg I. P. for 14 days to induce hepatotoxicity in the rats (10).

EXPERIMENTAL DESIGN

Each experimental group consisted of eight rats, which were randomly divided into six groups. Group A, serving as the positive control**,** consisted of healthy rats that received no treatment. To induce liver injury, Groups B through F were administered carbon tetrachloride (CCl_4) intraperitoneally at a dose of 1 mL/kg for 14 consecutive days. Group B functioned as the negative control and received only CCl_4 without any therapeutic intervention.

Groups C, D, E, and F were treated with varying concentrations of *Myrtus communis* extract. Specifically, Group C was administered 100 mg/kg CCl_4 along with silymarin (as the reference standard), while Groups D, E and F received *Myrtus communis* extract at doses of 200 mg/kg, 300 mg/kg, and 400 mg/kg, respectively, in combination with CCl_4.

After three weeks of treatment, liver tissues and blood samples were collected for biochemical analysis to evaluate the hepatoprotective potential of the aqueous extract of *Myrtus communis*. Approximately 5 ml of blood was drawn from each rat via the tail vein. The serum was separated by centrifugation at 1500 RPM for 10 minutes and stored at −60°C until further biochemical analysis.

Liver enzyme activities—including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)—were measured using commercially available diagnostic kits (Biomerieux, USA), following the manufacturer's protocols.

PREPARATION OF LIVER HOMOGENATE AND MEASUREMENT OF OXIDATIVE STRESS PARAMETERS

The liver tissues were then pulverised using liquid nitrogen. The tissues were homogenised on ice using a Ultra-Turrax homogenizer which was set at 9500 rpm after washing the tissues with HEPES buffer in superoxide dismutase (SOD) activity measurement and with RIPA for measuring malondialdehyde (MDA). The supernatants obtained after homogenates' centrifugation were used for measuring the quantities of SOD and MDA with the help of the chemical reaction in the ELISA kits designed for rats (Cayman Chemical for SOD and TBARS Assay STA-330 for MDA). Using Sedlak and Lindsay technique the GSH levels in the liver tissues were established to be reduced. Two millilitres of fifty millilitres of Tris-HCl buffer at pH 7.5, twenty millimolar EDTA and 0. To homogenise the liver tissue 2 millimeters of sucrose were applied. 5. Precipitate of 5-dithiobis (2-nitrobenzoic acid) was added to the homogenate and was then centrifuged for 40 minutes at 4°C at 4200 rpm. The GSH of the supernatant was determined next. The level of GSH was assessed using spectrophotometer at wavelength of 412 nm. SOD activity was represented by the expression's U/mg tissue, serum GSH was represented by the expression of μM/mg tissue, and MDA was also represented by the expression of μM/mg tissue (11).

HISTOPATHOLOGICAL EXAMINATIONS

The rats' livers were then fixed in 10% formalin for a whole day. Following fixation, the tissues were washed with distilled water and then dehydrated with 100% Ethyl alcohol before washing again in tap water. After washing the specimens in xylene the specimens were autoclaved at 50°C and kept in paraffin for a day. The tissues which were treated were later embedded in paraffin blocks, from which sections of a thickness of 4 μm were prepared using sledge microtome and later placed on the glass slides. For histological assessment the slides containing tissues were deparaffinized, stained with Haematoxylin and Eosin and the tissues were then analyzed every two, four and six weeks (4).

STATISTICAL ANALYSIS

Data was analyzed using a program known as GraphPad Prism (GraphPad Software Inc., La Jolla, CA USA) for all the statistical tests. Statistical analysis was done with analysis of variance (ANOVA), and all the results were given as mean ± standard deviation. Each contribution was considered statistically significant where p-value was ≤ 0. 05.

RESULTS

PHYTOCHEMICAL SCREENING OF *MYRTUS COMMUNIS* FOR PRIMARY AND SECONDARY METABOLITES IDENTIFICATION

Phytochemical screening of *Myrtus communis* revealed the presence of a substantial quantity of both primary and secondary metabolites, as detailed in Tables I and II. Among the primary metabolites, carbohydrates were detected using Molisch's test, indicated by the formation of a violet ring at the interface, with a moderate intensity (++). Lipids were confirmed through the Sudan III solubility test, which produced a turbid mixture, suggesting a strong presence (++). Proteins, particularly glycine, were identified by the Biuret test, evidenced by a violet coloration and a corresponding intensity of (++).

The plant also demonstrated a rich profile of secondary metabolites. Alkaloids showed strong positivity (+++) when tested with Wagner's reagent. Tannins were confirmed using the ferric chloride test, indicating their presence. Glycosides were identified using the Keller-Kiliani test, with a notable intensity of (+++). Furthermore, the foam test and the olive oil emulsion test both confirmed a high presence of saponins.

These findings highlight the presence of multiple bioactive compounds in *Myrtus communis*, supporting its potential pharmacological applications.

Table I. Phytochemical screening of *Myrtus communis* for primary metabolites identification

Class of primary metabolite	Test name	Observation	Intensity	Detection
Carbohydrates	Molisch's test	A violet ring at the interface	+++	Carbohydrates exist
Lipids	Solubility test (Sudan III Test)	Turbid(cloudy) mixture is formed	+++	Lipids are Present
Proteins	Biuret test	presence of a violet color	+++	Indicates glycine presence

+++ indicates strong presence

Table II. Phytochemical screening of *Myrtus communis* for Secondary metabolites detection

Name of Secondary metabolites	Test name	Results
Alkaloids	Wagner's test	+++
Tannins	Ferric chloride test	+++
Glycosides	Keller kiliani test	+++
Saponins	Foam test test	+++
	Emulsion test with Olive Oil	+++

+++ indicates strong presence

Table III presents the quantitative analysis of total flavonoid content in *Myrtus communis,* expressed as quercetin equivalents (QE) per gram of extract. The results indicate variation among the samples analyzed. Sample 1 was diluted to an absorbance of 0.560, corresponding to a flavonoid content of 30.0 mg QE/g of extract with a standard deviation of ±0.25. This relatively high standard deviation suggests notable variability, indicating that the flavonoid content in this sample is above average. Sample 2 exhibited an absorbance of 0.478, with a flavonoid content of 25.5 mg QE/g and a standard deviation of ±0.19. This represents the lowest flavonoid concentration observed among the samples analyzed. Sample 3 recorded the highest flavonoid concentration, with an absorbance of 0.612, yielding 32.0 mg QE/g and a standard deviation of ±0.22. This result is consistent with the trend observed across the selected samples, reinforcing the high flavonoid presence in *Myrtus communis* . Sample 4 showed an absorbance of 0.500, corresponding to a flavonoid content of 28.0 mg QE/g, while Sample 5 recorded an absorbance of 0.530 and a flavonoid content of 29.7 mg QE/g, with a standard deviation of ±0.18. Overall, the flavonoid content in all samples demonstrated relatively minor variations, confirming a consistent and strong presence of flavonoids in *Myrtus communis* extracts.

Table III. Total flavonoid content in *Myrtus communis*

Sample ID	Absorbance at 420 nm	Quercetin Equivalent (mg/g of extract)	Standard Deviation (±SD)
Sample 1	0.560	30.2 mg	± 0.25
Sample 2	0.478	25.5 mg	± 0.19
Sample 3	0.612	32.8 mg	± 0.22
Sample 4	0.500	28.6 mg	± 0.21
Sample 5	0.530	29.7 mg	± 0.18

ANTIOXIDANT ACTIVITY OF MC EXTRACT

The percentage inhibition of the *Myrtus communis* extract (Fig. 1) was determined at different concentrations (10, 20, 30, 40 and 50 µg/ml) and compared with the standard antioxidant ascorbic acid. At the conc of 10µg/ml ascorbic acid had 36±2% anti-oxidant, crude *Myrtus communis* had 39±2%, and *Myrtus communis* extract had 43±1% anti oxidant activity. Concentration of 20 µg/ml was observed to enhance the antioxidant activity of ascorbic acid to 46% (±2), crude extract to 45% (±2) and the extract to 47% (±2). At 30 µg/ml the activity of ascorbic acid increased to 62% (±3. 1), with the crude extract activity at 52% (±3. The IC 50 compared as follows: ascorbic acid at 40 µg/ml showed 71 % activity (±2), the crude extract 65% activity (± 2) and the extract 77% (±2) activity. Lahore water-bath showed maximum activity of 82 % ± 1 at 50 µg/ml, ascorbic acid and *Myrtus communis* extract were found to be 73 ± 1 % and 80 ± 1 % respectively. These

outcomes confirm the correlation between the concentration of *Myrtus communis* extract and its antioxidant activity its efficacy is nearly the same as ascorbic acid at higher concentration indicating the high antioxidant properties of the plant extract.

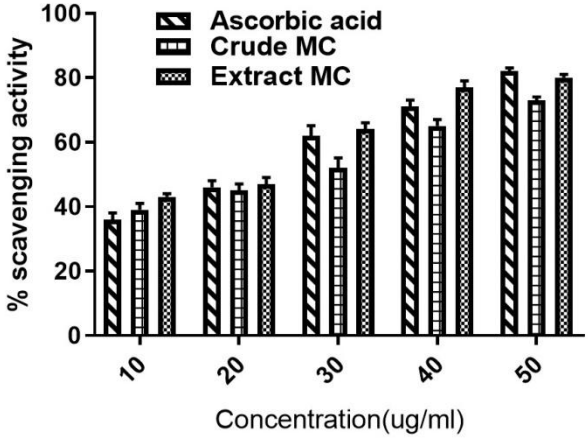

Fig. 1. Antioxidant activity of *Myrtus communis* (MC)

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

FT-IR spectra of *Myrtus communis* extract has shown different bands at 3284 cm^{-1} attributed to O–H stretching vibrations and at 2967 cm^{-1}/Si C–H and CH_2 vibrations of aliphatic hydrocarbons Fig. 2 (a & b). Moreover, C=O stretching vibrations are confirmed by the presence of peak 1693 cm^{-1} and C=C stretching vibrations are confirmed by the band 1518 cm^{-1}. Basically, the band starts at 1020. 19 cm^{-1} is O–H deformation accountable for hydrogen bonding in the HCH complex. There is a very sharp peak at 3244. In the final form the value is expressed in the dilutions of 12 cm^{-1} pointing to the O–H groups' presence in the chemical compound. Other signals that can be associated with C–H asymmetric stretching, C=O of aromatic rings, and C – OH bending are also present at 1605.91 cm^{-1} and 1025.53 cm^{-1}, respectively.

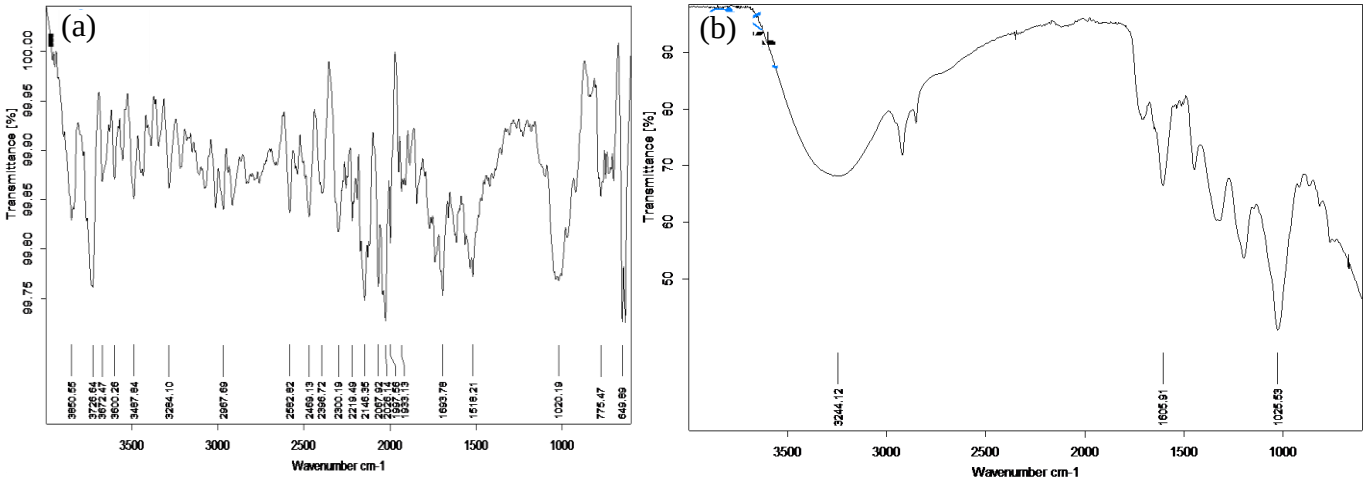

Fig. 2 (a). FTIR of Crude MC; **(b).** FTIR of final form of extract

HEMOSTATIC EFFECT OF MC EXTRACT IN LIVER

Haemostatic effect of MC extract was compared to chitosan and a control group (Fig. 3 a & b). The control group required 315 seconds for haemostasis with standard deviation of ±15 which implied that clotting process is slow. In contrast, due to chitosan`s well-known haemostatic properties, the duration of coagulation decreased to 170 sec with lesser variability and was no more than ±10. In this case, *Myrtus communis* extract has a greater impact as Attestation time was reduce to 110 seconds using a standard variation of ±12. Such research outcomes indicate the possibility of using *Myrtus communis* extract as a natural blood clotting promoter since it exerts a highly haemostatic effect that is significantly greater than that of chitosan and the control.

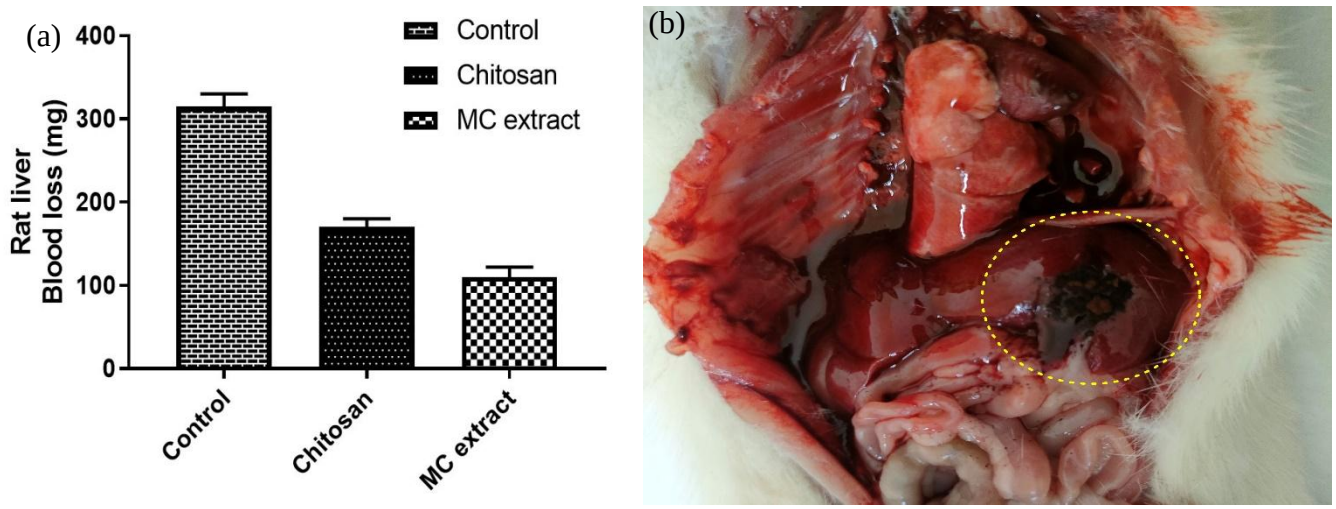

Fig. 3 (a & b). Hemostatic effect of MC resin in liver

EFFECTS OF *MYRTUS COMMUNIS* EXTRACT ON LIVER ENZYMES IN RATS (BIOCHEMICAL ANALYSIS)

The data for liver enzymes, including ALT, AST, and ALP are presented in Table IV. Elevated serum levels of these enzymes are typically indicative of hepatic injury, as they reflect the leakage of hepatocellular enzymes into systemic circulation. In the present study, a significant reduction in ALT, AST, and ALP activities was observed in rats treated with *Myrtus communis* (MC) extract. This suggests that the extract may exert a hepatoprotective effect, potentially by preventing the leakage of these enzymes from hepatocytes into the bloodstream, thereby indicating no apparent hepatocellular damage.

Table IV. Effects of *Myrtus communis* extract on liver enzymes in rats

Groups	ALT (IU/L)	AST(IU/L)	ALP(IU/L)
Control	26.01±1	55±2.5	80±6.5
Standard	14.4±1	42±1.5	72±5.5
MC extract (100 mg/kg)	20.4±1	52±1.5	78±5.5
MC extract (200 mg/kg)	18±1	48±1.5	74±5.5
MC extract (300 mg/kg)	16±1	46±1.5	70±5.5
MC extract (400 mg/kg)	15.5±1	44±1.5	65±5.5

Table V illustrates the enhancement of the antioxidant defense system following administration of *Myrtus communis* (MC) extract, as evidenced by increased superoxide dismutase (SOD) activity and elevated glutathione (GSH) levels, along with a marked reduction in malondialdehyde (MDA) levels. Given the polyphenol-rich composition of MC extract, these findings support the conclusion that its hepatoprotective effects are largely attributable to its antioxidant properties.

Table v. Effects of *Myrtus communis* L. berries on liver tissue SOD activity and GSH, MDA levels

Groups	SOD	GSH	MDA
Control	12.01±1.08**	2.77 ±0.09**	23.54 ± 0.97**
MC extract (1000 mg/kg)	13.52±0.92**	2.86 ±0.09**	21.79 ± 1.03**

Results are mean±SD, MC: *Myrtus communis* extract, DM: *: p<0.05, **: p<0.001

HISTOPATHOLOGICAL EXAMINATIONS

Fig. 4 (a, b, c) illustrates distinct histological differences among the livers of rats treated with *Myrtus communis* extract, untreated controls, and those exposed to carbon tetrachloride (CCl_4). Fig. 4 (a) presents the normal hepatic architecture characterized by well-organized hepatocytes without signs of inflammation or cellular injury. In contrast, Fig. 4 (b) reveals significant pathological alterations in the CCl_4-treated group, including hepatocellular necrosis, inflammatory cell infiltration, and fatty degeneration—hallmarks of acute hepatotoxicity. Notably, Fig. 4 (c) demonstrates substantial histological recovery in rats treated with *Myrtus communis* extract post-CCl_4 exposure. The liver tissue shows evident regeneration, reduced inflammation, and restoration of cellular integrity, indicating the hepatoprotective potential of *Myrtus*

communis. This effect is likely mediated through the promotion of hepatocyte regeneration and repair mechanisms.

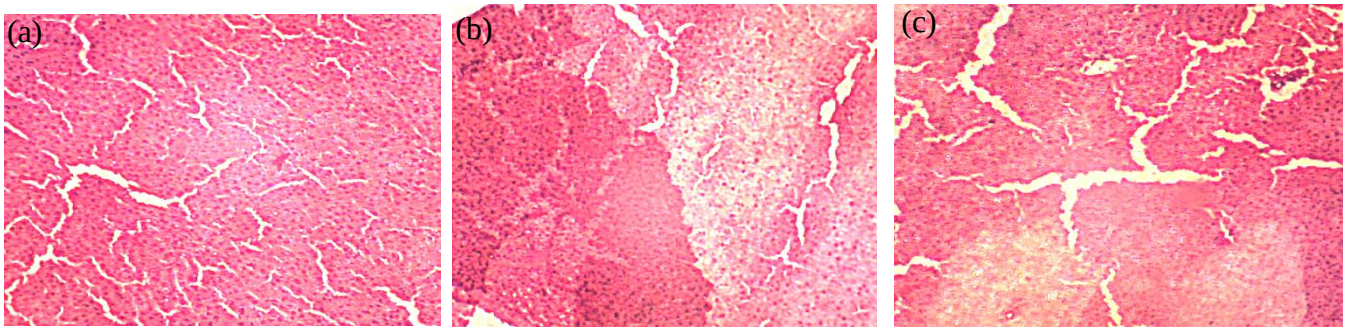

Fig. 4 (a). Healthy rat liver; **(b)**. CCL_4 induced rat liver; **(c).** Rat treated with MC extract

DISCUSSION

It was found from FT-IR spectra of *Myrtus communis* extract, it has their own characteristic peaks that belong to different functional groups. The band at 2967 cm^{-1} is characteristic for C–H and CH_2 vibrations that point to aliphatic hydrocarbons presence; Powerful band at 3284 cm^{-1} is attributed to O–H stretching vibrations proving the existence of hydroxyl groups. This is noticed by the peak at 1693 cm^{-3} attributed to carbonyl group and this result is as a result of C=O stretching vibrations. The band for 1518 cm^{-3} is attributed to C=C stretching which is probably due to the presence of aromatic rings. Further, there is a perfect match for the width and centroid scaling of the O-H deformation mode with the peak position at 1020.19 cm^{-1}. In the final form of the extract, the spectra in relation to C-H asymmetric stretching and C-OH bending are presented at 1605.91 cm^{-1} and 1025, respectively, and a broad peak at 3244 CM^{-1}. at 12 cm^{-1} is also attributed to O-H groups (12). In accordance to the above mentioned findings, the presence of flavonoids and polyphenolic chemicals are confirmed, and which are in line with the FT-IR data. In the phytochemical screening, *Myrtus communis* was reported to consist of a high concentration of primary and secondary metabolites (13). There were the proteins, the lipids and the carbohydrates clearly outlined which denote the structure and nutrients of the plant respectively. There was also presence of secondary metabolites which included saponins, glycosides, tannins and alkaloids, all of which were present in high concentrations (14). Some of these have also been reported in prior studies with emphasis on their roles to the medicinal value of the plant such as anti-inflammatory and antioxidant properties. Translating the total flavonoid values into quercetin equivalent, it was observed that the total flavonoid concentrates of *Myrtus communis* was not constant with sample variation between 25.33ug QE/g to 5mg QE/g to 32.8 mg QE/g. This goes well with other studies which reveal that *Myrtus communis* has high flavonoids, which are compounds with antioxidant properties. The plant extract displayed relatively potent free radical quenching activity particularly at high concentrations and performed almost as efficiently as ascorbic acid which is a standard antioxidant (15). The statistical data ensuing from the antioxidant activity confirm this. This is true as demonstrated by the plant's high antioxidant activity which as has been established in various researches on polyphenolic content of the plant.

Reflecting on the hemostatic effectivity, the *Myrtus communis* extract significantly decreased the clotting time as compared to the control and the standard hemostatic compound – chitosan. Haemostasis is the clotting that occurs when the blood vessels are damaged, and the extract further reduced this time from 170 seconds when using chitosan to about one minute, thirty-five seconds. This outcome strengthens the extract's strong hemostatic properties that are in accord with the traditional uses of this plant for the healing of wounds as well as what was noted in other experiments involving plant-based hemostatic remedies. Liver enzyme analysis revealed that rats' levels of ALT, AST and ALP dropped significantly meaning that the rats were shielded from hepatic injury by the *Myrtus communis* extract. These findings are consistent with earlier studies that have reported that *Myrtus communis* has hepatoprotective significance, for which the plant presumably helps to avert by reducing the degree of enzymically leakage into the blood-stream. Furthermore, the fluidity of the antioxidant system was improved and was demonstrated by increased

levels of SOD activity, increased GSH levels and reduced MDA levels in the rats treated with *Myrtus communis* extract. This is in agreement with earlier finding which identified the importance of polyphenolic constituents in the enhancement of the body antioxidant status and suggest that the observed hepatoprotective effects of the extract are as a result of its antioxidant properties. The hepatoprotective activity of *Myrtus communis* is further evidenced by histological examination study. Oxidative changes such as necrosis, inflammation, fatty changes that ensued from CCl_4 intoxication were significantly less in the group that received *Myrtus communis* extract. The extract seems to promote liver repair and remodeling in the experimental animals as a result of extensive improvement in liver architecture, including reduction in inflammation in rats treated with the extract. These results are in parallel with prior studies that identified M. communis for its hepatoprotective impact in liver injury models.

CONCLUSION

Myrtus communis contains significant hepatoprotective properties within a selected scope, and particularly against liver injury proceeded by CCl_4. Phytochemical screening confirmed the existence of flavonoids, alkaloids tannins as well as saponins which were important bioactive substances that enhanced the plant's efficacy for medicinal use. Other functional groups associated with these bioactive substances were identified using FT-IR and they included functional groups relating to anti-inflammatory and antioxidant activity. *Myrtus communis* shows relatively high free radical scavenging activity comparable to ascorbic acid based on the antioxidant assays pointing to the plant's strong antioxidant potential. As a result of the biochemical analysis, there was significantly less amount of the liver enzymes such ALT, AST and ALP in the rats receiving *Myrtus communis* extract, implying that the extract has a good potentiation against hepatic injuries. Moreover, an increase in antioxidant markers in the liver and kidney which is shown by the increase in SOD activity, increase in GSH levels and decrease in MDA levels confirms that hepatoprotective effect of *Myrtus communis* is due to antioxidant potential. Histological assessments further confirmed the effectiveness of *Myrtus communis* extract in halting CCl_4-mediated hepatotoxicity because there were alterations in the architectural pattern of liver, decreased inflammation and reduction in the area of necrosis. These findings concur with other findings that shed the possible of the plant as a hepatoprotective agent. Taking all these factors into consideration, this study thus calls for further research on bioactive compounds of *Myrtus communis* so as to come up with safer natural plant based hepatoprotective drugs as well as endorsing the use of this plant as a natural remedy for ailments affecting the liver.

Authors' contribution:

SAF and MS Conceptualization and conduct research; AS and MAK Methodology; ZA and SN Formal Analysis; MU and AR Writing, Review & Editing; MN and HUA Supervision.

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