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Phytochemical and Biological Evaluation of *Cassia tora,* L. Seeds

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Abstract:*In the present study the total and the defatted alcoholic extracts of seeds of Cassia tora Linn. (Leguminosae) were screened for hepatoprotective activity using adult Wister albino rats (120-170 g) as the experimental animals. Hepatic injury caused by carbon tetra chloride, was analyzed through estimation of AST, ALT, ALB and platelets in blood samples taken from the veins of orbital plexus of each animal as well as the histopathological examination of the liver.The effects of the extracts were comparable with standardhepatoprotective drug Silymarin. On the other hand GC-MS analysis was performed on the fatty acid composition of the lipoidal fraction for the seeds. The separated fatty acids were converted to their methyl ester and then subjected to the analysis.*

Keywords:*Fatty acids, Hepatoprotective, Silymarin, Carbon tetra chloride, Cassia tora*.

I. Introduction

There is much interest created by natural resources such as herbs and seeds for their therapeutic activity and treatment of diseases in man. Liver disorders are a world problem, despite its frequent occurrence, high morbidity and high mortality, its medical management is currently in adequate[1].*Cassia tora* (C. tora) (sub-family: Caesalpinioideae;Family: Leguminosae/Fabaceae)is a well-known oriental herb or undershrub, found as a rainy season weed throughout India[2],[3].The seeds of *Cassia tora,*L. (Leguminosae) have been conventionally used throughout the Asian region of several countries .They are reputed in Chinese and Ayurvedic medicine to treat night blindness, hypertension, hypercholesterolemia and constipation. Its roasted seeds are popularly used as a tea in Korea. Seeds of *Cassia tora,*L. are reported to possess various pharmacological activities including hypolipidemic, antihepatotoxic, hypoglycemic, antimutagenic and antifungal[4]. Few reports have been traced concerning the fatty acid composition of the lipoidal fraction for the seeds. This encouraged the authors to investigate them as a trial to complete the phytochemical picture of *C.tora,* L. species so the lipid fraction obtained after defatting the alcoholic extract was saponified and the fatty acids fractions were converted to their methyl esters and were analyzed by GC-MS.Dealing with the biological study, most of the reports proved the different parts of the plant to havehepatoprotective and antihepatotoxic activity[3-6].Therefore, it was worthy to investigate the hepatoprotective activity of the seeds and compare it with the widely used hepatoprotective drug Silymarin where significant hepatoprotective effects were observed.The lipid fraction obtained after defatting the alcoholic extract was saponified and thefatty acidsfractions wereconverted to their methyl esters and were analyzed by GC-MS.

II. Materials And Methods

2.1 Plant materials:

The seeds of *Cassia tora,* L. were cultivated in the farms of the Arab Company for Pharmaceuticals and Medicinal Plants, Egyptafter they were bought from the Islamic Center for Medical Sciences, Kuwait. These seeds were imported to Kuwait from MaxoCompany, India.

2.2 Preparation of the Extracts:

The air dried powdered seeds of *Cassia tora* L. (500 g), were defatted with n-hexane till exhaustion. The n-hexane was removed under vacuum at 40º C to give semisolid lipid extract (CaL). This lipid fraction was used for the phytochemical investigation of the fatty acids content.The defatted powderwas exhausted with ethanol 70% till complete extraction. The solvent was removed in each case under vacuum at 40º C, to give a soft defatted extract (CaD).The air dried powdered seed (50 g), was exhausted with 70% ethanol till complete extraction. The solvent was removed to give a soft total extracts (CaT).Part of each of the prepared extracts CaDand CaT, were used for the biological study.

2.3 Preparation of the Fatty Acids Methyl Esters:

The fatty acids in the fraction (CaF) were converted to their methyl esters by reflux with methanol saturated with HCl gas for 6 hours. The obtained solution was cooled then diluted with water and exhausted with n-hexane. The n-hexane extract was dehydrated over anhydrous sodium sulfate and then concentrated under vacuum at 40ºC to 10 ml and used for GC-MS analysis using a Shimadzu GC-MS, Model QP-2010 Ultra; under the following condition:

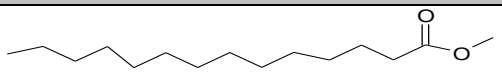
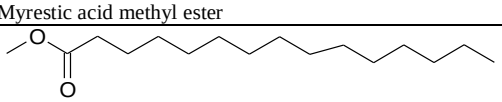
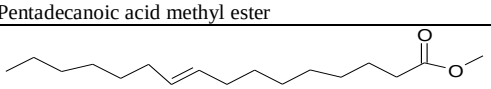
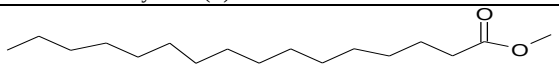
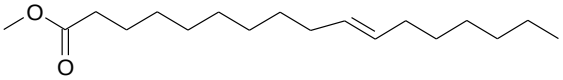
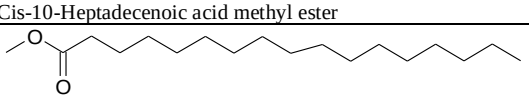
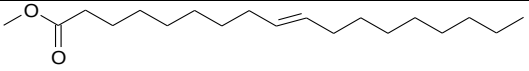
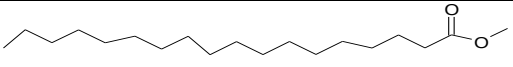
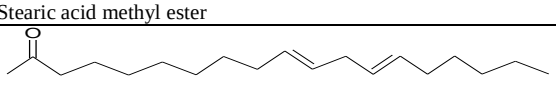
Column: Rtx-MS 30 meter length, 0.25mm ID, 0.25 um film thickness.
Detector: MS
Carrier gas: Helium.
Injector temperature: 240 º C.
Detector temperature: 240 º C.
Column temperature program:
Total program time: 26min.
Injection volume: 1 ul
Split ratio: Split ratio 1:50

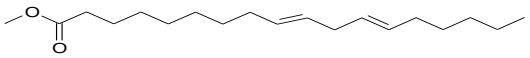
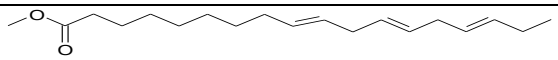
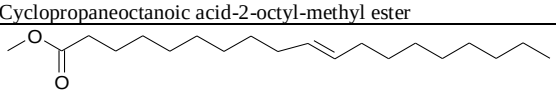
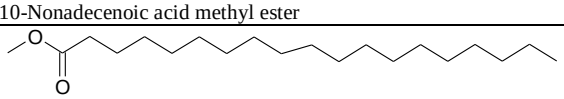
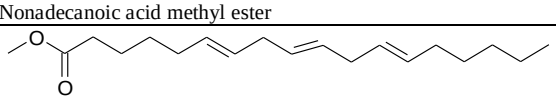
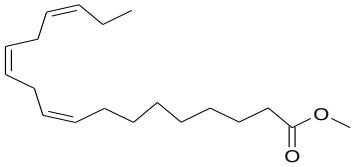
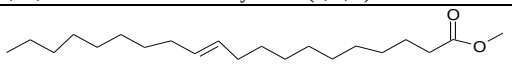
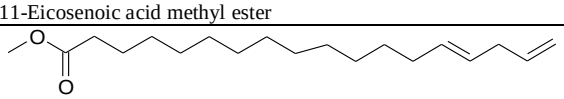
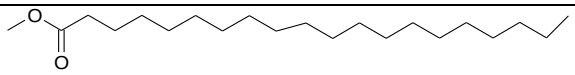
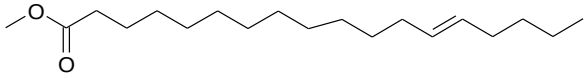
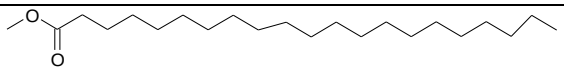
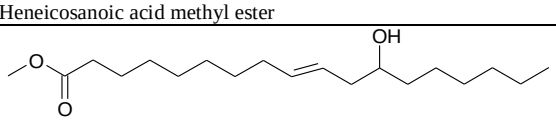
Rate	Temp.	Hold time (min)
—	70 º C.	0
20	130 º C.	0
3	180 º C.	0
20	220 º C.	5

III. Results

A:GC-MS analysis of the fatty acids methyl esters:

Table1: GC-MSAnalysis of the Fatty Acids Methyl Esters of *Cassiatora*, L. Seeds.

No.	Rt min	Formula	Relative%	Structure
1	3.05	$C_{15}H_{30}O_2$	0.22	 Myrestic acid methyl ester
2	3.79	$C_{16}H_{32}O_2$	0.16	 Pentadecanoic acid methyl ester
3	4.67	$C_{17}H_{32}O_2$	1.04	 Palmitoleic methyl ester(Z).
4	5.055	$C_{17}H_{34}O_2$	14.91	 Palmitic acid methyl ester
5	6.01	$C_{18}H_{34}O_2$	0.20	 Cis-10-Heptadecenoic acid methyl ester
6	6.35	$C_{18}H_{36}O_2$	0.32	 Heptadecanoic acid methyl ester= Margaric acid methyl ester
7	8.61	$C_{19}H_{36}O_2$	5.52	 Oleic acid methyl ester
8	8.96	$C_{19}H_{38}O_2$	10.53	 Stearic acid methyl ester
9	9.20	$C_{19}H_{34}O_2$	0.62	 9,12-Linoleic methyl ester Methyl Linolelaidate

10	9.37	$C_{19}H_{34}O_2$	0.37	 9,12-Linoleic methyl ester (Z, Z)
11	9.73	$C_{19}H_{32}O_2$	0.11	 9, 12, 15-Octadecatrienoic acid methyl ester
12	10.63	$C_{20}H_{38}O_2$	0.05	 Cyclopropaneoctanoic acid-2-octyl-methyl ester
13	10.77	$C_{20}H_{38}O_2$	0.06	 10-Nonadecenoic acid methyl ester
14	11.49	$C_{20}H_{40}O_2$	0.11	 Nonadecanoic acid methyl ester
15	12.26	$C_{19}H_{32}O_2$	0.15	 Gamma-Linolenic acid methyl ester (Cis)
16	12.53	$C_{19}H_{32}O_2$	0.17	 9,12,15-Linolenic acid methyl ester (z, z, z)
17	14.56	$C_{21}H_{40}O_2$	1.58	 11-Eicosenoic acid methyl ester
18	14.77	$C_{19}H_{34}O_2$	0.30	 14, 17- Octadecadienoic acid methyl ester
19	15.94	$C_{21}H_{42}O_2$	4.90	 Eicosanoic acid methyl ester= Arachidic acid methyl ester
20	16.21	$C_{19}H_{36}O_2$	0.10	 13E-Octadecenoic acid methyl ester
21	20.49	$C_{22}H_{44}O_2$	0.59	 Heneicosanoic acid methyl ester
22	20.82	$C_{19}H_{36}O_3$	1.13	 9-Octadecenoic acid, 12-hydroxy, methyl ester (Z)

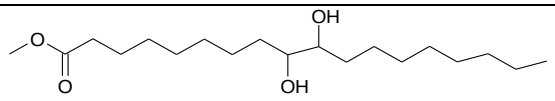
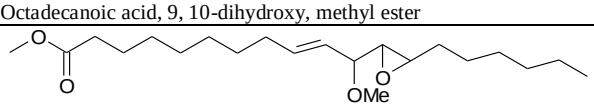
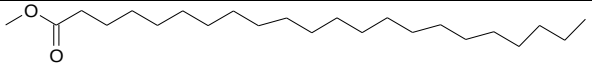
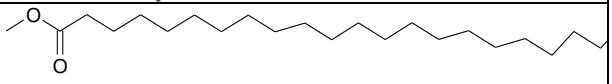
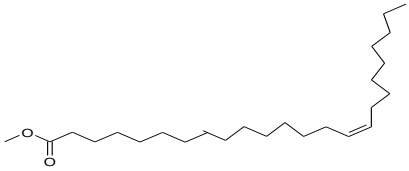
23	21.61	$C_{19}H_{38}O_4$	0.10	 Octadecanoic acid, 9, 10-dihydroxy, methyl ester
24	21.99	$C_{20}H_{36}O_4$	0.59	 12,13-epoxy-11-methoxy-9-octadecenoic acid methyl ester
25	22.16	$C_{23}H_{46}O_2$	1.13	 Behenic acid methyl ester
26	23.10	$C_{24}H_{48}O_2$	0.10	 Tricosanoic acid methyl ester
27	23.75	$C_{25}H_{48}O_2$	0.59	 15-Tetracosenoic acid metyl ester.

Table 1 shows that the analysis of the fatty acids fraction of *Cassia tora* L. seeds revealed the presence of 27 fatty acids which were identified through the fragmentation pattern given by the MS and the data obtained from the library saved on the computer of the MS. Analysis resulted in 12 saturated fatty acids and 15 unsaturated fatty acids.

B: Biological Study (Hepato-protective Activity):

B.1: Chemicals and Reagents: Carbon tetrachloride (CCl_4) and all other chemicals and solvents used were of analytical grade and obtained from Sigma Chemicals Co., USA. Biochemical enzymatic kits were procured from ERBA, Diagnostics Mannheim GmbH, Germany.

B.2: Experimental Animals (Hepatoprotective Assay):[7-9]

Forty five adult female Wister albino rats (120-170 gm) were used in this study, purchased from the Animal House, EL- Nile chemical company, Egypt. Rats were housed Mansoura University and left to acclimatize for 7 days to laboratory conditions before the commencement, during the acclimatization, with free access to standard laboratory chow diet and water ad-libitum. The animals were housed at a temperature of 25 ± 1 ^{0}C within a 12 hr light/ dark cycle. The experiments were conducted according to the ethical norms approved by Institutional Animal Ethics Committee (IAEC) guide lines for animal care and were adhered to as recommended by CPCSEA guidelines for the use and care of experimental animals[10-12].

B.3: In-vivo Experimental Design:[10]

The test and standard drug Silymarin were suspended in 0.5% w/v carboxymethyl cellulose (CMC) for oral administration. The toxicant 50% CCl_4 in corn oil (2 ml/kg, i.p.) was given on 4th and 5th day, 2 hrs after the test and standard drug administration. Each group was placed into a separate cage. Rats were randomly divided into 5 groups each of 9 rats as follows:

Group 1 control healthy received distilled water orally (1 ml per day) for 6 weeks, the other four groups were given Carbon tetra chloride(CCl_4) (1 ml/kg b.wt. s.c) during the last five days of the experiment.

Group 2 intoxicated-non-treated: CCl_4only was used as a control positive.

Group 3Intoxicated-treated: CCl_4 + Silymarin: received Silymarin orally at a dose of 100 mg/kg b.wt. for 6 weeks. Group 4 CCl_4 + *C.tora*, L.total extract (CTT) were given orally at doses of 500 mg/kg b.wt. three times a week for 6 weeks.Group 5CCl_4 + *C.tora*, L.defatted extract (CTD) were given orally at doses of 500 mg/kg a week for 6 weeks.

B.4: Analysis of Hepatic Injury and Statistical Analysis:

At the end of experiment, blood samples were taken from the veins of orbital plexus of each animal with anticoagulant at the end of the experimental period. Serum samples were separated by centrifugation at 3000 rpm for 10 min. These samples were used for estimating the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB) and platelets (Platelets).

The data of studies were expressed as mean ± SD and mean ± SEM of triplicate experiments, respectively. The data was analyzed by one-way ANOVA followed by Tukey's multiple comparison analysis as post-hoc test using GraphPad Prism 4 (GraphPad Software Inc., CA, and USA). The $p<0.05$ was considered to be statistically significant

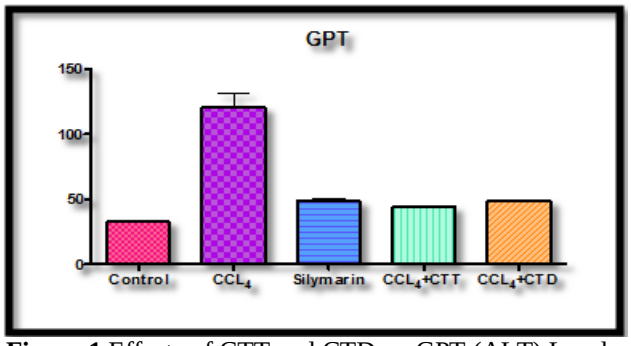


Figure 1 Effects of CTT and CTD on GPT (ALT) Levels

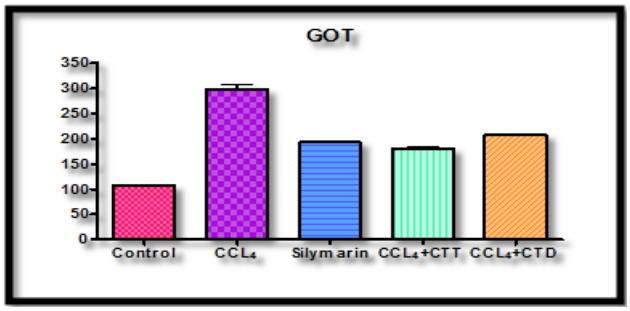


Figure 2 Effects of CTT and CTD on GOT (AST) Levels.

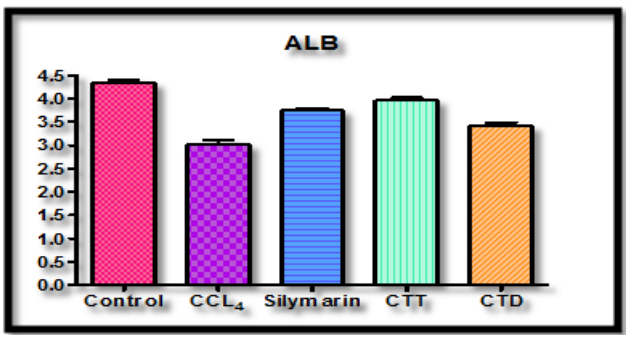


Figure 3 Effects of CTT and CTD on Albumin Levels

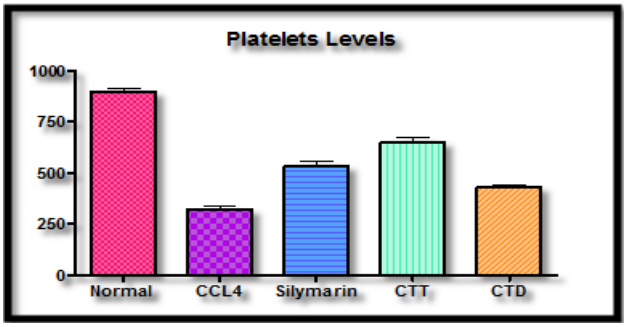


Figure 4 Effects of CTT and CTD on Platelets count

B.5: Comment:

The studies of one way ANOVA using Tukey's Multiple Comparison Test showed values of P<0.001 in groups from 3 to 5 when compared to the group 2 which indicates significance in reducing GPT and GOT levels increased by toxicity of CCl_4.The studies of one way ANOVA using Tukey's Multiple Comparison Test showed values of P<0.001 in groups from 3 to 5 when compared to the group 2 which indicates significance in increasing albumin levels decreased by toxicity of CCl_4 and inincreasing platelets count decreased by toxicity of CCl_4.CTT was found to be more effective than Silymarin the standard drug in reducing the levels of GPT and GOT enzyme levels.CTD was found to be close to the effect of the standard drug Silymarin in reducing the levels of GPT and GOT enzyme levels increased by toxicity of CCl_4.CTT was found to be more effective than Silymarin the standard drug in increasing the albumin levels and inincreasing platelets count decreased by toxicity of CCl_4.CTD was found to be close to the effect of the standard drug Silymarin in increasing the albumin levels and inincreasing platelets count decreased by toxicity of CCl_4.

B.6: Discussion: Number of Dead Animals DuringExperiment:

Group	Time
Group 1	None
Group 2: 3	After 10, 22, 35 days
Group 3:1	After 18 days
Group 4	None
Group 5: 1	After 12 days

Group 1: normal (control Group) showed no deaths during the whole time of the experiment.
Group 2:showed deaths due to toxicity of the CCl_4 at 10, 22, 35 days of the experiment.
Group3: Silymarin Group (Standard group) showed death which was higher than group 1 and fewer than group 2 compared to both the control group and the CCl_4 group respectively.
Group 4:*Cassia tora,* L. total (CTT) showedno deaths during the whole time of the experiment which is very important in evaluating the hepatoprotective effect of the drug which was higher than the Silymarin used as standard drug.
Group 5:*Cassia tora, L.*defatted (CTD) showed deaths which occurred after 12 days of the experiment.

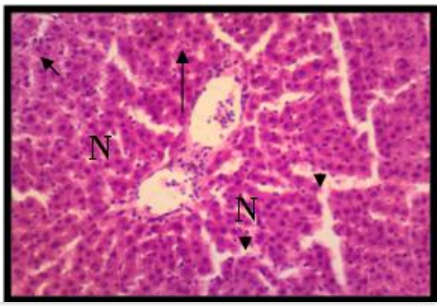
C: Histopathology:

The livers were immediately removed and the tissues were fixed in 10% formalin, dehydrated in ethanol (50–100%), cleared in xylene and embedded in paraffin wax. These were then cut into 4–5 μm thick sections in rotary microtome and stained with haematoxylineosin for photomicroscopicassessment**.**

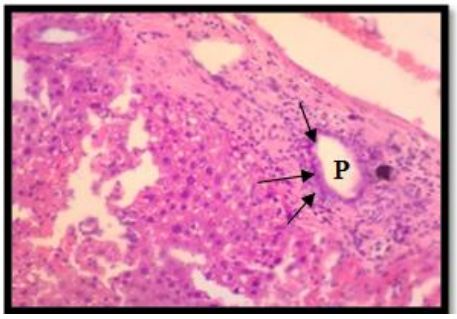
C.1: H&E Stain:

Normal control group: 100 x

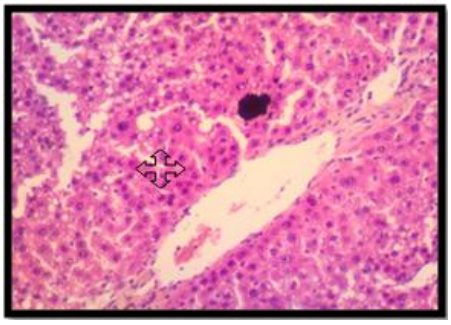
Photomicrograph of a section in the control liver showing typically arranged hepatocytes in a single cell plate radiating from the central vein. The hepatocytes appear polygonal in shape, joined to one another in anastomosing plates (N). The cells appear to have granular eosinophilic cytoplasm and rounded vesicular nuclei with prominent one or two nucleoli (short arrows). The blood sinusoids, in between the hepatocytes cords, are radiating as distensible vascular channels lined with endothelial cells (arrow heads) and phagocytic Kupffer cells (longarrows), which appeared larger than endothelial cells.

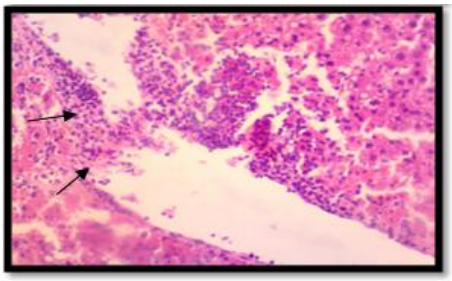


CCl_4:100and 400x

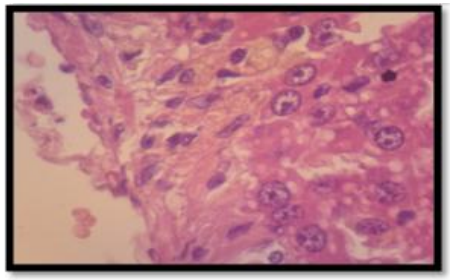


Silymarin(S):100and 400x



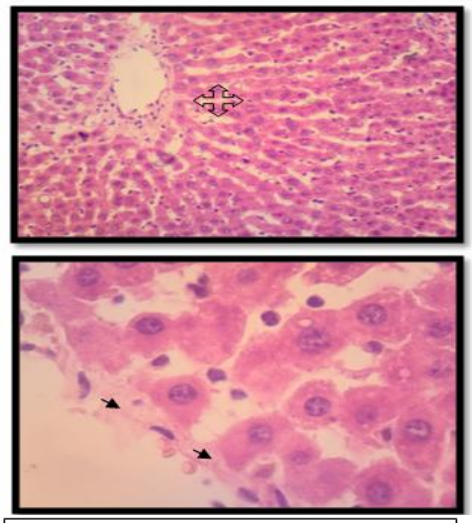


Photomicrograph of a section in the carbon tetrachloridegroup liver showing a congested and dilated portal vein (P) with portal and periportal inflammatory cells infiltration. The inflammatory cells (neutrophils and plasma cells)(arrows) invades the necrotic peripheral limiting plate of hepatocytes surrounding the portal triad (piecemeal necrosis).



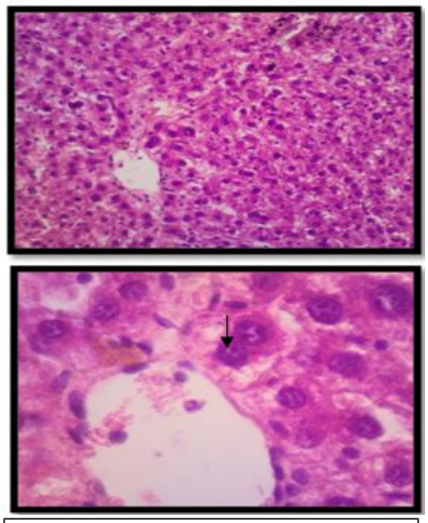
A photomicrograph of a section in the S group liver showing normally arranged hepatocytes radiating from central vein (arrows).

CTT: 100 and 400x



A photomicrograph of a section in the CTT liver showing normally arranged hepatocytes radiating from central vein with littleinflammatory cells infiltration of (Cross arrow) and fibroblast arealso seen (arrow heads)

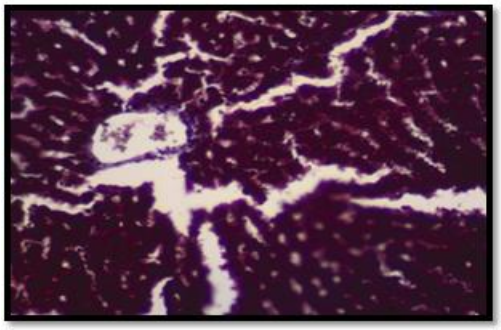
CTD: 100 and 400x



A photomicrograph of a section in the CTD group liver showing normally arranged hepatocytes with vacuolated cytoplasm and (short arrows), dilated central vein besides, diplocytes were seen (long arrow)

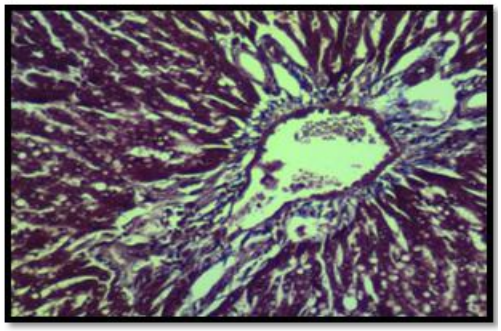
C.2:.Masson's trichrome Stain (Magnification 100x):

Normal control group: 100 x

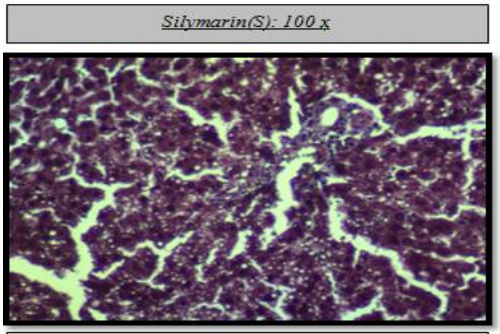


A photomicrograph of a section in the control liver showing minimal connective tissue between the hepatic lobules and inside the central vein.

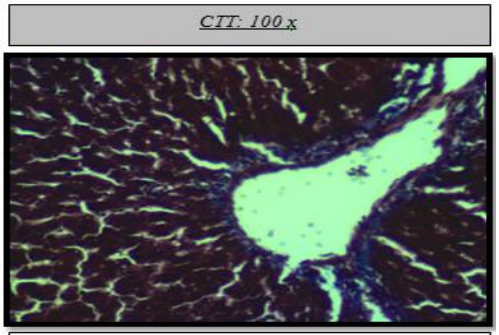
CCl_4:100 x



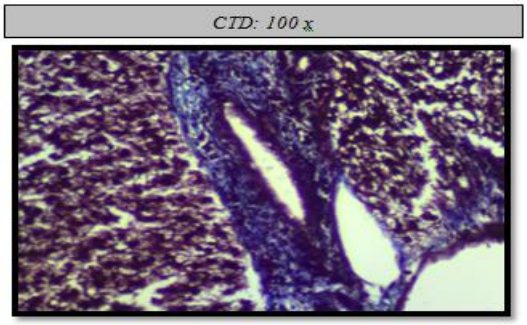
A photomicrograph of a section in the CCl_4 showing portal and periportal fibrosis.



A photomicrograph of a section in the(S) liver showing little increase of connective tissue between the hepatic lobules and inside the portal triad.



A photomicrograph of a section in the CTT liver showing moderate increase of connective tissue between the hepatic lobules and inside the portal triad.



A photomicrograph of a section in the CTD liver showing moderate increase of connective tissue between the hepatic lobules and inside the portal triad.

IV. Conclusion

Litrature review and preliminary phytochemical screening of *Cassiatora*, L. seeds, revealed the presence of carbohydrates, glycosides, anthraquinones, flavonoids, fats,saponins and gums.

Anthraquinones, flavonoids, saponins and their glycosides are well known for their anti-oxidant and hepatoprotective activities. In this study alcoholic extracts of the seeds (total or defatted), showed hepatoprotective effect against toxicity induced by CCl_4, this significant hepatoprotective effect confirms the folklore claim for *Cassia tora*, L. seeds as hepatoprotective remedy.It can be concluded that the total extract was more active than the defatted extract which may indicate the effect of the lipid fraction in the bioactivity of the drug.

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