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Phytochemical and Biological Evaluation of *Cichorium intybus* L. Seeds

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Abstract: *In this study, we aim to demonstrate, separately, the hepatoprotective activity of the total ethanolic extract as well as the defatted ethanolic extract of Cichorium intybus L., using adult Wister albino rats (120-170 g) as the experimental animals. The total as well as the defatted alcoholic extracts of Cichorium intybus L., seeds possess significant hepatoprotective activity; which may be attributed to the individual or combined effects of the phytoconstituents of each extract separately. In this study, hepatic injury caused by carbon tetra chloride, was analyzed through estimation of AST (GOT), ALT (GPT), albumin 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 standard drug Silymarin. On the other hand a 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: *Cichorium intybus L. seeds, hepatoprotective, carbon tetrachloride, Silymarin, fatty acids.*

I. Introduction

According to a report of world Health Organization three fourth of world population cannot afford modern medicine and rely on traditional medicine of plant origin [1]. Chicory (*Cichorium intybus* L.,) belongs to the family *Asteraceae* and it is a small aromatic biennial or perennial herb. The whole plant contains a number of medicinally important compounds such as inulin, esculin, volatile compounds (monoterpenes and sesquiterpenes), coumarins, flavonoids and vitamins [2]. It is used as an anti-inflammatory, digestive, diuretic and helps in treatment of spleen enlargement. The plant is used as liver tonic and as an alternative medicine in treating hepato-toxicity. It is used for local application in the treatment of acne, inflammation of throat, and in diarrhea and vomiting [3, 4]. Dealing with the biological study, most of the reports proved the different parts of the plant to have hepatoprotective and antihepatotoxic activity [5-11]. The present study was carried out to evaluate the hepatoprotective effect of the total and the defatted alcoholic extracts of *Cichorium intybus* L. seeds, comparable with standard Silymarin. The hepatoprotective activity of both extracts of the seeds, were separately evaluated through protection against the hepatic injury caused by carbon tetra chloride to the livers of Wister albino rates. A significant hepatoprotective effect was observed. The lipid fraction obtained by defatting *Cichorium intybus* L. seeds, was saponified and the fatty acids in the lipid fraction were converted to their methyl esters where they were analyzed by GC-MS.

II. Materials and Methods

2.1: Plant materials:

The seeds of *Cichorium intybus* L. were cultivated in the farms of the Arab Company for Pharmaceuticals and Medicinal Plants, Egypt.

2.2: Preparation of the Extracts:

The air dried powdered seeds of *Cichorium intybus* L. (1500 g) were defatted with n-hexane till exhaustion. The n-hexane was removed under vacuum at 40° C to give semisolid lipid extracts (CiL). This lipid fraction was used for the phytochemical investigation of the fatty acids content. The defatted powder was exhausted with ethanol 70% till complete extraction.

The solvent was removed under vacuum at 40°C, to give soft defatted extract (CID). The air dried powdered seeds (50 g), was exhausted with 70% ethanol till complete extraction. The solvent was removed to give soft total extracts (CIT).

Part of each of the prepared extracts CID (*Cichorium intybus* L. defatted,) and CIT (*Cichorium intybus* L. total) were used for the biological study.

2.3: Preparation of the Fatty Acids Methyl Esters:

The n-hexane extract was dehydrated over anhydrous sodium sulfate and then concentrated under vacuum at 40° C to 10 ml. and used for GC-MS analysis using a Shimadzu GC-MS, Model QP-2010 Ultra; under the following condition:

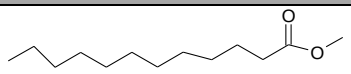
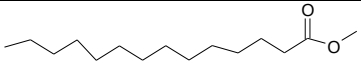
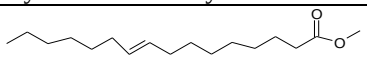
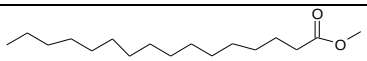
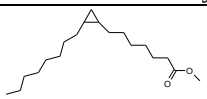
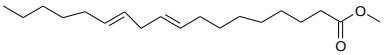
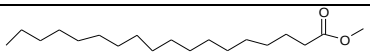
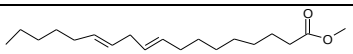
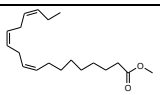
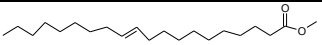
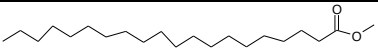
Column: Rtx-MS 30 meter length, 0.25mm ID, 0.25 um film thickness.
Carrier gas: Helium.
Injector temperature: 240 º C.
Detector temperature: 240 º C.
Column temperature program:
Total program time: 26min.
Injection volume: 1 ul
Split ratio: Split ratio 1:50

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 fatty acids methyl esters of *Cichorium intybus* L seeds:

Table 1: GC-MS Analysis of the Fatty Acids Methyl Esters of *Cichorium intybus* L. Seeds

No.	Rt min	Formula	Structure	Relative%
1	12.580	$C_{13}H_{26}O_2$	 Lauric acid methyl ester	0.13
2	15.317	$C_{15}H_{30}O_2$	 Myrestic acid methyl ester	0.06
3	17.662	$C_{17}H_{32}O_2$	 Palmitoleic methyl ester	0.38
4	18.342	$C_{17}H_{34}O_2$	 Palmitic acid methyl ester	19.77
5	21.525	$C_{19}H_{36}O_2$	 Methyl dihydromalvalate	0.44
6	21.700	$C_{19}H_{34}O_2$	 9,12- Linoleic methyl ester Z,Z (Cis)	58.980
7	22.050	$C_{19}H_{38}O_2$	 Stearic acid methyl ester	9.07
8	22.258	$C_{19}H_{34}O_2$	 9,12- Linoleic methyl ester (Trans), E,E Methyl Linolelaidate	0.59
9	23.500	$C_{19}H_{32}O_2$	 9,12,15- Linolenic acid methyl ester (Z,Z,Z)	0.81
10	24.383	$C_{21}H_{40}O_2$	 11-Eicosenoic acid methyl ester	0.65
11	24.825	$C_{21}H_{42}O_2$	 Eicosanoic acid methyl ester	1.69

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): [12-14]

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 in a wire mesh plastic cage, in the animal care center of Mansoura faculty of Pharmacy 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 ± 10°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 [15].

B.3: In-vivo Experimental Design:[13]

The test and standard drug Silymarin were suspended in 0.5% w/v Carboxymethyl cellulose (CMC) for oral administration. The toxicant 50% carbon tetra chloride (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 [12].

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) 6 weeks the other four groups were given CCl_4 (1 ml/kg b.wt. s.c) during the last five days of the experiment.
- Group 2 (intoxicated-non-treated: CCl_4 only) was used as a control positive.
- Group 3 (Intoxicated-treated: CCl_4 + Silymarin): received Silymarin orally at a dose of 100 mg/kg b.wt. for 6 weeks.
- Group 4 CCl_4 + *Cichorium intybus* L.total extract (CIT) were given orally at doses of 500 mg/kg b.wt. Three times a week for 6 weeks.
- Group 5 CCl_4 + *Cichorium intybus* L.defatted extract (CID) were given orally at doses of 500 mg/kg b.wt. Three times 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.

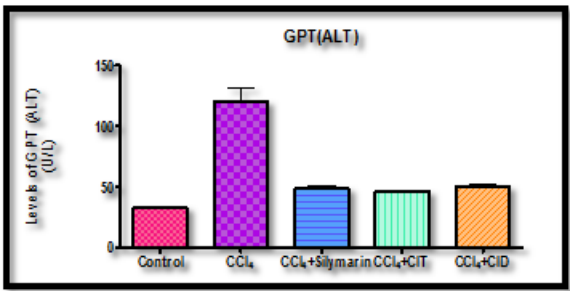


Figure 1 Effects of CIT and CID on GPT levels

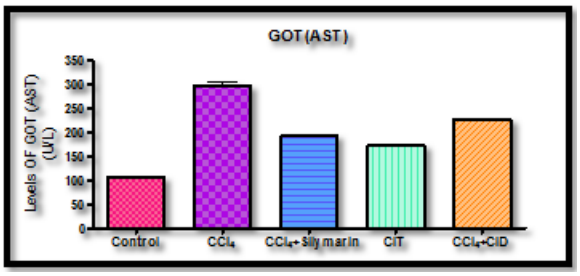


Figure 2 Effects of CIT and CID on GOT levels

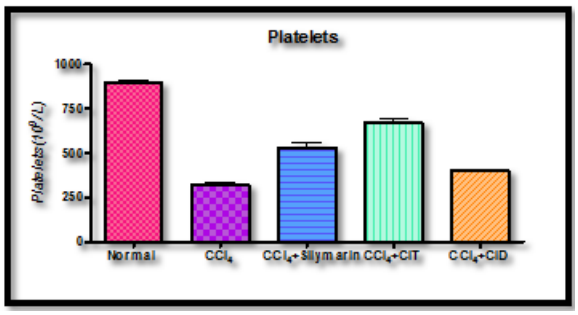


Figure 3 Effects of CIT and CID on Platelets count

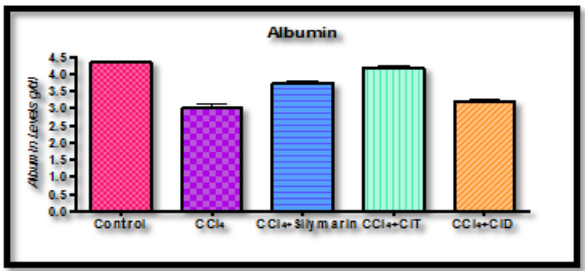


Figure 4 Effects of CIT and CID on Albumin levels

Comment:

- P value P<0.0001
- 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.
- CIT and CID were found to be nearly as effective as standard drug Silymarin in lowering the levels of GPT and GOT in blood when compared to it.
- 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 ALB levels Decreased by toxicity of CCl_4 and in increasing Platelets count decreased by toxicity of CCl_4.
- CIT was found to be more effective in restoring the levels of ALB in blood and in increasing Platelets count decreased by toxicity of CCl_4 when compared to the standard drug Silymarin.
- CID was found to be nearly as effective as standard drug Silymarin in restoring the levels of ALB in blood and in increasing Platelets count decreased by toxicity of CCl_4.

IV. Discussion

- Number of Dead Animals During Experiment:

Group	Time
Group 1	None
Group 2: 3	After 10, 22, 35 days
Group 3:1	After 18 days
Group 4: 1	After 23 days
Group 5: 2	After 20, 36 days

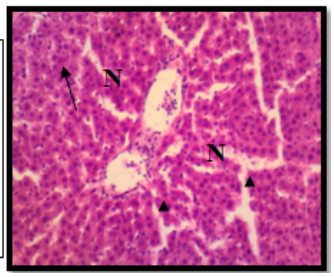
- (Group 1)The normal (control Group) showed no deaths during the whole time of the experiment.
- (Group 2) Showed deaths due to toxicity of the CCl_4 at 10, 22, 35 days of the experiment.
- (Group3) Silymarin Group (Standard group) showed death which was higher than group 1 and fewer than group 2 compared to both the control group and the CCl_4 group respectively.
- (Group 4) *Cichorium intybus* L. total (CIT) showed deaths which occurred after 23 days of the experiment.
- (Group 5) *Cichorium intybus* L. defatted (CID) showed deaths which occurred after 20, 36 days of the experiment.

C: Histopathological Studies: 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 photomicroscopic assessment.

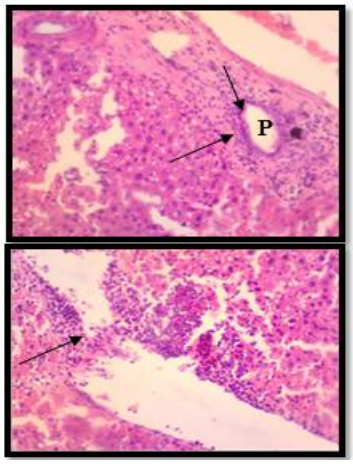
C.1: H&E Stain:

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 (long arrows). The cells appear to have rounded vesicular nuclei with prominent one or two nucleoli (short granular eosinophilic cytoplasm and 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 (arrow), which appeared larger than endothelial cells.

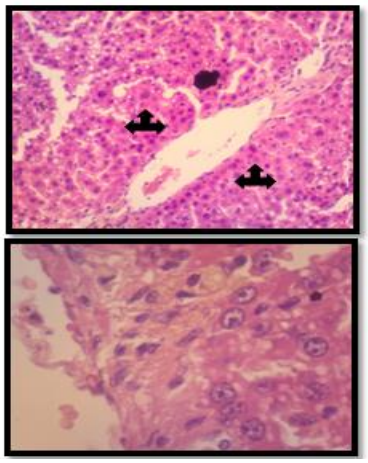


CCl_4:100x



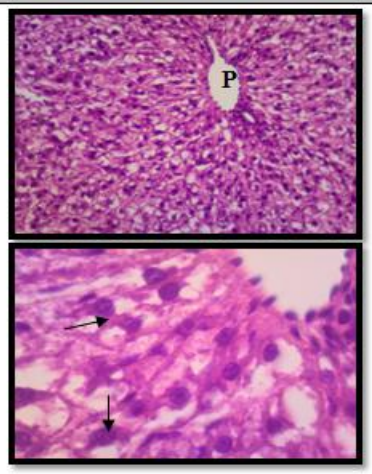
A photomicrograph of a section in the carbon tetrachloride group liver showing dilated portal vein (P) with portal and periportal inflammatory cells infiltration. The inflammatory cells (arrows) invade the necrotic peripheral limiting plate of hepatocytes surrounding the portal triad (piecemeal necrosis

Silymarin (S) 100x and 400x



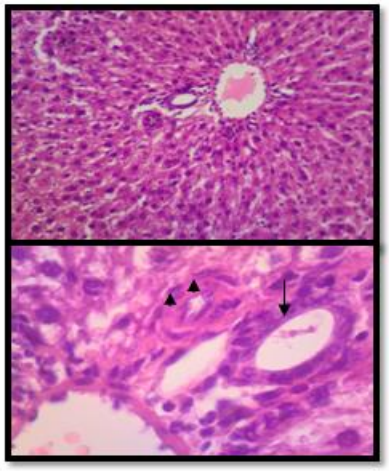
A photomicrograph of a section in the S group liver showing normally arranged hepatocytes radiating from central vein with little inflammatory cells infiltration (arrows).

CIT (*Cichorium intybus* L.total: 100 x and 400x



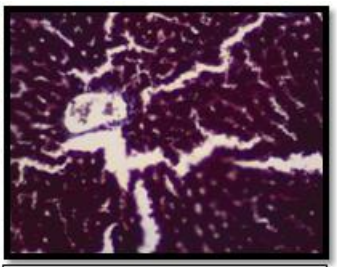
A section in the CIT showing typically arranged hepatocytes in a single cell plate radiating from the portal triad with congested and dilated portal vein (P). The cells appear to have granular eosinophilic cytoplasm and rounded vesicular nuclei with prominent one or two nucleoli

CID (*Cichorium intybus* L.total: 100 x and 400x



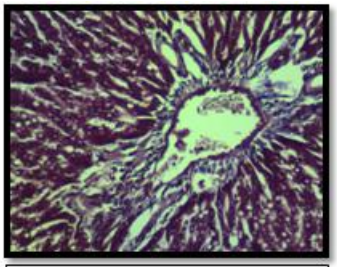
A section in the CID group liver showing normally arranged hepatocytes which appears vacuolated with pknotic nucleus (arrowheads) with little periportal inflammatory cells infiltration and ductal proliferation. (Arrows)

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



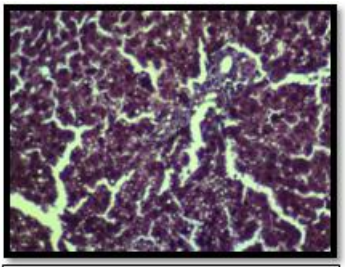
Normal Control Group

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



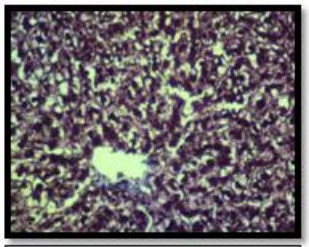
CCl_4:

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



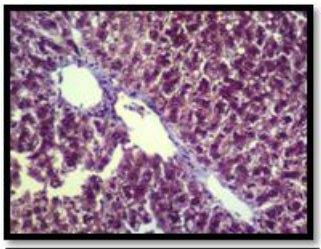
Silymarin:

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



CIT(Cichorium intybus total:)

A photomicrograph of a section in the CIT liver showing a little increase of connective tissue between the hepatic lobules and inside the central vein.



CID(Cichorium intybus defatted):

Photomicrograph of a section in the CID liver showing moderate increase of connective tissue inside the portal triad.

V. Conclusion

Literature review and preliminary phytochemical screening of *Cichorium intybus* L. seeds, revealed the presence of carbohydrates, glycosides, flavonoids, saponins, fats and gums.

Flavonoids, saponins and their glycosides are well known for their anti-oxidant and hepatoprotective activities. In this study alcoholic extract of the seeds (total or defatted), showed significant hepatoprotective effect against toxicity induced by CCl_4, which may be attributed to the individual or combined effect of phytoconstituents present in them. Based on the above results of the pharmacological screening, it can be concluded that the alcoholic seed extract of *Cichorium intybus* L. seeds, possess significant hepatoprotective effect. This result confirms the folklore claim for *Cichorium intybus* L. seeds as hepatoprotective remedy.

It could 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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