Available online on 15.08.2019 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Phytochemical and Pharmacological Evaluation of *Carthamus tinctorius* Leaves

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ABSTRACT

Hepatoprotective activity of methanolic extract of leaves of *Carthamus tinctorius* L. (MECT) was investigated against hepatotoxicity produced by administering a combination of two anti-tubercular drugs Isoniazid and Rifampicin for 24 days by Oral route in rats. MECT were administered at two graded dose i.e. 200 and 300 mg/kg p.o. 45 min after to anti-tubercular challenge for 24 days. MECT was evident in the all doses as there was a significant decrease in AST, ALT, ALP, and Total Bilirubin levels. MECT significantly decreased the level of these biochemical parameter as compared to control. HPTLC analysis showed the presence of B-sitosterol and Lupeol in MECT. Results showed that *Carthamus tinctorius* possess significant Hepatoprotective activity which might be due to flavonoids.

Keywords: *Carthamus tinctorius* leaves¹, hepatoprotective², Rifampicin³, Isoniazide⁴, SGPT⁵.

Article Info: Received 18 June 2019; Review Completed 24 July 2019; Accepted 30 July 2019; Available online 15 August 2019

Cite this article as:

Pant R, Irchhaiya R, Rao CV, Phytochemical and Pharmacological Evaluation of *Carthamus tinctorius* Leaves, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):285-294 <http://dx.doi.org/10.22270/jddt.v9i4-s.3277>

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1. INTRODUCTION

The Indian subcontinent is endowed with a rich expertise in local health traditions. The traditional medicine in India functions through two social streams. One is the local folk stream, which is prevalent in rural and tribal villages of India. The carriers of these traditions are ions of housewives, thousands of traditional birth attendants, bone setters, practitioners skilled in acupressure, eye treatment or treatment of snakebites, the traditional village level herbal physicians (the "Vaidyas") and tribal physicians in the tribal areas. These local health traditions thus represent an autonomous community supported system of health delivery at the village level, which runs parallel to the state supported system. (Kalia A.N. 2005)

The term "Traditional Medicine" refers to protecting and restoring health that existed before the arrival of modern medicine. As the term implies, these approaches to health belong to the traditions of the each country, and have been handed down from generation to generation. Traditional system in general has had to meet the needs of the local communities for many centuries. China, India, for example, have developed very sophisticated system such as acupuncture

and ayurvedic medicine. In practice, the term "Traditional Medicine" refers to following components: acupuncture, traditional birth attendants, mental healers and herbal medicine. (Treas and Evans, 2004)

Modern society has inherited knowledge about the herbal treatment of liver disease from many cultures. The dominant Western medical culture has largely ignored this knowledge until recently. Research into plants traditionally used in the treatment of liver diseases has significantly advanced in the past 15 years, and much of what has been discovered supports traditional knowledge. Still, there continues to be a need for safe, effective treatments of liver disease. Much research is yet to be done, but these plants appear to have a place in the treatment of liver poisoning, viral hepatitis, and cirrhosis of the liver. The research done for liver diseases so far on plants is much less complete, the situational background explained above warrants for developing a safety, effective and scientifically validated hepatoprotective agent/s taking lead from traditional medicine which is affordable for the rural poor population. Thus in this context the present investigation was under taken to study the tissue level molecular mechanisms involved against chemical/drug -induced hepatocellular damage of the

selected medicinal plant, traditionally used in the treatment of liver diseases.

Liver plays a vital role in regulation of physiological processes. It is involved in several functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailments. They may be classified as acute or chronic hepatitis (inflammatory liver disease), hepatosis (non-inflammatory disease) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). (Orisakwe E.O., 2018, MDPI)

Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidized oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbon, etc.) excess consumption of alcohol, infection and autoimmune disorder. Most of the hepatotoxic chemicals damage liver cells mainly by including lipid peroxidation and other oxidative damages in liver. A strict hepatological delineation of these disorders or systematic classification of liver therapeutic agents is practically impossible. Probably, this is the reason for the existence of nearly six hundred commercial therapeutic preparations against hepatitis alone, therefore, it clearly indicates that an actual curative therapeutic agent has not yet been found. Most preparations support or promote the process of healing or regeneration of the liver.

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailments. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidized oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune disorder. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Enhanced lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis. It has been estimated that about 90% of the acute hepatitis is due to viruses. The major viral agents involved are Hepatitis B, A, C, D (delta agents), E and G. Of these, Hepatitis B infection often results in chronic liver diseases and cirrhosis of liver. Primary liver cancer has also been shown to be produced by these viruses. It has been estimated that approximately 14-16 million people are infected with this virus in South East Asia region and about 6% of the total population in the region are carriers of this virus. A vaccine has become available for immunization against Hepatitis B virus. Hepatitis C and Hepatitis E infections are also common in countries of South East Asia region. (C. Hari Kumar, 2011 IJPSR, Vol.2-3)

Herbal medicines have been used to treat liver disorders for thousands of years and have now become a promising therapy for various pathological liver conditions. In India, over 40 polyherbal commercial formulations reported to have hepatoprotective action are being used along with 160 phytoconstituents from 101 plant families. A list of plants reported to have significant hepatoprotective activity is shown in Table 1 in alphabetical order of the plant scientific

name together with the part of the plant used, kind of extract used or compound isolated, including model, type of assay and mechanism of action involved. (Sayed S.H., 2014, Vol. 4)

Carthamus tinctorius L. is commonly known as Safflower. *C. tinctorius* extracts and oil are important in drug development with numerous pharmacological activities in the world. This plant is cultivated mainly for its seed, which is used as edible oil. For a long time *C. tinctorius* has been used in traditional medicines as a purgative, analgesic, antipyretic and an antidote to poisoning. It is a useful plant in painful menstrual problems, post-partum hemorrhage and osteoporosis. *C. tinctorius* has recently been shown to have antioxidant, analgesic, anti-inflammatory and antidiabetic activities. Carthamin, safflower yellow are the main constituents in the flower of *C. tinctorius*. Carthamin, isocarthamin, hydroxysafflower yellow A, safflower yellow A, safflorin C and luteolin are the main constituents which are reported from this plant. Caryophyllene, p-allyltoluene, 1-acetoxytetralin and heneicosane were identified as the major components for *C. tinctorius*.

Flowers' essential oil. Due to the easy collection of the plant and being widespread and also remarkable biological activities, this plant has become both food and medicine in many parts of the world. This review presents comprehensive analyzed information on the botanical, chemical and pharmacological aspects of *C. tinctorius*. (Asgarpanah J. 2013, CJIM)

A total of eight flavonoids (1-8), including a novel quercetin-7-O-(6''-O-acetyl)- β -D-glucopyranoside (6) and seven known flavonoids, luteolin (1), quercetin (2), luteolin 7-O- β -D-glucopyranoside (3), luteolin-7-O-(6''-O-acetyl)- β -D-glucopyranoside (4), quercetin 7-O- β -D-glucopyranoside (5), acacetin 7-O- β -D-glucuronide (7) and apigenin-6-C- β -D-glucopyranoside (8), have been isolated from the leaves of the safflower (*Carthamus tinctorius* L.) and identified on the basis of spectroscopic and chemical studies. The antioxidative activity of these flavonoids was evaluated against 2-deoxyribose degradation and rat liver microsomal lipid peroxidation induced by hydroxyl radicals generated via a Fenton-type reaction. Among these flavonoids, luteolin-acetyl-glucoside (4) and quercetin-acetyl-glucoside (6) showed potent antioxidative activities against 2-deoxyribose degradation and lipid peroxidation in rat liver microsomes. Luteolin (1), quercetin (2), and their corresponding glycosides (3 & 5) also exhibited strong antioxidative activity, while acacetin glucuronide (7) and apigenin-6,8-di-C-glucoside (8) were relatively less active. (Lee J.Y. et al, 2002,)

2. PLAN OF WORK

The plan of work for the study of *Carthamus tinctorius* was carried out as follows.

1. Collection and authentication of plant.
2. Macroscopic and Histological evaluation
3. Pharmacognostical studies

3.1 Phytochemical Studies

- a. Ash value
- b. Extractive value
- c. Foreign matter

3.2 Preliminary phytochemical studies

- a. Preparation of extract

- b. Qualitative phytochemical studies
4. Pharmacological Evaluation: Investigation by Bio-chemical Parameters
 - a. Determination of Serum glutamic pyruvic transaminase (SGPT)
 - b. Determination of Serum glutamic oxaloacetic transaminase (SGOT)
 - c. Determination of serum alkaline phosphatase (SALP)
 - d. Determination of serum total bilirubin

3. MATERIALS AND METHODS

3.1 Selection of plant

The selection of a medicinal plant for Hepatoprotective activity, undergo discussion with a tribal medicine practitioner for the traditional and tribal uses of Leaf of *Cathamus tinctorius* which have not been evaluated for Hepatoprotective activity. The compiled list of plant must therefore be subjected to a literature survey to confirm that the plant has not been previously investigated for

Hepatoprotective activity. Hence, the present work involves Phytochemical and Pharmacological evaluation of Hepatoprotective property of plant *Cathamus tinctorius*.

3.2 Material

3.2.1 Equipment Used

Table-1 Instruments used

S.No	Legends	Manufactured By
1.	Digital melting Point Apparatus	Jyoti Scientific Laboratory
2.	pH Meter	Simtronics digital pH meter Model SE 962-P
3.	Digital Balance	Parkar TH 500
4.	Hot Air Oven	Universal hot air oven
5.	Electric Water Bath	Jyoti Scientific Laboratory
6.	Magnetic Stirrer	Remi 2 MLH
7.	Refrigerator	Whirlpool
8.	Heating Mantle	Labtech Sunbim.
9.	Digital Microscope	Olympus
10	Rota Vapour	Buchi U.S.A.

Table-2 List of material used & their manufacturer

S.no.	Name of material	Name & address of manufacturer
1	Hydrochloric acid	Qualigens@Fine Chemicals, Mumbai.
2	Methanol	Qualigens@Fine Chemicals, Mumbai.
3	Formic acid	Qualigens@Fine Chemicals, Mumbai.
4	Acetic acid	Qualigens@Fine Chemicals, Mumbai.
5	Sodium Hydroxide	Qualigens@Fine Chemicals, Mumbai.
6	n-Hexane	Qualigens@Fine Chemicals, Mumbai.
7	Benzene	Qualigens@Fine Chemicals, Mumbai.
8	Chloroform	Qualigens@Fine Chemicals, Mumbai.
9	Potassium iodide	Qualigens@Fine Chemicals, Mumbai.
10	Aceton	Merck Limited, Mumbai-400018.
11	Petroleum ether (60-80°C)	Qualigens@Fine Chemicals, Mumbai.
12	Silica Gel GF	Qualigens@Fine Chemicals, Mumbai.
13	Sodium nitroprusside	Qualigens@Fine Chemicals, Mumbai.
14	Potassium sodium (+) tartrate	Central drug house (p)Ltd. New delhi.
15	Alcohol (95%)	Qualigens@Fine Chemicals, Mumbai.
16	Ferric Chloride	Qualigens@Fine Chemicals, Mumbai.
17	Mercuric Chloride	Fine Laboratory Chemicals, Mumbai.
18	Dimethylsulphoxide	central drug house (p)Ltd. New delhi.

Table-3 List of Glassware used and their manufacturer

S.No	Legends	Capacity	Manufactured By
1.	Condenser	-	Asgi
2.	Pipette	1-5 ml	Borosilicate
3.	RBF	500 ml	Borosilicate
4.	Measuring Cylinder	10-100 ml	Borosilicate
5.	Test tubes	10-15 mm	Borosilicate
6.	Funnel	75 mm	Borosilicate
7.	Beakers	50-500 ml	Borosilicate
8.	Separating Funnel	250 ml	Borosilicate
9.	Petri dish	200 mm	Borosilicate
10.	Desiccators	150 mm	Jyoti Scientific Laboratory
11.	Glass rod	15 cm	Jyoti Scientific Laboratory
12.	Distillation Unit	3 lit.	Borosilicate

3.3 Collection of plant

The Leaves of plant *Cathamus tinctorius* (family - Asteraceae) were collected from botanical garden of National botanical research institute, Lucknow, India in

month of February 2019. The plant material was authenticated by Dr. Ch.V. RAO, Scientist and Taxonomist Dr.(Mrs.) Sayeeda Khatoon Pharmacognosy and Ethanopharmacology division of N.B.R.I. Lucknow, where

deposited in the departmental herbarium for future reference.

3.4 Preparation of crude drug for extraction

The freshly collected leaves (1kg) of *Cathamus tinctorius* were washed with distilled water and shade-dried. Then dried it in tray drier under the control condition and powdered with help of mechanical grinder. The powder was passed through sieve no. 40 and stored in an air tight container for extraction.

3.5 Preliminary phytochemical screening

3.5.1 Successive Phytochemical extraction

The defatted dried mark was successively extracted with non polar to polar solvents. The mark was extracted with petroleum ether by soxhlet extraction process. The mark was obtained dried and successive extraction with chloroform, benzene, methanol and water respectively. After each extraction the powdered drug was dried and then treated with next solvent. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA).

3.5.2 Preparation of petroleum ether extract of *Carthamus tinctorius* leaves

The mark left was dried and then further extracted with pet. ether for 72 hours maceration. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA). and then dried in lyophilizer under reduced pressure and low temperature. The extract obtained was further subjected to toxicological and pharmacological investigations.

3.5.3 Preparation of chloroform extract of *Carthamus tinctorius* leaves

The mark left was dried and then further extracted with chloroform for 72 hours by maceration. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA). and then dried in lyophilizer under reduced pressure and low temperature. The extract obtained was further subjected to toxicological and pharmacological investigations.

3.5.4 Preparation of benzene extract of *Carthamus tinctorius* leaves

The mark left was dried and then further extracted with benzene for 72 hours by maceration. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA). and then dried in lyophilizer under reduced pressure and low temperature. The extract obtained was further subjected to toxicological and pharmacological investigations.

3.5.5 Preparation of methanol extract of *Carthamus tinctorius* leaves

The mark left was dried and then further extracted with 50% methanol for 72 hours by maceration. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA). and then dried in lyophilizer under reduced pressure and low temperature. The extract obtained was further subjected to toxicological and pharmacological investigations.

3.5.6 Preparation of aqueous extract of *Carthamus tinctorius* leaves

The mark left was dried and then further extracted with distilled water for 72 hours by maceration. The extract was separated by filtration and concentrated on

rotavapour (Buchi, USA). and then dried in lyophilizer under reduced pressure and low temperature. The extract obtained was further subjected to toxicological and pharmacological investigations.

50% methanolic extract of *Cathamus tinctorius* was subjected to qualitative taste for the identification of various active constituents' carbohydrate, glycoside, alkaloids amino acids, flavanoids, fixed oil, tannins, gum and mucilage, phytosterols etc. according to Kokate, 1990 and Khandelwal, 2000.

3.6 Pharmacological Studies

3.6.1 Experimental Set up

The animals (Sprague-Dawley rats weighing 170-200 g) was divided into 6 groups of 6 animals each.

Group I

Control animals were received 1 % carboxymethyl cellulose in distilled water (10 mL/kg b.wt.) and this served as solvent control.

Group II

Animal was received Rifampicin + Isoniazide (RIF + INZ) (50mg/Kg body wt. each, p.o). RIF and INH solutions were prepared separately in sterile distilled water, the pH of RIF solution was adjusted to 3.0 with 0.1 mol / L HCL. RIF + INH will administered orally for 24 days, (Ravinder pal et al, 2006). After 24 days hepatotoxicity was confirmed with the help of biochemical studies and this group of animals was used for detailed investigation.

Group III

Animals were received RIF + INH (50mg/Kg body wt. each, p.o) and 50% EtOH extract of *Carthamus tinctorius* (100 mg/kg body wt. p.o) for 24 days.

Group IV

Animals were received RIF + INH (50mg/Kg body wt. each, p.o) and 50% EtOH extract of *Carthamus tinctorius* (200 mg/kg body wt. p.o) for 24 days.

Group V

Animals were received RIF + INH (50mg/Kg body wt. each, p.o) and 50% EtOH extract of *Carthamus tinctorius* (400 mg/kg body wt. p.o) for 24 days.

Group VI

Animals were received (RIF + INZ) (50mg/Kg body wt. each, p.o) and Silymarin (100mg/kg body wt. p.o) for 24 days.

After completion of the treatment, animals were weighed and sacrificed by cervical decapitation. Blood samples were collected and serum was separated. Liver was removed immediately, washed in saline, weighed and homogenized in Tris-HCl buffer 0.1M pH 7.4. The serum and liver homogenate were used for the biochemical analysis.

3.7 Bio-Chemical parameters Investigated

Serum was analyzed for the following parameters: Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), Total bilirubin, total protein and Albumin, and enzymatic parameters like- lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD) by liver homogenate.

3.7.1 Determination of Serum Glutamic Pyruvic Trausaminase (SGPT)

Method using SGPT (Span diagnostic reagent kit).

3.7.2 Determination of Serum glutamic oxaloacetic transaminase (SGOT)

Method using SGOT (Span diagnostic reagent kit).

3.7.3 Determination of serum alkaline phosphatase (SALP)

The alkaline phosphates level was estimated by King and Armstrong, (1965) method alkaline phosphatase (Span diagnostic reagent kit).

3.7.4 Determination of serum total bilirubin

Total bilirubin level in serum was determined by modified DMSO method using Agappe diagnostic kit (Mumbai).

4. RESULT AND DISCUSSION

Phytochemical investigation of *Carthamus tinctorius* leaves were found as follows-

4.1 Pharmacognostical Studies:

Macroscopy: The macroscopic characters of the *Carthamus tinctorius* leaf were shown in the figure -2.

Figure 1 Macroscopy of *Carthamus tinctorius* leaf

Histology: The microscopic characters of the *Carthamus tinctorius* leaf were shown in the figure -3 and 4.

Colour – Green

Odour- Characteristic smell

Taste- Slightly bitter

Size- 12-18cm in length, 4-7cm in width

Shape- Regular, Long,

4.2 Histology of *Carthamus tinctorius* Leaf

Figure 2 Histology of *Carthamus tinctorius* Leaf

Figure 3 Histological view of Vascular Bundle

Table-4 Nature and colour of *Carthamus tinctorius* leaves extract

S.no.	Name of extract	Colour	Consistency	Yield% W/W
1	Methanolic extract	Dark green	Sticky mass	5.9
2	Aqueous extract	Brown	Syrupy mass	3.9

4

3 Physico-Chemical Parameters

A. Ash Values:

Table-5

S.No	PARAMETER	% w/w
	ASH VALUES	
1.	Total Ash	3.5
2.	Water Soluble Ash	2.5
3.	Acid Insoluble Ash	1.0

B. Extractive Values:

Table-6

S.No	PARAMETER	% w/w
	EXTRACTIVE VALUES	
1.	Water Soluble Extractive	6.1
2.	Alcohol Soluble Extractive	7.5

C. Loss on Drying:

Table-7

S.No	PARAMETER	% w/w
1.	Loss on Drying	2.9

4.4 Preliminary Phytochemical Screening

Table-8. Preliminary phytochemical screening of the 50% Methanolic extract of *Certhmus tinctorius* leaves:

S. No.	Constituents	Tests	MeOH Extract	Aquousextract
1.	Carbohydrate	Fehling's test Molisch test Benedict's test	+ve +ve +ve	+ve +ve +ve
2.	Glycoside	Legal's test Borntrager test Baljet test	-ve --ve -ve	-ve -ve -ve
3.	Flavonoids	Aqueous NaOH test Conc. H ₂ SO ₄ test	+ve +ve	+ve +ve
4.	Phenolic and tannins	Alkaline test FeCl ₃ test	+ve +ve	+ve +ve
5.	Alkaloids	Dragendorff's test Mayer's test Hager's Test	-ve -ve -ve	-ve -ve -ve
6.	Terpenes	Liebermann-Burchard test	+ve	+ve
7.	Protein and amino acids	Ninhydrin test Biuret test	+ve +ve +ve	+ve +ve +ve
8.	Fatty-acids	Foam test	-ve	-ve

Effect of *Carthamus tinctorius* Extract RIF+INZ induced Hepatotoxicity:

Table 9: Biochemical Parameters in Rifampicin+Isoniazide induced hepatotoxicity model.

Treatment	SGOT(U/L)	SGPT(U/L)	ALP(IU/P)	Total bilirubin (mg/dl)
Normal control	61.57±9.42	69.52±1.16	75.35±1.45	0.52±0.053
Rifampicin+Isoniazide	132±3.84	159.5±2.66	132.33±5.20	1.61±0.04
Silymarin (100 mg/kg)	74.33±3.20	81.33±4.36	90.66±2.73	0.74±0.02
MCT(200mg/kg)	78.5±3.08	78±2.82	84.83±3.65	1.28±0.07
MCT(300mg/kg)	66.66±4.32	68.83±2.31	75.33±4.32	1.17±0.06

One way ANOVA Followed by NEWMAN-KEULS TEST values are means ±S.E.M. for six rats

Data 1

Graph-1. Effect of Methanolic extract of *Carthamus tinctorius* on SGOT level in Rifampicin+Isoniazide induced Liver damage in rats.

Graph-2. Effect of Methanolic extract of *Carthamus tinctorius* on SGPT level in Rifampicin+Isoniazide induced Liver damage in rats.

Graph-3. Effect Effect of Methanolic extract of *Carthamus tinctorius* on ALP level in Rifampicin+Isoniazide induced Liver damage in rats.

Graph-4. Effect of Methanolic extract of *Carthamus tinctorius* on Total Bilirubin level in Rifampicin+Isoniazide induced Liver damage in rats.

4.5 Analytical Profile

4.5.1 Preparation of crude extract

Accurately weighed 2.0 gm of the coarse powder of Plant sample extracted with methanol and sonicate for 30 minutes. The combined extracts were filtered and prepare 10mg / mL solution with analytical grade methanol.

4.5.2 High Performance Thin Layer Chromatography

HPTLC was performed on 20 cm × 10 cm TLC glass plates pre coated with 200-μm layer thickness of silica gel 60F₂₅₄ (s d fine-chem. Limited, Mumbai, India). Samples were applied as 6 mm band width using Camag 100 microlitre sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator

(Camag, Switzerland) under a flow of N₂ gas. The Linear ascending development was carried out with Toluene: Ethyl Acetate, [80: 20 v/v] for plant samples as mobile phase in a Camag glass twin trough chamber (20 x 10 cm). The chamber was previously saturated with mobile phase vapour for 8 minutes at room temperature (25 ± 2°C), 50 % ± 2 relative humidity and plates were developed at distance of

approximately 80 mm from the point of application. After development, plates were dried through air drier and scanning were performed using Camag TLC Scanner 3 at λ_{max} 600 nm in UV absorbance mode for Plant samples operated by win CATS Software [Version 3.2.1]. The slit dimensions were 4 mm x 0.45 mm and the scanning speed was 100 mm/s.

Table No.- 10 Percentage of compounds present in HPTLC profile

Plant	% β - Sitosterol	% Lupeol	Ursolic acid
<i>Crthamus tinctorius</i>	0.27	0.39	Absent

Figure No.-4 Chromatographic profile of *Carthamus tinctorius* leaves extract with standards

1= Plant sample, S= Beta sitosterol, 2= Lupeol

Densitometric scan profile at 600nm

Solvent system : Toluene: Ethyl Acetate:: 8:2

Spraying reagent : Anisaldehyde Sulfuric acid

Lupeol in 20 μ l of sample = 415.96ng

β sitosterol in 20 μ l of sample = 355.39ng

5. DISCUSSION

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects.

This is one of the reasons for many people in the world over including those in developed countries turning complementary and alternative medicine (CAM). Many traditional remedies employ herbal drugs for the treatment of liver ailments.

The present study showed for the first time, flavonoids and other compounds isolated from *Carthamus tinctorius* possess hepatoprotective activity as evidenced by the significant inhibition in the elevated levels of serum enzyme activities induced by Rifampicin+ Isoniazide. Earlier studies have shown that flavonoids, a major component of *Carthamus tinctorius* possesses potent antioxidant property.

In the present study, *Carthamus tinctorius* leaves extract was given orally (200-300 mg/kg), once daily for 24 days showed dose dependent hepatoprotective activity, and highly significant effect was seen with of 200 and 300 mg/kg bodyweight, against Rifampicin+ Isoniazide induced hepatic damage.

The administration of INH and RIF, the most common medication prescribed against tuberculosis, produces many metabolic and morphological aberrations in liver due to the fact that liver is the main detoxifying site for these antitubercular drugs. These antitubercular drugs induce hepatotoxicity by a multiple step mechanism. The exact mechanism responsible for liver injury caused by these drugs is not clear.

Isoniazid is acetylated and then hydrolyzed, resulting in isonicotinic acid and monoacetylhydrazine; the later compound can be activated to a toxic species by *cytochrome* P-450. *In vitro* studies indicated that metabolic oxidation of acetylhydrazine leads to a reactive acylating species that binds covalently to microsomal protein.

It is postulated that acetylhydrazine and hydrazine act as acetylating agents by binding covalently with liver cell macromolecules, causing hepatocyte injury. RIF, a powerful inducer of mixed-function oxidase, increases the hepatotoxicity of INH by enhancing the production of toxic metabolites from acetylhydrazine.

Rats show a similar genetically determined acetyltransferase activity as in humans and are more sensitive to INH-induced hepatotoxicity due to a high amidase activity, which results in release of large amount of acetylhydrazine, which induces hepatotoxicity. Antitubercular drugs mediated oxidative damage is generally attributed to the formation of free radicals, which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane (Georgieva et al., 2004). Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components [reduced glutathione (GSH)] have been reported in INH and RIF-induced hepatotoxicity.

INH-induced liver damage in rats. INH is metabolized in the liver primarily by acetylation and hydrolysis, and it is these acetylated metabolites that are thought to be hepatotoxins. The combination of INH and RIF was reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid peroxidation, and *cytochrome* P-450 was

thought to be involved in the synergistic effect of RIF on INH. However, its role in INH induced hepatotoxicity is unclarified, as INH itself is an inducer of CYP2E1.

Metabolism of chemicals takes place largely in the liver, which accounts for the organ's susceptibility to metabolism-dependent, drug induced injury. The drug metabolites can be electrophilic chemicals or free radicals that undergo or promote a variety of chemical reactions, such as the depletion of reduced glutathione; covalently binding to proteins, lipids, or nucleic acids; or inducing lipid peroxidation. In present study, toxic control group increased the level of SGPT, SGOT, ALP and Total Bilirubin level and silymarin treatments were indicating the extract is useful for the treatment of drug injury caused by INH + RIF.

Administration of MECT markedly prevented INH+RIF induced elevation of levels of serum AST, ALT, ALP, and Bilirubin, but increased the level of total protein. The results were compared with standard drug, silymarin. Treatment with MECT significantly reduced the INH+RIF induced hepatotoxicity. The reason for hepatoprotective effect of the extract may be that, the *Carthamus tinctorius* contain flavonoids and sesquiterpenoids, which might have scavenged the free radical offering hepato-protection. In summary the results obtained from the analysis of biochemical parameters and histopathological studies, enabled to conclude that the MECT were not able to revert completely hepatic injury induced by INH + RIF, but it could limit the effect of INH + RIF to the extent of necrosis. These results indicate that it is worth undertaking further studies on possible usefulness of the MECT in hepatotoxicity.

CONCLUSION

The above observations strongly indicate the hepatoprotective activity of *Carthamus tinctorius* leaves against RIF-INZ intoxicated rats. The activity of related to detoxification was increased towards normalization. In addition, the activity of *Carthamus tinctorius*, suppressing free radical and the content of GSH reduced by free radical were affected by flavonoids. Therefore, it is concluded that effects of *Carthamus tinctorius* leave extract on liver protection are related to glutathione mediated detoxification as well free radical scavenging activity.

Herbal medicines are getting more importance in the treatment of liver disease because the modern medicine does not find curative treatments. A large proportion of the Indian population for their physical and psychological health needs depend on traditional system of medicine. Medicinal plants have become the focus of intense study in term of conservation as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. Herbal medicine is free from side effects and less costly when compared to synthetic drugs. The present study was helped the industry to produce herbal drugs which are affordable and more effective in the treatment of hepatotoxicity.

ACKNOWLEDGMENT

Authors are thankful to Dr. Ch. V. Rao, Scientist 'E', Ethanopharmacology division of N.B.R.I. Lucknow, for providing necessary facilities.

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