Available Online at www.jddt.in

ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics 2 (19) 2014, 30-39

RESEARCH ARTICLE

Enhancement of Hepatoprotective Effect of Herbal Formulation (F_1) on Carbon tetrachloride and Ethanol Induced Liver Toxicity in Albino Rats

Munish Kumar*, Hayat M. Mukhtar[1], Rohit Goyal[2], Renu Goyal[3] and Vijay Singh Nain[4]

[*4]Swami Devi Dyal Institute of Pharmacy, Barwala, Haryana, Panchkula, India

[1]Shaheed Bhagat Singh College of Pharmacy, Patti, Tarntarn, Amritsar, Punjab, India

[2]School of Pharmaceutical Sciences, Shoolini University, Solan, H.P, India

[3]Indian Institute of Integrative Medicine, Department of Natural Product Chemistry, CSIR Lab, Jammu Tawi, J & K. India

Received 10 July 2014; Accepted 22 July 2014

ABSTRACT

There are several plants and formulations which are recommended for the treatment of liver diseases. The formulation F_1 was composed with *Andrographis paniculata* (Kalmegh), *Boerhavia diffusa* (Punarnava), *Eclipta alba* (Bhringraj) and *Picrorhiza kurroa* (Kutki). In present study, an Herbal formulation was evaluated for its hepatoprotective effects against carbon tetrachloride and alcohol induced hepatocellular injury in rats. Hepatotoxicity was induced in male Charles *foster* rats by administering CCl_4 (1ml/kg, p.o., diluted in liquid paraffin) and ethanol (36.6%) 30 ml/kg/day p.o. Herbal formulation at the doses of 100, 200 and 400 mg/kg and silymarin as standard 50mg/kg were administered p.o. The hepatoprotective assessment was done by estimating biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, alkaline phosphatase, albumin, triglycerides, lipid peroxidation (TBARS) and glutathione. The histopathological studies were also done to estimate the extent of tissue injury. Herbal formulation markedly reversed the toxic effects caused by CCl_4 and ethanol intoxication as evidenced with serum and tissue biochemical parameters; similar to silymarin. Herbal formulation has also restored the cellular integrity of liver architecture after CCl_4 and ethanol exposure. The results of the study strongly indicate that the Herbal formulation has potential hepatoprotective action against CCl_4 and ethanol induced hepatic damage in rats.

Keywords: Herbal formulation, Hepatoprotective and Carbontetrachloride and Ethanol

INTRODUCTION:

Herbal drugs are rapidly becoming popular in recent years as an alternative therapy. Numerous polyherbal formulations, which are combinations of different herbal extracts/fractions, are used for the treatment of liver diseases. Antioxidants that can protect liver from oxidative damages are included in polyherbal formulations. For developing a satisfactory hepatoprotective herbal formulation, there is a need to evaluate the formulation for desired properties such as antioxidant and hepatoprotective activity. The desired activities of the polyherbal formulations containing different plants/extracts have to be tested again in the formulation form[1]. Hence, there is a need to establish simple and sensitive screening method for antioxidant activity as a means of developing quality control of polyherbal formulations. Liver plays a pivotal role in metabolism, secretion and storage. Liver disease remains one of the serious health problems as cirrhosis, jaundice and hepatic failure etc. In recent years, the herbal drug for the treatment of liver disease has increased all over the world[2,3]. There about 600 commercial herbal formulations, which are claimed to have hepatoprotective activity. Previous reports Herbal formulation have been published hepatoprotective activity on paracetamol induced hepatotoxicity[4].

Andrographis paniculata (Family: Acanthaceae) is used extensively in the Indian traditional system of medicine[5,6] as hepatoprotective and hepatostimulative agent. Andrographolide, the main active constituent of *Andrographis paniculata* has excellent anti-inflammatory, anti-bacterial and anti-viral effects. *Andrographis paniculata* is an Indian herb, well known as 'King of Bitter'. This bitter herb generally has an affinity with heart and liver. New research has confirmed a host of

***Corresponding author:** Dr. Munish Kumar **|E-mail:** goyal.munish87@gmail.com*

pharmacological benefits of this herb for its enormous potential in far wide range of diseases[7].

Boerhaavia diffusa (Family: Nyctaginaceae) is a herbal plant, which is common in the tropics in both dry and rainy seasons. It is found in India, Nigeria and many other countries. The roots are reported to be diuretic and laxative and are given for the treatment of ascites and jaundice[8,9]. In earlier studies, the roots of B. *diffusa* have been found to have diuretic, anti-inflammatory, fibrinolytic, nephrotic syndrome and anti convulsant activities[10,11,12]. It is also reported the hepatoprotective activity of the aerial part of *Boerhaavia diffusa*[13].

Eclipta alba (Family: Asteraceae) is an annual herbs. In Ayurveda plant is used as alternative, good for the complexion, cures inflammations, hernias, bronchitis, asthma, leucoderma, diseases of skin, heart, itching and night-blindness. It is principally used as a tonic and deobstruent in hepatic and splenic enlargements and in various chronic skin diseases[14]. The chemical composition of *Eclipta alba* leaves contains coumestan derivatives such as wedelolactone (1.6%) and demethyl wedelolactone[15]. Alcoholic extract of the plant is known to show protective effect on experimental liver damage in rats and mice[16]. Studies revealed the anti hepatitis B virus properties of *E.alba*[17].

Picrorhiza kurroa (Family Scrophulariaceae) are the powerful players in the international medicinal botanical scene. Kutkin is the active principle of *Picrorhiza kurroa* and is comprised of kutkoside and the iridoid glycosides picrosides I, II and III[18]. The rhizomes and roots of picrorhiza have been traditionally used to treat warms, constipation, low fever, scorpion sting and ailments affecting the liver. Current research on *Picrorhiza kurroa* has focused on its hepatoprotective[19] anticholestatic, antioxidant[20] and immune modulating activity[21]. The anti-allergic, anti-inflammatory, antioxidant and free radical scavenging effects have also been reported[22].

The Traditional system of medicine referrers that the *Andrographis paniculata*, *Boerhaavia diffusa*, *Eclipta alba* and *Picrorhiza kurroa* have been reported to possess great potential to treat various hepatic ailments. Therefore, these plants were incorporated into a Herbal formulation to evaluate its hepatoprotective effects using CCl_4 and ethanol induced hepatic injury in rat. The present communication substantiates the therapeutic utility of the formulation as hepatoprotective agents.

Individually single plant extract has been reported to have hepatoprotective activity. However, literature survey shows that no sufficient scientific data have been submitted on pharmacological evaluation of these plants in combined form. So it was decided to prepare and evaluate the formulation for its protective effects against the hepatotoxins like Carbontetrachloride (CCl_4) and ethanol.

MATERIALS AND METHODS:

Collection and Authentification of plant materials:

The fresh leaves of *Andrographis paniculata*, root of *Boerhavia diffusa*, whole plant of *Eclipta alba* and rhizome part of *Picrorhiza kurroa* were collected from the botanical garden of Indian Institute of Integrative Medicine (Regional Research Laboratory), Jammu. It was botanically identified and authenticated. A voucher specimen (Collection No. 50028, 50029, 50030 and 50031) has been kept in our laboratory for future reference. The plants were shade dried, powdered, sieved through 40 mesh and stored in a tightly closed container for further use.

Preparation of the Plant Extract:

The four powdered plant materials (200gm) was extracted successively with different solvents like Methanol (*Andrographis paniculata*), 80% ethanol (*Boerhavia diffusa*), 80% ethanol (*Eclipta alba*) and 50% ethanol (*Picrorhiza kurroa*) for twenty hours in round bottom flask and filtered. The marc was macerated with same solvent for 24 hour and filtered. This process was repeated and all the filtrates were collected and concentrated in rotary evaporator. The remaining solvent was evaporated in a water bath to obtain the extract.

Preparation of Formulation:

The extracts of the plants parts of *Andrographis paniculata*, *Boerhaavia diffusa*, *Eclipta alba* and *Picrorhiza kurroa* were employed to prepare formulation in different proportions as 1:1:1:1 (Same proportion to prepare Herbal formulation).

Animal Used:

Male Charles *foster* rats weighing 150-175gm were procured from Indian Institute of Integrative Medicine (Regional Research Laboratory) Jammu Tawi, India. They were maintained under uniform laboratory conditions in standard steel cages and provided with food (Lipton India Ltd., Bombay, India) and water ad libitum. The experiments were conducted according to the norms approved by Institutional Animal Ethics Committee guide lines for animal care and were adhered to as recommended by the Indian National Science Academy, New Delhi (1992).

EXPERIMENTAL PROTOCOL:

Carbon tetrachloride Induced hepatotoxicity:

Liver injury was induced by administration of Carbontetrachloride (CCl_4) (1ml/kg, p.o) mixed with

Liquid Paraffin (5 fold dilution)**.** Herbal formulation was administered to rats continuously for three days. On day fourth the rats were administered with herbal formulation followed by administration of toxicant after two hour. Again herbal formulation was administered on the fifth day, the rats were sacrificed to collect the blood and liver samples for blood analysis[23]. The experimental animals were divided into six groups, each group comprising six animals.

Table 1:

Sr. No.	Groups
1	Group 1: Normal Control
2	Group 2: CCl_4 Control
3	Herbal formulation (100mg/kg, *p.o.*) + CCl_4 (1ml/kg, *p.o.*)
4	Herbal formulation (200mg/kg, p.o.) + CCl_4 (1ml/kg, p.o.)
5	Herbal formulation (400mg/kg, p.o.) + CCl_4 (1ml/kg, p.o.)
6	Silymarin (50mg/kg, p.o.) + CCl_4 (1ml/kg, p.o.)

Ethanol Induced Hepatotoxicity:

Animals were divided into the five groups of six rats. Group I received normal feed and distilled water, Group II served as control, received ethanol (36.6% v/v) 30 ml/kg/day p.o., Group III received standard silymarin 50 mg/kg along with ethanol 30 ml/kg/day while Groups IV, V and VI received herbal formulation at the dose of 100, 200 and 400 mg/kg with 36.6% ethanol 30 ml/kg/day for 20 days[24]. All the animals received their respective treatment for 20 days by oral administration. Blood was collected on the 21st day.

Table 2:

Sr. No.	Groups
1	Group 1: Normal Control
2	Group 2: Ethanol Control (36%, 30ml/kg/day p.o.)
3	Silymarin (50mg/kg, *p.o.*) + Ethanol (36%, 30ml/kg/day p.o.)
4	Herbal formulation (100mg/kg, p.o.) + Ethanol (36%, 30ml/kg/day p.o.)
5	Herbal formulation (200mg/kg, p.o.) + Ethanol (36%, 30ml/kg/day p.o.)
6	Herbal Formulation (400mg/kg, p.o.) + Ethanol (36%, 30ml/kg/day p.o.)

Biochemical Estimations:

At the end of the experimental period, the animals were killed by cervical dislocation. Blood was collected in the glass tubes from orbital sinus to obtain haemolysis free clear serum for the analysis for the estimation of (SGOT) Serum Glutamate Oxaloacetate Transminases and (SGPT) Serum Glutamate Pyruvate Transminases[25], Bilirubin[26] and Triglycerides[27], All the animals were sacrificed by decapitation and livers were quickly excised freed from any adhering tissues, washed and perfused with chilled normal saline, minced and homogenized in ice bath using Potter-S-homogenizer (B. Braun, MelsungenAG, Germany, 1100 rpm for 2 min) in chilled 10mM Tris–HCl buffer (pH 7.4) to obtain 10% liver homogenate for the estimation of (GSH) glutathione[28], (LP) lipid peroxidation[29] and estimation of Albumin was used standard kit (Bayer Diagnostic Ltd., Gujarat, India).

Histopathological Investigation:

The liver tissues were excised out, washed with the cold saline, fixed in 10% buffered formalin for 12 hours and processed and stained with hematoxylin and eosin dye for photomicroscopic observations.

Statistical Analysis:

Data ± SEM, p value ≤ 0.001 considered statistically significant; One way ANOVA followed by Dunnett's multiple comparison test as post hoc analysis.

RESULT:

Table No. 4 and 5. Show hepatotoxication with CCl_4 and alcohol caused significant ($p<0.05$) increase in serum transaminases: ALT, AST, ALP, triglyceride and bilirubin levels in comparison to saline control rats. Treatment with Herbal formulation (F_1) at dose level 100, 200, 400 mg/kg p.o. and silymarin 50 mg/kg p.o. significantly ($p<0.05$) attenuated the increase in these serum markers in a dose dependent manner. All the hepatotoxicant control groups showed significant ($p<0.05$) increase in

lipid peroxidation products (TBARS) as compared to SC. Treatment with Herbal formulation (F_1) at dose 100, 200 and 400mg/kg p.o. and SILY50 produced significant ($p<0.05$) decrease in TBARS level in a dose-dependent manner as compared to hepatotoxicants. The effect of SILY50 was not significantly ($p>0.05$) different from that of saline treated control rats (Fig 1,4).

CCl_4 or alcohol intoxications caused significant ($p<0.05$) decrease in tissue GSH level as compared to SC. Treatment with Herbal formulation (F_1) 100, 200 and 400mg/kg p.o. and SILY50 significantly ($p<0.05$) restored the levels of GSH in a dose-dependent manner. The increase in GSH level on treatment with Herbal formulation (F_1) 400mg/kg p.o. and SILY50 against alcohol intoxication, which indicate the most active dose 400mg/kg p.o (Fig 2,3).

In histological study, (A) liver sections from saline control group showed normal lobular architecture and hepatic cells. (B) Histological examination of livers challenged with CCl_4 and alcohol revealed obvious fatty degeneration with displacement of the nucleus (C,D,E) Treatment with Herbal formulation (F_1) 100, 200 and 400 showed normal lobular structure with slight fatty changes against CCl_4; and little dilatation in blood sinusoids and vacuolization against alcohol dose dependently. (F) SILY50 group also caused marked reversal in histological changes against hepatotoxicants (Fig 5,6).

Table 3: Hepatoprotective activity of Herbal formulation (F_1) against carbon tetrachloride (CCl_4) Induced Hepatotoxicity (SERUM PARAMETERS)

Group	**SGOT** (U/l)	**SGPT** (U/l)	**Albumin** (g/dl)	**Bilirubin** (mg/dl)	**Triglyceride** (mg %)
Control	94.36±7.55	103.67±6.53	3.35±0.20	0.34±0.04	103.34±6.14
CCl_4 1ml/kg, p.o	1328.04±126.38[a]	811.89±34.84[a]	2.35±0.10[a]	1.12±0.09[a]	147.29±8.27[a]
Herbal formulation 100mg/kg, p.o	852.96±61.54[*]	601.46±27.38[**]	2.57±0.13[***]	0.79±0.05[**]	131.09±3.44[*]
Herbal formulation 200mg/kg, p.o	757.12±60.44[***]	523.91±31.50[***]	2.70±0.11[**]	0.75±0.05[*]	129.23±3.00[**]
Herbal formulation 400mg/kg, p.o	697.60±38.84[***]	482.13±17.89[***]	2.86±0.19[***]	0.70±0.06[***]	125.14±2.76[***]
Silymarin 50mg/kg, p.o	630.59±50.29[***]	470.47±22.49[***]	2.89±0.20[***]	0.65±0.04[***]	124.35±5.02[***]

(Values as Mean ± S.D, n=6)

[a]p≤ 0.0015 vs Normal control, [*]≤0.05, [**]≤0.01 [***]≤0.001vs Carbontetrachloride (CCl_4) control group

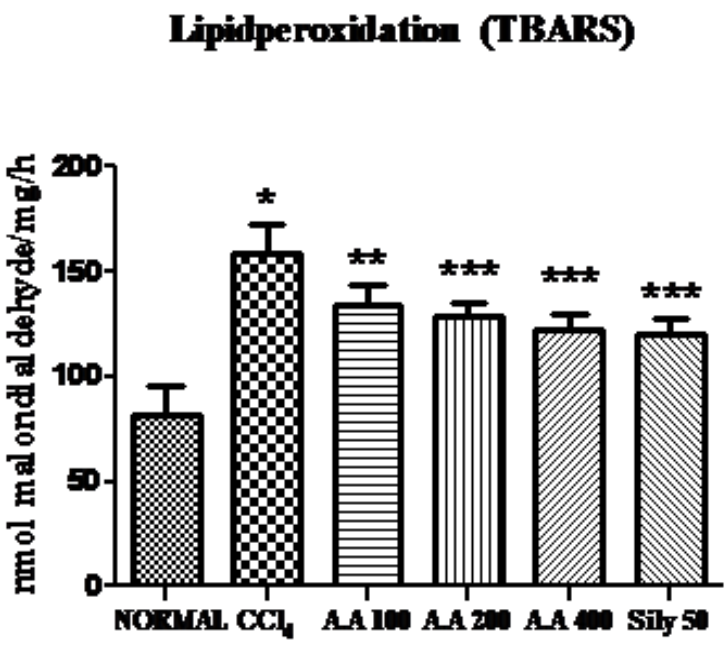

Fig1: Results: Mean±SD, *p≤0.05 vs Normal **p≤0.01 and ***p≤0.001 vs CCl_4 (n=6)

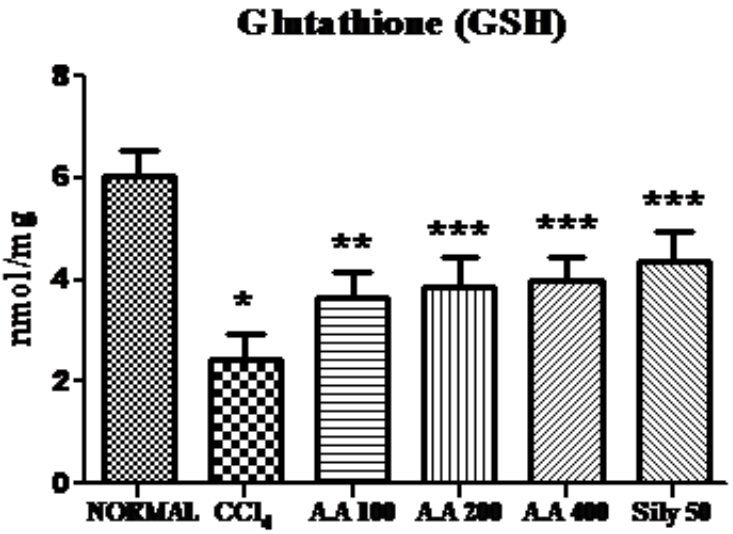

Figure 2: Result: Mean ± SD, *p≤0.05 vs Normal **p≤0.01 and ***p≤0.001 vs CCl_4 (n=6)

Table 4: Hepatoprotective activity of Herbal formulation (F_1) against Ethanol Induced Hepatotoxicity (SERUM PARAMETERS)

Group	**SGOT** (U/l)	**SGPT** (U/l)	**Albumin** (g/dl)	**Bilirubin** (mg/dl)	**Triglyceride** (mg %)
Control	64±3.5	56±1.2	5.10±1.10	1.97±0.42	127.1±2.05
Ethanol (36.6% v/v) 30 ml/kg/day p.o	216±10.1	204±5.2	4.36±1.92	3.01±1.08	163.0±2.10
Herbal formulation 100mg/kg, p.o	130±7.91***	126.6±4.32**	4.65±1.71*	2.66±1.72**	150.91±2.01**
Herbal formulation 200mg/kg, p.o	112.4±4.78***	102.1±2.09***	4.85±1.21**	2.41±1.63*	145.14±2.12***
Herbal formulation 400mg/kg, p.o	90±1.32**	94.02±1.13***	4.97±1.08***	2.16±1.52**	136.02±2.21**
Silymarin 50mg/kg, p.o	70±6.1***	87±0.08***	5.04±1.67***	2.05±1.03***	130.18±2.35***

(Values as Mean ± S.D, n=6)

[a]p≤ 0.0015 vs Normal control, *≤0.05, **≤0.01 ***≤0.001vs Ethanol control group

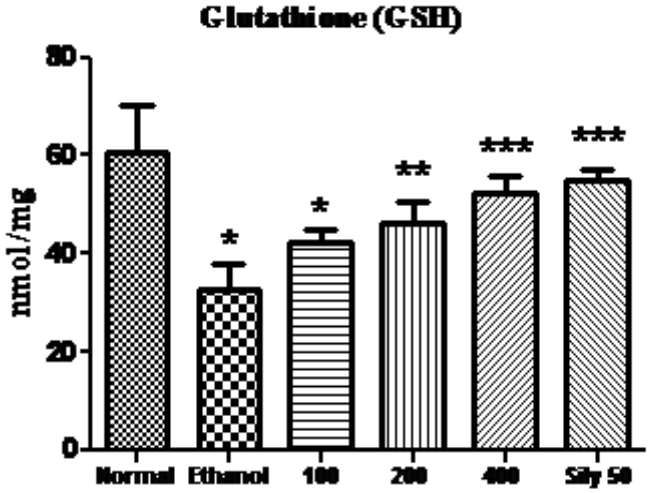

Figure 3: Result: Mean ± SD, *p≤0.05 vs Normal *p≤0.05, **p≤0.01 and ***p≤0.001 vs Ethanol (n=6)

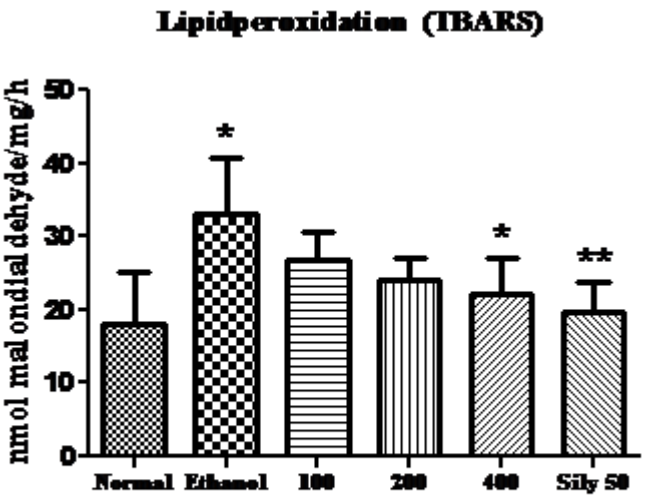

Fig 4: Results: Mean±SD, *p≤0.05 vs Normal **p≤0.01 and ***p≤0.001 vs Ethanol (n=6)

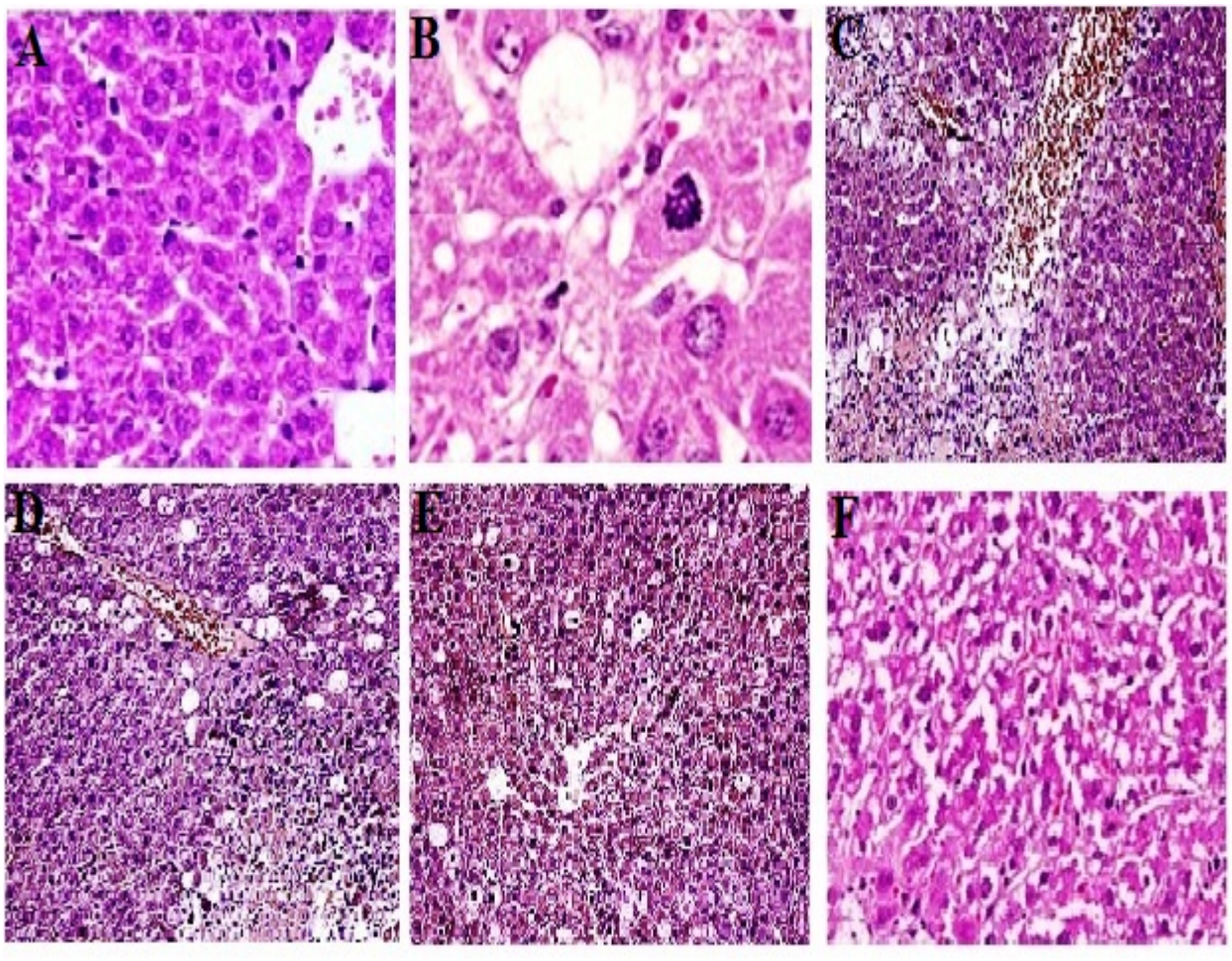

Figure 5: Hepatoprotective activity of Herbal formulation (F_1) against Carbontetrachloride (CCl_4) Induced Hepatotoxicity

Effect of Herbal formulation (F_1) (100, 200 and 400 mg/kg, p.o.) on histopathological characteristics: A: Saline Control (SC); B: CCl_4 Control; C: Herbal formulation 100+CCl_4; D: Herbal formulation 200+CCl_4; E: Herbal formulation 400+CCl_4; F: SILY50+CCl_4 (10X).

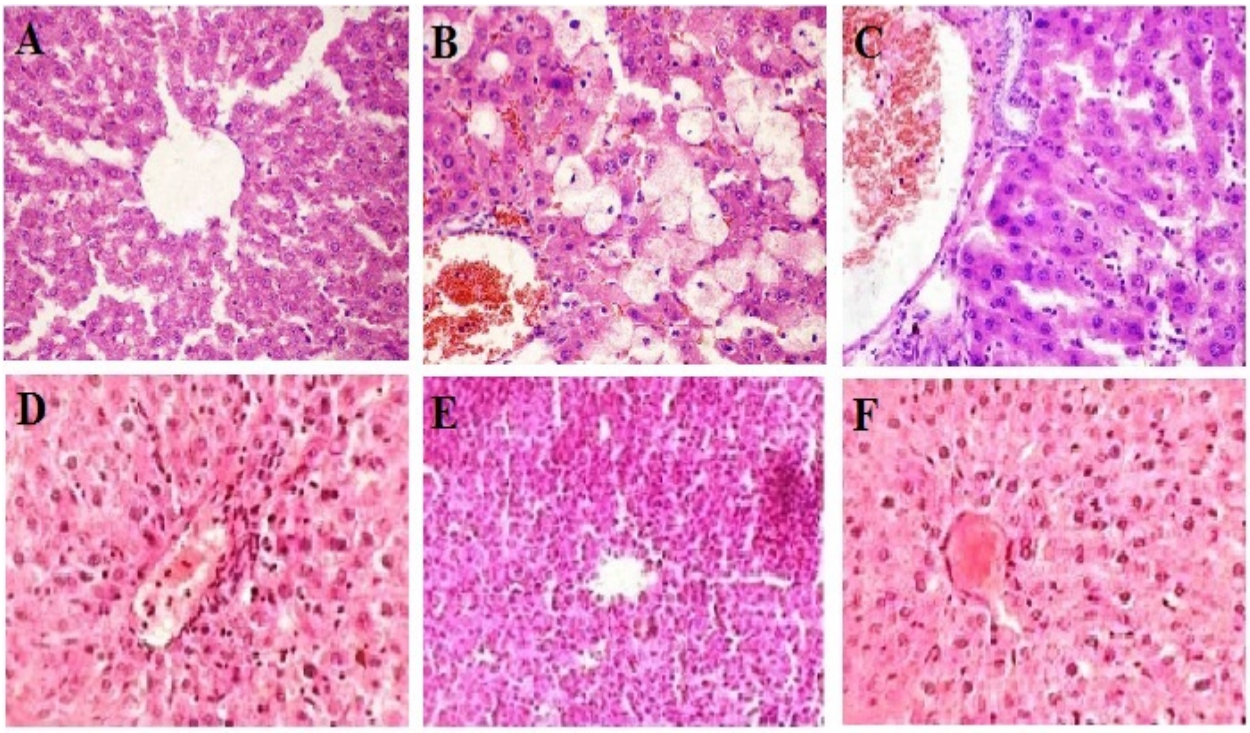

Figure 6: Hepatoprotective activity of Herbal formulation (F_1) against Ethanol Induced hepatotoxicity

Effect of Herbal formulation (F_1) (100, 200 and 400 mg/kg, p.o.) on histopathological characteristics: A: Normal Control; B: Alcohol Control; C: Herbal formulation100+Alc.; D: Herbal formulation200+Alc.; E: Herbal formulation 400+Alc., and F: SILY50+Alcohol (10X).

DISCUSSION:

The study was taken up with an aim to determine the efficacy of marketed hepatoprotective agents. The present work dealt with the comparative study of selling Polyherbal hepatoprotective formulations. A monoherbal standardized extract of *Andrographis paniculata*. The monoherbal extract of Andrographis paniculata was used to determine whether addition of many plants in a formulation is superior to the use of one single herb for hepatoprotection. From the results, it is evident *Andrographis paniculata* produced better effect. In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and Polyherbal formulations claimed to have hepatoprotective activities. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity[30,31,32]. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations. Some of the Polyherbal formulations are verified for their hepatoprotective action against chemical induced liver damage in experimental animals[33,34]. In most of these studies, marginal and moderate levels of hepatoprotective activities were observed. It is believed that efficacy is not sufficient enough to use these agents as effective drugs[35]. Besides, most of the reported studies described the beneficial effects of the drugs against few hepatotoxic chemical-induced subclinical level of hepatotoxicity. It is not known whether or not these drugs exhibit any beneficial effects against severe liver damage.

Further, the herbal products manufactured in India have been criticized by regulatory agencies in both India and abroad. A recent study conducted by 'National Commission of Macroeconomics and Health (NCMH)' set up by the Ministry of Health to review the state of the country's health, concluded that "Liv-52 is a useless liver drug"[36]. Another report published by Dhiman and Chawla also reported that Liv.52 is not effective for treatment of alcohol induced liver damage[37].

The present study was carried out using four different models of liver damage to determine the effects of drugs. The different drugs used in the present study showed variable effects in different models of hepatotoxicity. This difference could probably be due to the different mechanisms by which hepatotoxicity is induced.

Hepatic cells participate in metabolic activities and contain host of enzymes. In tissue, asparate aminotransferase (AST) and alkaline aminotransferase (ALT) were found to be in higher concentrations in cytoplasm, and AST exists in mitochondria. In liver injury, transport function of the hepatocytes gets disturbed, resulting in the leakage of plasma membrane and thereby causing an increased enzyme level in serum[38]. The elevated activities of these enzymes are indicative of cellular leakage and the functional integrity of the cell membranes in liver. ALP is excreted by liver via bile in the liver injury due to hepatotoxins, which results in a defective excretion of bile by the liver and is reflected in their increased levels in serum. In drug induced liver toxicity, the level of LDH, and TB and DB get elevated. The present study was carried out to find out the effect of the Herbal formulation (F_1) on the CCl_4 and ethanol induced hepatotoxicity.

Carbon tetrachloride (CCl_4) is one of the most powerful hepatotoxin in term of severity of injury. It causes necrosis, which leads to biochemical changes having clinical features similar to those of acute viral hepatitis[39]. It has been assumed that CCl_4 is biotransformed by the cytochrome P450 system to produce the •CCl3 free radical. This free radical may react again with oxygen to form a trichloromethyl peroxyl radical, which may attack lipids[40]. Thus, rapid breakdown of the structure and in turn function of the endoplasmic reticulum is due to decomposition of the lipid. Plasma membrane damage is thought to be caused by relatively stable fatty aldehydes, which are produced by lipid peroxidation in the SER but are able to act at distant sites. This is followed by massive influx of calcium and cell death[41].

In chronic Alcohol induced hepatotoxicity caused by oxidative stress is one of the major factors in the etiology of ethanol injury mainly by Kupffer cell derived from ROS. Chronic consumption of ethanol causes injury to the liver cells. Increased level of serum albumin, AST, ALT, and ALP in alcohol-treated rats can be attributed to the damaged structural integrity of the hepatic cells because of the enzymes ALP located in the cytoplasm and released in the circulation after cellular damage. Alcohol consumption causes both plasma and organelle membrane damage[42]. Alcohol consumption is known to cause fatty infiltration and cirrhosis. It enhanced lipid peroxidation produced during the microsomal metabolism of ethanol. It will directly generate free radicals such as O_2 (superoxide), H_2O_2, OH (Hydroxy free radicals), and CH_3CHOH (Hydroxy ethyl free radicals) through activation of cytochrome P450 $2E_1$ enzymes. Oral administration of ethanol at a dose of 30 ml/kg/day significantly increased the SGOT, SGPT, ALP, Bilirubin, triglycerides and lipidperoxidation (TBARS) and decreased the levels of albumin and glutathione (GSH).

Treatment with Herbal formulation (F_1) reversed the activity of transaminases, and restored them towards normal depicting a marked protective effect. These findings are also substantiated by the administration of plant extracts of *Artemisia absinthium*, *Mamordia subangulata* Turmeric and *Calotropis procera* that showed significant reduction in toxicant induced rise in the enzymatic levels. In our study treatment with of Herbal formulation (F_1) significantly ($p \leq 0.01$) reversed the increased lipid peroxidation and albumin level by carbon tetrachloride and ethanol. Therefore it is possible that the fractions could elevate glutathione levels, thereby increasing the formation of extract GSH-containing radical adducts and thus explain the reduced CCl_4 and ethanol injury following herbal formulation treatments.

Treatment with Herbal formulation (F_1) significantly reversed CCl_4 and ethanol induced serum markers like AST, ALT, Bilirubin, triglyceride and albumin and liver tissue like lipidperoxidation (TBARS) and glutathione (GSH) levels indicating protective effects against fatty liver. Formulation (F_1) at the dose 400mg/kg, p.o. showed maximum protection, which is well comparable to silymarin in CCl_4 and ethanol. Hence, from the results of the study, it is recommended that the manufacturers should concentrate on using standardized monoherbal extracts containing known amount of active constituent than mixing more than one drug in the formulation. Further, it is recommended that standardization of herbal drugs, proper preclinical studies and clinical studies should be carried out before marketing the herbal drugs.

CONCLUSION:

The above data may reveal that the Herbal formulation composed with *Andrographis Paniculata*, *Boerhaavia diffusa*, *Eclipta alba* and *Picrorhiza kurroa* has hepatoprotective potential against Carbon tetrachloride (CCl_4) and ethanol induced hepatotoxicity in rat. In this study, the Herbal formulation (400mg/kg, p.o.) produced excellent hepatoprotective activity on Wister albino rats. The dose levels selected were 100mg/kg, p.o, 200mg/kg, p.o and 400mg/kg, p.o. Histopathological examination of the liver sections of the rats treated with toxicant showed necrosis and the fatty material changes. Thus it was concluded that the Herbal formulation 400mg/kg, p.o exhibited significant dose dependent hepatoprotective activity.

ACKNOWLEDGEMENT:

Thank to Division of Pharmacology, I.I.I.M (CSIR Lab.), Jammu and I.S.F. College of Pharmacy, Moga, Punjab, and

the authors great fully acknowledge the valuable contributions/suggestions of Dr. N. Mahadevan and Dr. B.K Chandan.

REFERENCES:

1. Subramoniam, A and Pushpangadan, P. (1999). Indian Journal of Pharmacology, 31: 166-175.
2. Girish C and Pradhan S.C. (2008). Drug development for liver diseases; focus on Picroliv, ellagic acid and curcumin, *Fundam Clin Phamcol*, 22: 623.
3. Handa, S.S., Sharma, A and Chakarborti, K.K. (1986). Natural products and plants as liver protecting drugs. Fitoterapia, 57 (5): 307-351.
4. Kumar, M., Hayat, M.M, Munshi, S.K, Raman, S and Davinder K. (2011). Potential Hepatoprotective effect of herbal formulation on Paracetamol Induced hepatotoxicity in Mice. Pharma Research, 4 (2): 186-192.
5. Chen, J and Jiang, H. (1980). Morphological and histological studies on *Andrographis Paniculata* and comparison with four other Acanthaceae herbs. Yao Xye Xue Bao, 15 (12): 750-760.
6. Hu C.Q and Zhou, B.N. (1982). Isolation and structure of two new diterpenoid glucosides from *Andrographis Paniculata* Nees. Yao Xye Xue Bao, 17 (6): 435-440.
7. Sreeparna, N., Subhasis, D., Santanu K.M, Nirjal, M, Somenath R. (2008). Amelioratory effect of *Andrographis paniculata* Nees. on liver, kidney, heart, lung and spleen during nicotine induced oxidative stress. Environmental Toxicology and Pharmacology, 25: 321-328.
8. Kirtikar, K.R and Basu, B.D. (1933) Indian Medicinal Plants. Lalit Mohan Basu, Allahabad, India.
9. Chopra, R.N., Nayar, S.L and Chopra, I.C. (1956) Glossary of Indian Medicinal Plants. C.S.I.R., New Delhi, India.
10. Bhalla, T.N., Gupta, M.B and Bhargava, K.P. (1971) Anti-inflammatory activity of *Boerhaavia diffusa* L. Journal of Research in Indian Medicine 6 (1): 11-15.
11. Mudgal, V. (1975) Studies on medicinal properties of *Convolvulus pluricaulis* and *Boerhavia diffusa*. Planta Medica 28(1): 62-68.
12. Satyavati, G.V., Raina M.K and Sharma, M. (1976) Medicinal Plants India 1. Indian Council of Medicinal Research, New Delhi.
13. Chandan, B.K., Sharma, A.K and Anand, K.K., 1991. *Boerhaavia diffusa*: a study of its hepatoprotective activity. Journal of Ethnopharmacology 31, 299-307.
14. Kirtikar K.R and Basu B.D. (1975). Indian Medicinal Plants. Lalit Mohan Basu, 4, Leaders Road Allahabad, 1364-1365.
15. Meena, A.K., Raol, M.M., RP Meena, R.P., Panda, P and Renu. (2011). Pharmacological and Phytochemical Evidences for the Plants of Wedelia Genus- A Review. Asian Journal of Pharmacy Research, 1 (1): 7-12.
16. Singh B, Saxena K, Chandan B, Aggarwal S, Bhatia M.S and Anand K.K. (1993). Hepatoprotective effect of ethanolic extract of *Eclipta alba* on experimental liver damage. Phytother Res, 7: 154-158.
17. Jayaram S, Thyagrajan S, Panchanadam M, Subramanian S. (1987). Anti-hepatitis B virus properties of *Phyllanthus niruri* Linn and *Eclipta alba* Hassk. in vitro and in vivo safety studies. Bio-Medicine, 7: 9-16.
18. Stuppner, H and Wagner, H. (1989). Planta Medica, 55: 559-563.
19. Anandan, R and Devaki, T (1999). Hepatoprotective effect of *Picrorhiza kurroa* on tissue defence system in D-galactosamine induced hepatitis in rats. Fitoterapia, 70: 54-57.
20. Chander R, Kapoor N.K and Dhawan B.N. (1992). Picroliv, Picroside-I and kutkoside from *Picrorhiza kurroa* are scavengers of superoxide anions. Biochem Pharmacol, 44: 180-183.
21. Sharma, M.L., Rao, C.S and Duda, P.L. (1994). Immunomodulatory activity of *Picrorhiza kurroa* leaf extract. J Ethanopharmacolgy, 41: 185.
22. Baruah, C.C., Gupta, P.P., Nath, A., Patnaik, L.G and Dhawan, B.N. (1998). Anti-allergic and anti-anaphytic activity of picroliv- a standardized iridoid Glycoside fraction of *Picrorhiza kurroa*, Pharmacology Research, 38: 487.
23. Bramanti, G., Murmann, W., Pierini, P., Comporti, M. (1978). Effect of cicloxilic acid on CCl_4 induced liver injury. Arzneimettelforsch 28: 1212-1217.
24. Grange, L.L., Wang, M., Watkins, R., Ortiz, D., Sanchez, M.E and Konst, J. (1999). Protective effects of the flavonoid mixture, silymarin, on fetal rat brain and liver. J Ethnopharmacol, 65: 53-61.
25. Reitman, S and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. American Journal Clinical Pathology, 28: 56-63.
26. Malloy, H.T and Evelyn, K.A. (1937). The determination of bilirubin with the photoelectric colorimeter. Journal of Biological Chemistry, 119: 481-490.

27. Neri, B.P and Frings, C.S. (1973). Improved method for determination of triglycerides in serum. Clinical Chemistry, 19: 1201.

28. Ellman, G.L. (1959). Tissue sulfhydral groups. Archives of Biochemistry and Biophysics 82: 70-77.

29. Buege, J.A and Aust, S.D. (1978). Microsomal lipid peroxidation. In: Sidney Fleischer, Leister Packer (Eds.), Methods in Enzymology, vol. 52. Academic Press, New York, San Francisco, London, 302-310.

30. Scott Luper, N.D. (1998). A review of plants used in the treatment of liver disease: part I. Altern. Med. Rev, 3 (6): 410-421.

31. Bhatt AD, Bhatt NS. (1996). Indigenous drugs and liver disease. Indian J. Gastroenterol, 15 (2): 63-67.

32. Nadeem M. (1996). Hepatoprotective activity of some herbal formulations available in India. Indian Drugs, 33 (8): 390-396.

33. Karunakar N. (1997). Further studies on the antihepatotoxic activity of Jigrine. Indian J. Pharmacol, 29: 222-227.

34. Mitra, S.K., Venkataranganna, M.V., Sundaram, R and Gopumadhavan, S. (1998). Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. J. Ethnopharmacol, 63: 181-186.

35. Sane, R.T. (1995). Too many hepatoprotective drugs and little hepatoprotection. Current Sci, 69 (11): 898-899.

36. Retrieved from:- http://www.hindustantimes.com /2005/Oct/25/5922_1528820,0015002100000000.htm on 04-01-07.

37. Dhiman, R.K and Chawla, Y.K. (2005). Herbal Medicines for Liver Diseases. Digestive Diseases and Sciences, 50 (10): 1807-1812.

38. Rajagopal S.K., Manickam, P., Periyasamy, V and Namasivayam, N. (2003). Activity of Cassia auriculata leaf extract in rats with alcoholic liver injury. J. Nutr. Biochem, 14: 252-258.

39. Vogel, G. (1977). In New Natural Products and Plant Drugs with Pharmacological, Biological and Therapeutically activity. H Wagner and P Wolff (eds). Springer Verlag, Berlin: 249-265.

40. Ahmad, F.F., Cowan, D.L and Sun, A.Y. (1987). Detection of free radical formation in various tissues after acute carbontetrachloride administration in gerbil. Life Sci, 40: 2469-2475.

41. Raucy, J.L., Kraner, J.C and Lasker, J.M. (1993). Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. Crit. Rev. Toxicol, 23 (1): 1-20.

42. Jafri, M.A., Subhani, M.J., Javed, K and Singh, S. (1999). Hepatoprotective activity of leaves of *Cassia occidentalis* against paracetamol and ethyl alcohol intoxication in rats. J. Ethanopharmacol, 66: 355-361.

