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Research Article

Comparative Evaluation of Antioxidant and Hepatoprotective Activities of Some New Polyherbal Formulations

Kalam Sirisha*, Puchchakayala Goverdhan**,** Guggulothu Hima bindu, Meesa Madhavi
Vaagdevi College of Pharmacy, Kishanpura, Hanamkonda, Warangal-506 001, Andhra Pradesh, India

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Abstract

The present study was aimed at comparatively evaluating the hepatoprotective activity against paracetamol induced liver damage and antioxidant activities of some new polyherbal formulations in rats. Polyherbal formulations F1 and F2 were developed by exploiting the knowledge of traditional system of medicine and evaluated for antioxidant (*in vitro* and *in vivo*) and hepatoprotective activities using acute liver toxicity induced by paracetamol in rats. Rats were monitored for morphological changes in biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum bilirubin (total and direct), total protein and histopathological studies. *In vitro* and *in vivo* antioxidant activity of F1 and F2 were studied. Present investigations revealed that the animal groups treated with formulations F1 and F2 (500 mg/kg) showed significant antioxidant and hepatoprotective activities, which were comparable to Liv52 (1ml/kg). Activity of these two formulations was compared relatively and with the standard formulation Liv52.This study concludes that formulation F2 is more potent than formulation F1.

1. INTRODUCTION

Liver is an essential organ that plays an important role in regulating various physiological processes in the body. Any change in the anatomy or function of liver is characterized as liver disease. Liver has tremendous capacity to detoxify toxic principles and synthesize useful principles. Therefore damage to the liver by hepatotoxic agents is of grave consequence. Various types of liver disorders are characterized by cirrhosis, jaundice, tumours, metabolic and degenerative lesions, liver cell necrosis, hepatitis etc. Besides viruses, liver disorders can arise due to xenobiotics, excessive drug therapy, environmental pollution and alcohol intoxication[1, 2].

Liver is rich in antioxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) that can metabolize reactive oxygen species. Antioxidant enzymes eliminate superoxide anions and hydroperoxides that can oxidize cellular substrates. However, these enzymes also exert protective effects by scavenging the oxidized forms of the non enzymatic antioxidants vitamin E, vitamin C and glutathione[3]. Reactive oxygen species (ROS) are well recognized for the pathogenesis of various diseases such as cancer, atherosclerosis, inflammation, diabetes, parkinsonism etc. Thus, a balanced intake of antioxidant with scavenging action of ROS is important for prevention of the disease[4].

The management of liver diseases is still a challenge to the modern medicine. In spite of the tremendous advances in modern medicine, no effective drugs are available to stimulate liver functions and offer protection to the liver from the damage, although some of these drugs adversely affect the liver function.[5]

In the absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief. Herbal principles are coming up as an effective source of disease treatment. Ayurvedic system of medicine has always used this hidden potential, but it is very essential to mould this system with modern standards.[6] A great deal of research has been carried out to evaluate scientific basis for the claimed hepatoprotective activity of herbal agents as single agent or in formulation.[7] The herbal formulations (F1 and F2) under study contain plant ingredients like *Aloe barbadensis* (Liliaceae), *Azadirachta indica* (Meliaceae), *Cinnamomum zeylanicum* (Lauraceae), *Curcuma longa* (Zingiberaceae) and *Emblica officinalis* (Euphorbiaceae). The form of extract whether aqueous or ethanolic and content in dose is based on the traditional knowledge and reports present on these plants. The main objective of this study is to evaluate the efficacy of these formulations in rats in which acute hepatotoxicity was induced by paracetamol treatment.

2. MATERIALS AND METHODS

2.1 Preparation of Formulations

Two polyherbal syrup formulations F1 and F2 are a uniform mixture of dried and pulverized herbal extracts (Table 1). It contains mixture of ethanolic extract of *Aloe barbadensis, Azadirachta indica, Cinnamomum zeylanicum, Curcuma longa* and aqueous extract of *Emblica officinalis*. All crude materials were procured from market and authenticated by Dr. Vastavaya S. Raju, Head, Department of Botany, Kakatiya University, Warangal.

2.2 Chemicals

Liv.52 was purchased from Himalaya Drug Co; Bangalore and paracetamol from Finar chemicals Ltd; Chennai.

2.3 Choice of Animal

Male albino rats (strain Wistar) with weight range 150-200gm were obtained from Mahaveer Enterprises, Hyderabad. All animals were maintained under standard conditions at room temperature and 12 hr light/dark cycle. They had been given standard pellet diet and water *ad libitum* throughout the course of study. The rats were

Corresponding Author:
Dr. Kalam Sirisha,
Associate Professor,Vaagdevi College of Pharmacy, Kishanpura, Hanamkonda- 506001, A.P., India.
Tel: +91 9949024247, Fax: +91 870 2544949
Email: ragisirisha@yahoo.com

randomly selected and were divided into different groups with five animals in each group.

2.4 Acute Toxicity Studies

Acute toxicity study was carried out in adult male albino rats by the "fixed dose" method of OECD guideline no.420. In fixed dose method, test procedure with a starting dose of 2000mg/kg body weight, was adopted[8]. The animals were fasted overnight and on the next day formulations F1 and F2 (syrup) were administered orally at a dose level of 2000mg/kg. Then the animals were observed continuously for 3 hrs for behavioural, neurological and autonomic profiles and then every 30 min for next 3 hrs and finally for mortality after 24 hrs till 14 days.

2.5 Screening for In Vitro Antioxidant Activity

2.5.1 Reductive Ability

Extract solution (2ml), phosphate buffer (2ml, 0.2M, pH 6.6) and potassium ferricyanide (2ml, 10mg/ml) were mixed, and then incubated at 50°C for 20min. Trichloroacetic acid (2 ml, 100 mg/ml) was added to the mixture. A volume of 2ml from each of the above mixtures was mixed with 2ml of distilled water and 0.4ml of 0.1% (w/v) ferric chloride in a test tube. After 10 min reaction, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated a high reducing power[9].

2.5.2 Hydrogen Peroxide Scavenging Activity

Formulations F1 and F2 (4ml) prepared in distilled water at various concentrations were mixed with 0.6ml of 4mM H_2O_2 solution prepared in phosphate buffer (0.1M, pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2[10].

2.5.3 DPPH Radical Scavenging Activity

To 0.1ml of 0.1 mM solution of DPPH(2,2-diphenyl-1-picrylhydrazyl) in methanol, add 3ml of formulations suspension in water at different concentrations (10-100µg/ml). After 30 min the absorbance was measured at 517 nm[11]. Lower absorbance indicates higher activity. Ascorbic acid was used as reference material. Percentage inhibition (IC_{50}) was calculated as:

$$IC_{50} = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

2.6 In Vivo Animal Model for Hepatoprotective Activity

2.6.1 Paracetamol Induced Toxicity

Albino rats were divided into five groups with 5 animals in each group. Animals were overnight fasted and treated as follows-

Group-I (Normal control): vehicle simple syrup 1ml/kg p.o. for 7 days.

Group-II (Disease control): simple syrup 1ml/kg p.o. for 7 days and paracetamol 2gms/kg p.o. on 5th day

Group-III (Standard control): Liv.52 (1ml/kg, p.o.) for 7 days and paracetamol 2gms/kg p.o. on 5th day

Group-IV (F1): polyherbal formulation1 (F1) 500 mg/kg, p.o. for 7 days and paracetamol 2 gms/kg, p.o. on 5th day

Group-V (F2): polyherbal formulation2 (F2) 500 mg/kg, p.o. for 7 days and paracetamol 2 gms/kg, p.o. on 5th day

After 48h of paracetamol feeding, blood was collected from each rat for serum analysis. The biochemical parameters estimated include SGOT, SGPT, SALP, total protein and serum bilirubin (total and direct) [12].

2.6.2 Histopathological Studies

The animals were sacrificed; liver of each animal was removed and fixed in 10% formalin for histopathological studies of the liver to determine the degree of hepatic damage.

2.7 Screening of In Vivo Antioxidant Activity

2.7.1 Preparation of Tissue Homogenate

The tissues were weighed and 10% tissue homogenate was prepared with normal saline and centrifuged at 3000 rpm for 15 min. The supernatant was collected and used for estimation of catalase activity, lipid peroxidation and glutathione levels.

2.7.2 Catalase Activity

To 0.4ml of hydrogen peroxide (0.2M) was added 1ml of 0.01 M phosphate buffer (pH 7) followed by the addition of 0.1ml of clear supernatant of liver homogenate (10% w/v) and gently swirled at room temperature. The reaction of the mixture was stopped by adding 2ml of potassium dichromate acetic acid reagent (5 % $K_2Cr_2O_7$ prepared in glacial acetic acid). The changes in the absorbance was measured at 620 nm and recorded after 3 min interval[13]. Percentage inhibition was calculated by using the equation.

$$\% \text{ inhibition} = \frac{A_C - A_S}{A_C} \times 100$$

$A_C \rightarrow$ absorbance of control
$A_S \rightarrow$ absorbance of sample

2.7.3 Estimation of Lipid Peroxidation (Malondialdehyde)

To 0.5ml of liver homogenate, 0.5ml of 30% trichloro acetic acid (TCA) was added to precipitate the proteins and vortexed for 30 sec. Clear supernatant was taken after centrifuging at 3000 rpm for 10 min. To the supernatant, 500µl of 1% TBA (thiobarbituric acid) solution and 500µl of water was added and this solution was heated for 1 hr at 98°C. The solutions were cooled to room temperature and kept in ice for 5 min. Then the absorbance of the pink solution was measured at 532 nm using spectrophotometer. Standard graph was plotted using TEP (1, 1, 3, 3-tetraethoxy propane) [14].

2.7.4 Estimation of Glutathione

Glutathione forms a colored complex with DTNB (5, 5^1-(dithio-bis-2-nitro benzoic acid)), which is measured spectrophotometrically. To 0.5ml of liver homogenate, 0.5ml of 5% TCA solution was added to precipitate the proteins and centrifuged at 3000 rpm for 20 min. To 0.1ml of supernatant, 1ml of sodium phosphate buffer and 0.5ml of DTNB reagent was added. The absorbance of the yellow color developed was measured at 412 nm. The glutathione content was determined from the standard graph by using pure glutathione[15].

2.8 Statistical Analysis

The data were expressed as mean ± standard deviation (SD). Statistical comparisons were performed by one-way ANOVA followed by Dunnett's test using Graph Pad Prism version 6.0.

3. RESULTS

3.1 Acute Toxicity Study

Animals showed good tolerance to single dose of formulations F1 and F2 in dose as high as 2000 mg/kg body weight and were non-lethal. 500 mg/kg body weight of both the formulations was used to test the hepatoprotective and antioxidant activities.

***3.2 In vitro* Antioxidant Activity**

3.2.1 Reducing Power Method

Reducing power of formulations were found to be more than the reducing power of standard ascorbic acid and F2 exhibited greater reducing power than F1 (Figure 1).

3.2.2 Hydrogen Peroxide Scavenging Activity

Standard (ascorbic acid) was found to be more potent than the polyherbal formulations F1 and F2 (Figure 2). H_2O_2 scavenging activity was in the order of ascorbic acid>F2>F1.

3.2.3 DPPH Assay

The IC_{50} value for ascorbic acid was found to be very low when compared to IC_{50} values of F1 and F2 (Figure 3). F2 exhibited higher potency than F1 but the results are not comparable with that of the standard.

3.3 In vivo Antioxidant Activity

3.3.1 Catalase Activity

Catalase activity was determined by measuring the percentage inhibition of H_2O_2. Percentage inhibition of H_2O_2 was found to be more in standard, F1 and F2 treated groups when compared to toxic control group (Figure 4).

3.3.2 MDA and Glutathione Levels

Both the formulations reduced the levels of MDA when compared to toxic control. Glutathione levels were found to be near to the normal levels in standard and formulations treated groups (Figure 5).

3.4 Paracetamol Induced Hepatotoxicity

Paracetamol (2gm/kg body weight) given once orally showed hepatotoxicity after 48 h as evident from biochemical and histopathological parameters of the study. It significantly increased the levels of SGOT, SGPT, SALP, serum bilirubin and significantly decreased the levels of total proteins (Table 2). Formulations F1 and F2 significantly lowered paracetamol induced levels of SGOT, SGPT, and SALP also showed significant increase in total protein levels. However, the bilirubin levels (total and direct) were markedly reduced by F2 and standard but the reduction was found to be less significant with F1. Results of histopathological studies provided supportive evidence for biochemical analysis**.** Histology of liver sections of treated groups showed the hepatoprotective activity of the standard Liv52, F1 and F2 when compared to the normal and toxic control groups (Figure 6).

Table 1: Composition of formulations F1 and F2

Name of the plant	Parts used	F1 (gm/100ml)	F2 (gm/100ml)
Aloe barbadensis	Leaves	2	3
Azadirachta indica	Leaves	3	2
Cinnamomum	Bark	3	2
zeylanicum	Rhizome	2	3
Curcuma longa	Fruits	2	3
Emblica officinalis		12%	13%
% of formulation			

Table 2: Paracetamol induced liver toxicity in rats

Groups (n=5)	Dose	SGOT (IU/L)	SGPT (IU/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (gm%)	Alkaline phosphatase (U/L)
Control	----	56.3±19.9	52.9±5.7	0.7±0.2	0.2±0.1	6.0±0.1	490.5±11.7
Toxic Control (paracetamol)	2gm/kg	119±18.9	144.2±35.8	1.4±0.2	0.5±0.1	3.7±0.1	933.3±20.5
Standard drug Liv 52	1ml/kg	76.7±4.2**	75.7±6.1**	0.9±0.3*	0.2±0.1**	5.4±0.2**	592.5±15.8**
Formulation 1	500mg/kg	89.0±10.6**	85.5±10.3**	0.9±0.3^{ns}	0.3±0.1^{ns}	4.9±0.2**	602.8±18.6**
Formulation 2	500mg/kg	84.9±8.4**	78.2±7.8**	0.8±0.4*	0.3±0.1*	4.7±0.1**	606.7±19.5**

n=5, all values were expressed as mean ± SD, *P<0.05, **P<0.01 significant, ns=non significant, when compared with toxic control.

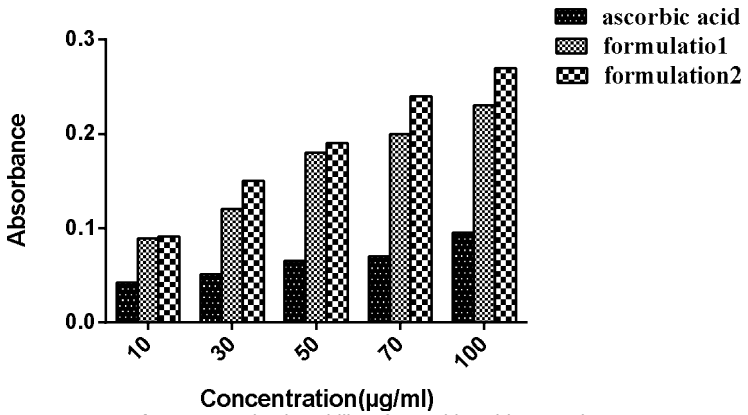

Figure 1: Reductive ability of ascorbic acid, F1 and F2

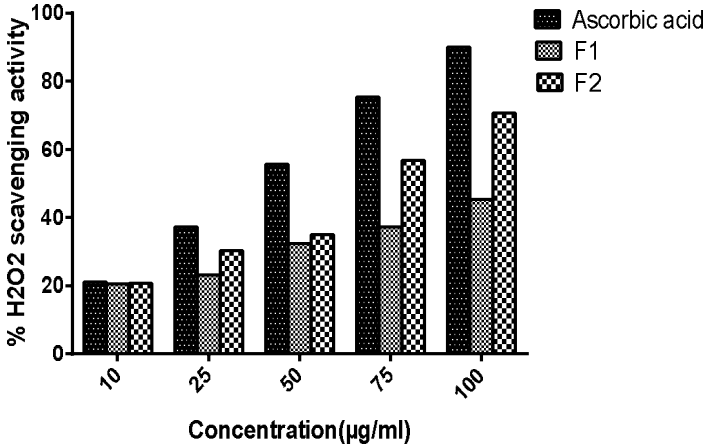

Figure 2: Percentage H_2O_2 inhibition of ascorbic acid, F1 and F2

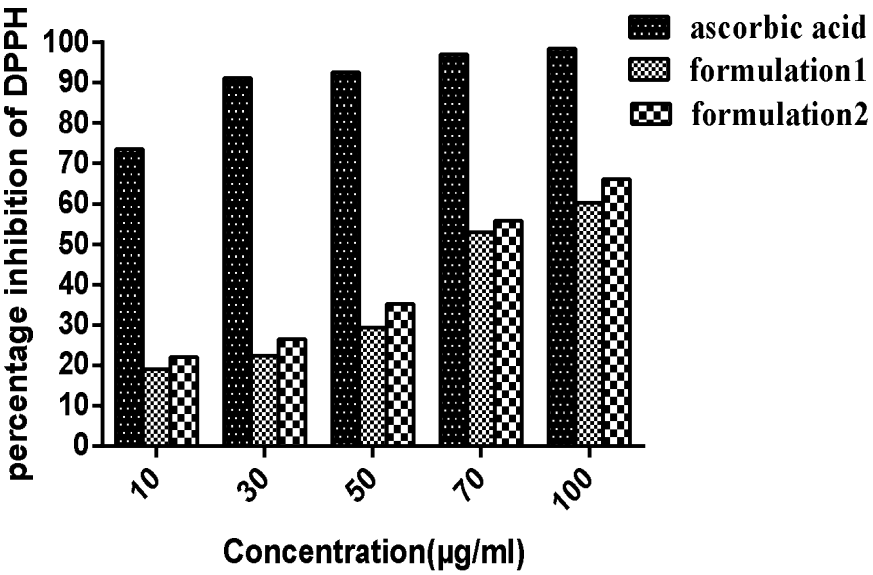

Figure 3: Percentage inhibition of DPPH radical by ascorbic acid, F1 and F2

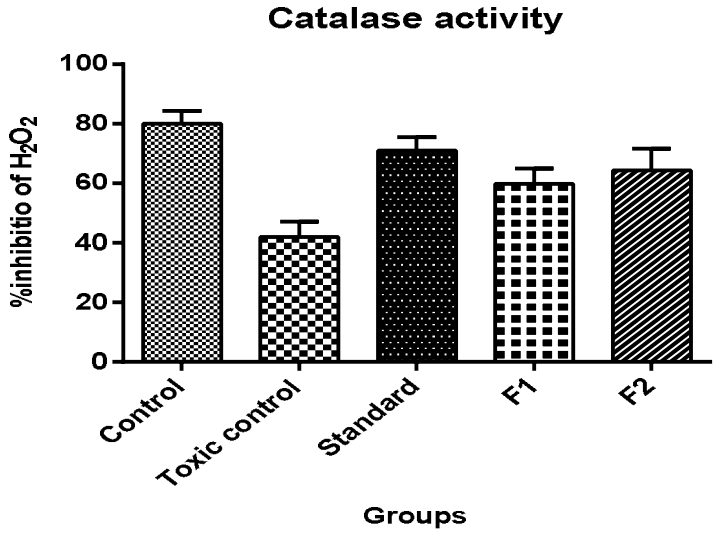

Figure 4: Effect of F1 and F2 on catalase activity in paracetamol induced hepatotoxic rats

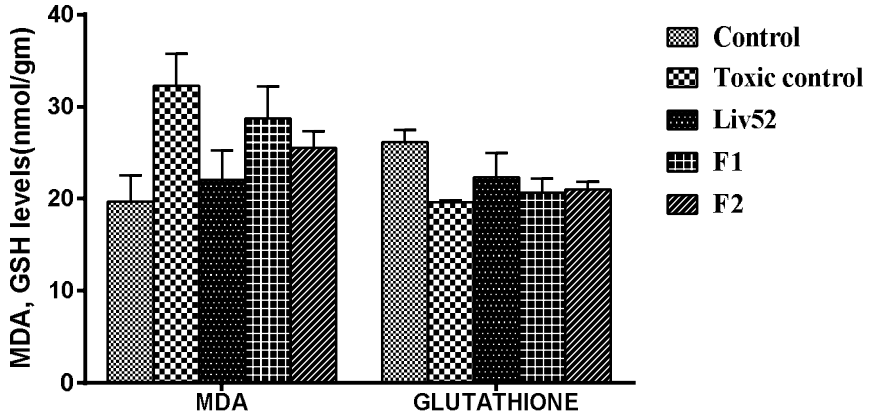

Figure 5: Effect of F1 and F2 on MDA, glutathione levels in paracetamol induced hepatotoxic rats

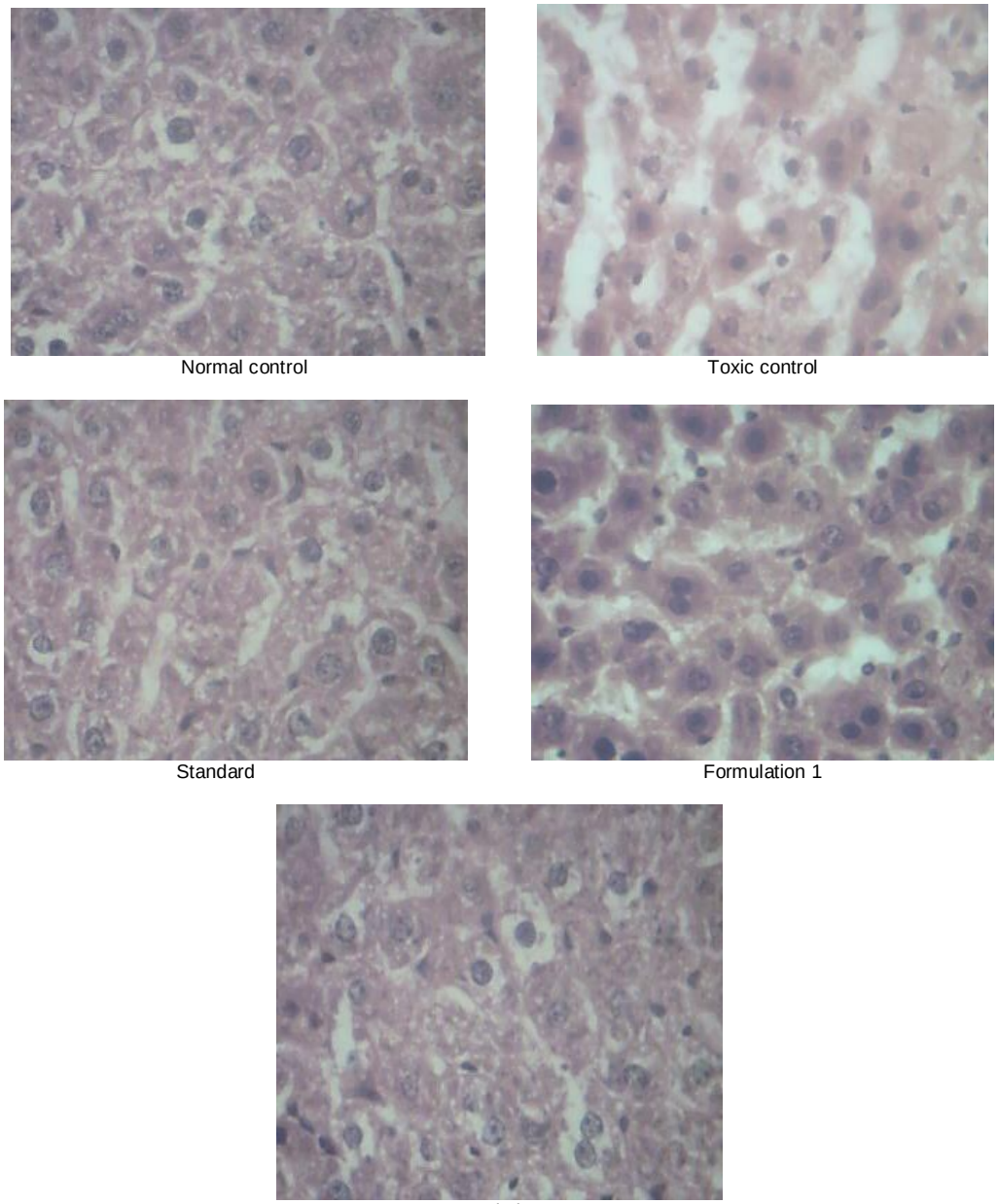

Figure 6: Histopathologsy of liver sections

3. DISCUSSION

Multi-component drug formulations F1 and F2 contain the extracts of five medicinal plants that contain specific therapeutically active principles and are traditionally used in liver disorders and screened for various hepatoprotective mechanisms. Combined action of all the ingredients helps to normalize the liver function and thus cure complex liver disorders. In the present study an attempt has been made to evaluate the hepatoprotective and antioxidant activities (*in vitro* and *in vivo*) of polyherbal formulations F1 and F2 against paracetamol induced hepatotoxicity in albino rats in comparison with LIV52 as standard. *In vitro* free radical scavenging activity of the polyherbal formulations were evaluated by reducing power method, H_2O_2 scavenging activity and DPPH assay. Reducing power of formulations was found to be more than the reducing power of standard ascorbic acid (0.094) at a concentration of 100µg/ml. Further F2 (0.27) was found to be more potent than F1 (0.23). For H_2O_2 scavenging activity IC_{50} values were found to be 44.28µg/ml (ascorbic acid), 84.54µg/ml (F1) and 65.31µg/ml (F2). Potency was in the order of ascorbic acid > F2 > F1. Ascorbic acid was found to be highly potent, having IC_{50} value 7.84µg/ml whereas IC_{50} values were found to be 77.18 µg/ml (F1), 68.84 µg/ml (F2) for DPPH radical scavenging activity.

In the present study, rats were treated with paracetamol to develop a significant hepatic damage and oxidative stress, which was observed from a substantial increase in the activities of SGOT, SGPT, SALP, total and direct bilirubin. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Administration of polyherbal formulations F1 and F2 has decreased the elevated levels of these biochemical markers.

The levels of SGOT, SGPT, SALP, total and direct bilirubin has been elevated to 119±18.99IU/l, 144.27±35.84IU/l, 933.32±20.55U/l, 1.49±0.29mg/dl and 0.57±.10mg/dl in paracetamol treated group from the normal levels 56.32±19.93IU/l, 52.94±5.76IU/l, 490.54±11.73U/l, 0.78±0.28mg/dl and 0.26±0.10mg/dl in normal control group respectively. Pre-treatment with Liv52, F1 and F2 has decreased the elevated levels of biochemical markers SGOT (76.77±4.27IU/l, 89.04±10.60IU/l and 84.95±8.40IU/l), SGPT (75.77±6.18IU/l, 85.56±10.39IU/l and 78.22±7.84IU/l), SALP (592.52±15.82U/l, 602.85±18.65U/l and 606.72±19.54U/l) respectively when compared to toxic control

group. Potency order of Liv52, F1 and F2 were found to be Liv52>F2>F1 in reducing the elevated levels of SGOT, SGPT and SALP. F2 has significantly reduced the levels of direct (0.32±0.15mg/dl) and total (0.86±0.40mg/dl) bilirubin respectively, whereas the reduction was not significant with F1 (0.37±0.17mg/dl and 0.93±0.36mg/dl). Further F2 was found to be more potent than standard Liv52 (0.91±0.33mg/dl) in reducing the total bilirubin levels. These observations suggest that the polyherbal formulations F1 and F2 possessed significant hepatoprotective activity against paracetamol induced hepatotoxicity.

A major defence mechanism involves the antioxidant enzymes including CAT, GSH which convert active oxygen molecules into nontoxic compounds. *In- vivo* antioxidant activity was assayed by estimation of CAT, MDA and GSH levels. Catalase activity (% inhibition of H_2O_2) of F2 (64.28±4.29) was found to be greater than F1 (59.82±5.15) and it was comparable with that of Liv52 (70.98±4.46). The reduction of MDA levels was more significant with F2 (25.51±1.80nmol/gm) than F1 (28.77±3.42nmol/gm). Further, F2 was found to elevate the depleted hepatic GSH levels (21.03±0.82nmol/gm) to a greater extent than F1 (20.67±1.52nmol/gm). These results indicate that F2 is more potent antioxidant *in vivo* than F1. However, its antioxidant potency was less than standard Liv52.

Histopathology of liver sections showed that the normal control group shows normal hepatic cells, where as administration of paracetamol in toxic control caused gross necrosis, periportal infiltration and the architecture was partly distorted. Treatment with Liv52 and formulations showed regenerative changes. F1 (500mg/kg) treated group showed that architecture was partly restored. Whereas Liv52 (1ml/kg) and F2 (500mg/kg) treated groups showed regenerating hepatocytes and showed that the overall structure of rat liver was near to normal. These observations suggest that the Liv52 and polyherbal formulations possessed significant hepatoprotective activity against paracetamol induced hepatotoxicity, further F2 is exhibiting more hepatoprotective activity than F1.

4. CONCLUSION

On the basis of results, it can be concluded that polyherbal formulations F1 and F2 combinations of five herbal plants, exert significant hepatoprotective and antioxidant effects when compared to the standard formulation Liv52. Formulation F2 was found to be more potent hepatoprotective and antioxidant than F1.These could be due to different types of active principles, each with single or diverse range of biological activities, which shows synergistic hepatoprotective action. The present study shows that the polyherbal formulations not only possesses hepatoprotective properties but also reduces oxidative stress in paracetamol induced toxicity in rats. This study emphasized the need for the preparation of polyherbal formulations making use of standardized extracts and to carry out in-depth pharmacological evaluation of these formulations and ascertain their claims in the light of modern scientific understanding such that their potentials may be tapped for better use as alternate and safe herbal drugs.

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REFERENCES

1. Meyer SA, Kulkarni AP, Hepatotoxicity. *In*: Hodgson E, Smart RC, editors. Introduction to biochemical toxicology. 3rd edn. New York, John Wiley & Sons. 2001,487-490.
2. Mohan H. The liver, blilary tract and exocrine pancreas. Text book of pathology, 4th edn. New delhi: Jaypee Publishers: 2002, 569-630.
3. Soylu AR, Altaner S, *et al.* Effects of vitamin E and C supplementation on hepatic glutathione peroxidase activity and tissue injury associated with ethanol ingestion in malnourished rats. *Current Therapeutic Research*, 2006, 67:118-137.
4. Aniya Y, Ohtani II, *et al.* Dimerumic acid as an antioxidant of the mold, *Monascus anka*. *Free Radical Biology and Medicine*, 2000, 28:999-1004.
5. Thabrew MI, Joice PDTM, Rajatissa WA. Comparative study of efficacy of *Paetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction. *Planta Medica*, 1987, 53:239-241.
6. Bhagwat KM, Rahul DK, Rangari VD. Hepatoprotective activity studies of herbal formulations. *International Journal of Green pharmacy*, 2008, 2:147-151.
7. Vekiari SA, Oreopoulou V, Tzia C, Thomopoulos CD. Oregano flavonoids as lipid antioxidants. *Journal of the American Oil Chemists Society* 1993, 70:483–487.
8. OECD/OCED. OECD guidelines for testing of chemicals. Acute oral toxicity-fixed dose procedure. 420, modified, Adopted 23rd Mar. 2006.
9. Oyaizu M. Antioxidative activities of browning products of glucosamine fractionated by organic solvent and thin layer chromatography. *Journal of the Japanese Society for Food Science and Technology* 1988, 35:771-775.
10. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea. *Carcinogenesis*, 1989, 10:1003-1008.
11. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*, 1958, 181:1199-1200.
12. Chattopadhyay R, Sarkar SK, *et al*. Hepatoprotective activity of *Azadirachta indica* leaves on paracetamol induced hepatic damage in rats. *Indian Journal of Experimental Biology*, 1992, 30:738-740.
13. Sinha AK. Colorimetric assay of catalase. *Analytical Biochemistry*, 1972, 47:389-394.
14. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 1979, 95:351-358.
15. Ellman GL. Tissue sulfhydryl groups, *Archives of Biochemistry and Biophysics*, 1959, 82:70-77.