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Hepatoprotective activity of a polyherbal mixture in ferrous sulphate and ethanol induced hepatotoxicity experimental animals

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ABSTRACT

Protective effect of a polyherbal mixture was evaluated for their hepatoprotective activity against ferrous sulphate and ethanol intoxication. Biochemical estimations for AST, ALT, ALP, Total bilirubin and Total protein were carried out at the end of treatment schedule for respective models. The mixture of equivalent amounts the extracts of Picrorhiza kurrao, Embilica officinalis, Andrographis paniculata, Eclipta alba to constitute total doses T50 mg was prepared. The hepatoprotective effects polyherbal mixture were estimated by liver function test and serum profile. The results revealed that polyherbal mixture produced significant hepatoprotective effect by decreasing serum transaminase like AST, ALT, ALP (alkaline phosphatase) and total bilirubin, but also significantly increased the levels of total protein. Morphological parameter like liver weight was restored by the polyherbal mixture. The effects of polyherbal mixture were comparable with standard drug silymarin.

Keywords: Hepatoprotective, polyherbal mixture, ferrous sulpahte, ethanol.

INTRODUCTION

Liver diseases remain one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders in Ethan medical practices as well as in traditional systems of medicine in India. Liver disease is worldwide problem. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Hence there is worldwide trend to go back to traditional medicinal plants. Many natural products of natural origin are used for treatment of liver ailments [1]. The Liver is very important organ of our human system having various multifunctional activities like metabolism and excretion. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [2].Often the efficacy of crude herbal preparation is found to be better than individual chemical constituents, possibly owing to the synergistic effect of the various components present. Mono and polyherbal preparations with potent antihepatotoxic activity in various liver disorders, made from traditionally used herbs with proven efficacy, have been described. More than 700 mono and polyherbal preparations in the form of decoction, tincture, tablets, and capsules from than 100 plants are in clinical use. Herbal drugs like *Eclipta alba, Emblica officinalis, Picrorrhiza Kurroa, Andrographis paniculata* have been proved to be potent hepatoprotective, each having different mode of action. But they have been never reported in combination. Our previous work using the same polyherbal mixture had demonstrated a significant hepatoprotective activity against CCL_4 and D-galactosamine induced hepatotoxicity [3].The present study was aimed to evaluate the hepatoprotective activity of the same herbal mixture in ferrous sulphate and ethanol induced hepatotoxicity experimental animals.

MATERIALS AND METHODS

Procurement of Herbal Extracts:

Picrorhiza kurrao	Methanolic extract
Embilica officinalis	Water extract
Andrographis paniculata	Water extract
Eclipta alba	Water extract

The above extracts were supplied as gift sample by Natural Remedies Pvt. Ltd. Bangalore.

Drugs and chemicals:

Silymarin	SILYBON, Micro Labs Ltd.
Ferrous sulphate	Sankalp Healthcare and Allied Products Pvt. Ltd.
Ethanol (40% v/v)	

Animals:

Rats:Albino rats of Wistar strain weighing 100-200 g were obtained from National Toxicology Center (NTC), Pune. Animals of either sex were housed in groups of four under standard laboratory conditions with free access to standard pellet diet and water *ad libitum.*

Preparation of Herbal Mixture:

1) Herbal mixture T-50: Dose (50mg/kg)

It contained 12.5 mg of all the four extracts to give 50mg of herbal mixture.

Dose selection of the herbal mixture:

With reference to our previous work the dose of 50mg/kg body of herbal mixture which showed significant hepatoprotective against carbon tetrachloride induced hepatotoxicity was used as the test dose for the present study [3].

Statistical Analysis:

All observations were presented as Mean ± SEM. The data was analyzed by one-way ANOVA followed by Dunnett's test for in-vivo study. $p < 0.05$ was considered as significant and $p < 0.001$ as highly significant.

Screening models:

Table No 1: Ferrous sulphate induced hepatotoxicity in rats [4]
Experimental Protocol (Grouping, Treatment and Observations)

Group (N = 6)	Treatment and Dose/ Day.	Observations
I	Control (Distilled Water p.o.)	1) Biochemical Parameters on 10^{th} day.
II	Control (Distilled Water p.o.) for 9 days)+Ferrous sulphate (30 mg/kg) i.p. on day 10	
III	Ferrous sulphate (30 mg/kg) i.p. on day 10) +Silymarin (100 mg/kg) p.o. from day 1 to day 9 o.d.	
IV	Ferrous sulphate (30 mg/kg) i.p. on day 10 +Herbal mixture (50mg/kg) p.o. from day 1 to day 9 o.d.	

Group I: Control group.
Group II: Negative control group.
Group III: Silymarin treated group.
Group IV: T-50 – Herbal Mixture (50 mg/kg) treated group.

Table No. 2: Ethanol induced hepatotoxicity in rats [5]
Experimental Protocol (Grouping, Treatment and Observations)

Group (N = 6)	Treatment and Dose/ Day.	Observations
I	1% Gum acacia in water (vehicle).	1) Biochemical Parameters on day 22. 2) Morphological Parameters on day 22.
II	Ethanol (40%V/V) 3ml/100gm bd.wt/day in two divided doses for 21 days.	
III	Ethanol (40%V/V) 3ml/100gm bd.wt/day in two divided doses for 21 days+ Silymarin (100 mg/kg) p.o. for 21 days o.d.	
IV	Ethanol (40%V/V) 3ml/100gm bd.wt/day in two divided doses for 21 days+ Herbal mixture (50mg/kg) p.o. for 21 days o.d.	

Group I: Control group.
Group II: Negative control group.
Group III: Silymarin treated group.
Group IV: T-50 – Herbal Mixture (50 mg/kg) treated group.

Biochemical and Morphological estimations:

At the end of the treatment the serum was isolated and subjected for estimation of AST, ALT, Alkaline phosphatase (ALP), Total protein (TP) and Total Bilirubin (TB). The liver was isolated and the weight of the liver was measured.

RESULTS

Table No. 3: Effect of herbal mixture treatment on different Biochemical parameters in $FeSO_4$ induced hepatotoxicity.

Sr. No.	Serum Biochemical parameters	Groups			
		Control	Negative control	Silymarin	T-50
1	AST (U/ml)	42.8 ±1.777	120## ±1.667	67.44** ±2.351	58** ±1.52
2	ALT (U/ml)	36.5 ±2.02	88.6## ±1.562	54.8** ±1.98	50.8** ±1.035
3	ALP (KA units/dl)	7.2 ±0.175	20.5## ±1.23	10.4** ±1.02	8.6** ±0.4
4	TP (gm/dl)	14 ±0.807	8.4## ±0.523	11** ±0.347	12.3** ±0.346
5	TB (mg/dl)	1.02 ±0.09	11## ±0.458	5.6** ±0.678	5.02** ±0.7

n=6. Values are expressed as Mean ±S.E.M.
** = p < 0.05, **=p < 0.01 when compared with Negative control*
= p < 0.01, when compared with control
Statistically analyzed by One Way ANOVA followed by Dunnett test.

Table No. 4: Effect of herbal mixture treatment on different Biochemical parameters in ethanol induced hepatotoxicity.

Sr. No.	Serum Biochemical parameters	Groups			
		Control	Negative control	Silymarin	T-50
1	AST (U/ml)	45.8 ±0.984	140## ±1.985	60.25** ±1.325	55.36** ±2.547
2	ALT (U/ml)	36.52 ±2.232	102.3## ±2.568	58.5** ±2.65	48..65** ±2.035
3	ALP (KA units/dl)	8.52 ±1.174	24.3## ±2.25	12.56** ±0.986	10.25** ±0.865
4	TP (gm/dl)	15.9 ±0.807	9.85## ±0.523	12.3** ±0.347	11.95** ±0.346
5	TB (mg/dl)	2.14 ±0.986	15## ±1.023	5.99** ±0.865	5.09** ±0.985
6	Liver weight (gm)	3.95 ±0.23	5.26## ±0.125	4.106** ±0.142	4.025** ±0.178

n=6. Values are expressed as Mean ±S.E.M.
** = p < 0.05, **=p < 0.01 when compared with Negative control*
= p < 0.01, when compared with control
Statistically analyzed by One Way ANOVA followed by Dunnett test.

DISCUSSION

Iron overload is associated with liver damage, characterized by massive iron deposition in hepatic parenchymal cells, leading fibrosis and eventually, to hepatic necrosis [6]. A ferrous salt reacts with hydrogen peroxide derived by the action of the superoxide anion radical, to form the highly reactive radical hydroxyl (Fenton reaction). Hydroxyl ion attacks all biological molecules, including cell membrane lipids, to initiate LPO. The highly toxic peroxidative metabolite induces widespread cellular injury. Hepatic injury results in the leakage of cellular enzymes into the bloodstream, resulting in the augmented levels of serum enzymes .Serum levels of these enzymes are excellent indicator of hepatic parenchymal damage and dysfunction [4]. The AST, ALT, ALP, TB and TP level was brought to the normal level which was altered due to the $FeSO_4$ significantly ($p<0.01$) by herbal mixture pretreatment (T-50) on 10^{th} day. Silymarin pretreatment significantly restored the levels of AST, ALT, ALP, TB and TP to the normal level on 10^{th} day. Whereas when the serum levels of Silymarin treated group and T-50 herbal

mixture treated group was considered, the levels of biomarkers in T-50 treated group was restored to normal level than Silymarin group. From the current study in this model it can be said that herbal mixture dose (50mg/kg) showed the greater protective effect to that of Silymarin dose (100mg/kg) (Table No.3).

Chronic ethanol intake is known to produce hepatocellular damage. The alcoholic liver injury appears by the effects of ethanol metabolism and the toxic effects of the immune response to alcohol or acetaldehyde altered proteins. Ethanol is primarily metabolized by alcohol dehydrogenase with the formation of acetaldehyde. Several other pathways are cytochrome p-450 dependent microsomal ethanol- oxidizing system, catalase and non-enzymatic ethanol oxidation and the involvement of free radical species. Ethanol induced hepatic hypoxia is also one of the main causes of hepatotoxicity [7]. Ethanol increases Triglycerides and cholesterol levels thus inducing imbalance in lipid metabolism in liver, heart and kidney [8]. Chronic ethanol ingestion produces fatty liver, hepatomegaly, alcoholic hepatitis, fibrosis and cirrhosis [9]. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membranes. In the present study, the transaminase level increased by chronic ethanol treatment was significantly ($p<0.01$) reduced by herbal mixture treatment (T-50) on day 22. Cholestasis may also contribute to the increased levels of AST and ALT. ALP activity is related to functioning of the hepatocyte cells. This ALP level was reduced by significantly herbal mixture treatment (T-50) ($p<0.01$) on day 22. Herbal mixture treatment (T-50) increased serum TP level indicating its hepatoprotective activity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and production of the liver cells [10]. The total bilirubin level was also restored to the normal level upon ethanol intoxication by the herbal mixture treatment ($p<0.01$). This indicates that abnormal uptake of bilirubin from liver cells restored normal to normal level. The increased weight of liver by ethanol was restored by herbal mixture (T-50) up to the normal significantly ($p<0.01$) on day 22. This revels that herbal mixture treatment prevented the fatty deposition in the liver cells.

Silymarin significantly restored the AST, ALT, ALP, TP and TB and TP on day 22. Weight of liver restored to normal significantly while reduced significantly by Silymarin on day 22. Whereas when the serum levels of Silymarin treated group and T-50 herbal mixture treated group was considered, the levels of biomarkers in T-50 treated group was restored to normal level than Silymarin group. From the current study in this model it can be said that herbal mixture dose (50mg/kg) showed the greater protective effect to that of Silymarin dose (100mg/kg) (Table No.4).

CONCLUSION

In models which cause hepatotoxicity by generation of free radicals like in case of the heavy metal toxicity like in iron overload which generates free radicals which causes the lipid peroxidation of the cell membrane, the herbal mixture showed better results than that of Silymarin. In chronic ethanol consumption the herbal mixture showed the similar effects to that of Silymarin. This shows that polyherbal mixture preserved the structural integrity of the hepatocelluar membrane and liver cell architecture and also prevented fatty deposition in the liver.

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REFERENCES

[1] Bafna A.R, Mishra S.H, *Ars Pharm*, **2004**, 45(4), 343-351.
[2] Anu W, Rajat M, Satish S, Vipin S, Sumeet G, *Der Pharmacia Sinica*, **2011**, 2 (5) , 288-299.
[3] Deshpande A.P, Nimbalkar V.V, Tarkase K.N, *Der Pharmacia Sinica*, **2012**, 3 (4), 437-442.
[4] Bhattacharya A, Ramanathan M, Ghosal S, Bhattacharya K, *Phytother. Res*, **2000**, 568-570.
[5] Gulati R.K, Agarwal S. Agrawal S.S, *Ind. J. Exp. Biol*, **1995**, *33***,** 261–268.
[6] Reddy A.C, Lokesh B.R, *Toxicology*, **1996**, 107 (1), 39-45.
[7] Devaki T, Shirashangari K.S, *J. of Natural Remedies*, **2004**, 4(2), 109-115.
[8] Mahendran P, Shyamala Devi C.S, *Indian J. of Pharmacol*, **2001**, 33, 87-91.
[9] Thurman R.G, Bradford B.U, *Frontiers in Bioscience*, **1999**, 4, 42-46.
[10] Nadana S, Srinivasagam R, Namasivayam N, *J.of. Pharm and Pharmac*, **2007**, 59 (3), 445–453.