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Hepatoprotective activity of a polyherbal mixture in carbon tetrachloride and D-galactosamine induced hepatotoxicity in experimental animals.

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ABSTRACT

Protective effect of a polyherbal mixture was evaluated for their hepatoprotective activity against carbon tetrachloride, D-galactosamine intoxication. Biochemical estimations for AST, ALT, ALP, Total bilirubin and Total protein were carried out at the end of treatment schedule for respective models. Three mixtures of equivalent amounts the extracts of Picrorhiza kurrao, Embilica officinalis, Andrographis paniculata, Eclipta alba to constitute total doses T25mg, T50 mg and T100 mg were prepared. Carbon tetrachloride induced hepatotoxicity was used as pilot model to determine effective dose. The pilot study showed significant ($p<0.01$) hepatoprotective activity at doses of T50 mg and T100 mg of mixture. Whereas T25 mg mixture dose was insignificant. The minimal effective dose of T50 mg of mixture was taken as the test dose for evaluation in D-galactosamine induced hepatotoxicity. In carbon tetrachloride induced hepatotoxicity, T50 mg and T100 mg mixture doses showed significant ($p<0.01$) restoration of SGOT, SGPT, ALP, Total bilirubin and Total protein levels at the end of treatment schedule. T50 mg mixture dose in D-galactosamine induced hepatotoxicity also showed significant ($p<0.01$) restoration of AST, ALT, ALP, Total bilirubin and Total protein levels at the end of treatment schedule. The above study suggest that T50 mg and T100 mg of mixture of extracts of above said plants have hepatoprotective against lipid peroxidation of hepatocytes against free radicals generated in carbon tetrachloride intoxication. In T-50 mg treated group the levels of ALT, ALP, TB were restored significantly ($p<0.01$) to the normal levels indicating the membrane stabilizing capacity of the herbal mixture in D-galactosamine induced hepatotoxicity.

Keywords: Hepatoprotective, polyherbal mixture, carbon tetrachloride, D-galactosamine

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INTRODUCTION

Traditionally trained practitioners of ayurvedic medicine recognize that balancing liver function is pivotal to ensuring overall health. In dealing with problems of the liver, the primary goal within the system of ayurveda is to enhance liver detoxification processes and help protect against further damage to the liver. Based on traditional use, herbs are selected and combined for their ability help promote "balance" within the body and to nourish the liver and related functions, including digestion and bile acid secretion [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. The present study was aimed to evaluate the hepatoprotective activity in combination as herbal mixture.

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.
D- Galactosamine	Hi-media
Carbon-tetrachloride	Qualigens Fine Chemicals, Mumbai.
Livfit®	Daubar Pharmaceutical Ltd.

Animals:

- Rats: Albino rats of Wistar strain weighing 100-200 g were obtained from National Toxicology Center (NTC), Pune.
- Mice: Swiss albino mice weighing 20-30 g were obtained from Serum India Pvt. Ltd. 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-25: Dose (25mg/kg)
It contains 6.25 mg of all the four extracts to give 25mg of herbal mixture.
2) Herbal mixture T-50: Dose (50mg/kg)
It contains 12.5 mg of all the four extracts to give 50mg of herbal mixture.
3) Herbal mixture T-100: Dose (100mg/kg)
It contains 25 mg of all the four extracts to give 100 mg of herbal mixture.

Dose selection of the herbal mixture:

Initial dose of the herbal mixture was randomly selected as 25mg/kg body weight, 50mg/kg body weight and 100mg/kg body weight. Carbon tetrachloride induced hepatotoxicity model was used as the model to determine the effective dose. From the above doses the dose that showed significant activity at minimal dose was taken as the test dose for evaluation of D-galactosamine induced hepatotoxicity.

Statistical Analysis:

All observations were presented as Mean ± SEM. The data was analyzed by student's t-test forin-vitro studies and one-way ANOVA followed by Dunnett's test for in-vivo study. $p < 0.05$ is considered as significant and $p < 0.001$ as highly significant.

Screening models:

Table No 1: Carbon tetrachloride induced hepatotoxicity in rats [3]: Experimental Protocol (Grouping, Treatment and Observations)

Group (N = 6)	Treatment and Dose/ Day.	Observations
I	Control (Distilled Water p.o.)	1) Biochemical Parameters on 8th day. 2) Histopathological examination on 8th day.
II	CCl_4 (0.7 ml/kg sc. alternate days)	
III	CCl_4 (0.7 ml/kg sc. alternate days) + Silymarin (100 mg/kg) p.o. from day 1to day 7 o.d.	
IV	CCl_4 (0.7 ml/kg sc. alternate days) + Herbal mixture dose (25 mg/kg) p.o. from day 1 to day 7 o.d.	
V	CCl_4 (0.7 ml/kg sc. alternate days) + Herbal mixture (50 mg/kg) p.o. from day 1 to day 7 o.d.	
VI	CCl_4 (0.7 ml/kg sc. alternate days) + Herbal mixture (100 mg/kg) p.o. from day 1 to day 7 o.d.	

Group I: Control group.
Group II: Negative control group.
Group III: Silymarin treated group.
Group IV: T-25 – Herbal Mixture (25 mg/kg) treated group.
Group V: T-50 – Herbal Mixture (50 mg/kg) treated group.
Group VI: T-100 – Herbal Mixture (100 mg/kg) treated group.

Table No. 2: D-galactosamine induced hepatotoxicity in mice [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 2nd and 9thday. 2) Histopathological examination on 9th day.
II	D-galactosamine (800mg/kg) i.p. on day 1 o.d.+ Distilled Water p.o. form day 2 to day 8.	
III	D-galactosamine (800mg/kg) i.p. on day 1 o.d. + Livfit(50mg/kg) p.o. form day 2 to day 8 o.d.	
IV	D-galactosamine (800mg/kg) i.p. on day 1 o.d.+ Herbal mixture (50mg/kg) p.o. form day 2 to day 8 o.d.	

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

RESULTS

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

Sr. No.	Serum Biochemical parameters	Groups					
		Control	Negative control	Silymarin	T-25	T-50	T-100
1	AST (U/ml)	**52.8 ±1.877**	**100.1## ±2.667**	**67.44** ±1.752**	**92.4 ±2.52**	**62.8** ±1.241**	**59.8** ±1.8**
2	ALT (U/ml)	**40.8 ±1.02**	**79.6## ±1.327**	**44.8** ±1.35**	**74.8 ±2.059**	**42.8** ±1.35**	**42** ±1.54**
3	ALP (KA units/dl)	**5.6 ±0.275**	**17.2## ±1.02**	**10.4** ±0.748**	**16.6 ±0.4**	**10** ±0.316**	**9.4** ±0.509**
4	TP (gm/dl)	**12 ±0.707**	**8.4## ±0.509**	**11** ±0.447**	**8.2 ±0.374**	**11.3** ±0.3**	**11.4** ±0.6**
5	TB (mg/dl)	**0.8 ±0.07**	**10## ±0.707**	**6.6** ±0.678**	**9 ±0.7**	**6.6** ±0.4**	**6.8** ±0.38**

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
Stastically analyzed by One Way ANOVA followed by Dunnett test.

Table No. 4 Effect of herbal mixture treatment on different Biochemical parameters in D- Galactosamine induced hepatotoxicity on day 2.

Sr. No.	Serum Biochemical parameters	Groups			
		Control	Negative control	Silymarin	T-50
1	ALT (U/ml)	**39.2 ±1.02**	**146.8$^{\#\#}$ ±2.871**	**149.6$^{\#\#}$ ±3.86**	**143.2$^{\#\#}$ ±3.611**
2	ALP (KA units/dl)	**7.5 ±0.3536**	**16$^{\#\#}$ ±0.2915**	**15.94$^{\#\#}$ ±0.3187**	**15.52$^{\#\#}$ ±0.1281**
3	TB (mg/dl)	**0.408 ±0.02**	**2.67$^{\#\#}$ ±0.096**	**2.554$^{\#\#}$ ±0.0102**	**2.476$^{\#\#}$ ±0.053.7**

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
Stastically analyzed by One Way ANOVA followed by Dunnett test.

Table No. 5: Effect of herbal mixture treatment on different Biochemical parameters in D- Galactosamine induced hepatotoxicity on day 9.

Sr. No.	Serum Biochemical parameters	Groups			
		Control	Negative control	Silymarin	T-50
1	ALT (U/ml)	**42 ±2.966**	**132$^{\#\#}$ ±2.55**	**61** ±1.99**	**78** ±3.038**
2	ALP (KA units/dl)	**6.06 ±0.010**	**14.3$^{\#\#}$ ±0.2**	**8.98** ±1.266**	**10.96** ±0.1166**
3	TB (mg/dl)	**0.378±0.012**	**2.38$^{\#\#}$ ±0.0141**	**0.606** ±0.052**	**1.522** ±0.08**

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
Stastically analyzed by One Way ANOVA followed by Dunnett test.

DISCUSSION

Various xenobiotics are known to cause hepatotoxicity one among them is carbon tetrachloride (CCl4). Steroids, vaccines and antiviral drugs have been used as therapies for liver pathologies; have potential adverse side effects, especially if administered chronically or sub-chronically. In the absence of reliable liver protective drugs in allopathic medicinal practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices as well as in traditional systems of medicine in India. CCl_4 is widely used experimentally as hepatotoxin which is biotransformed by the cytochrome p-450 system to produce the trichloromethyl free radical which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation disturbs calcium haemostasis and finally results in death of cell. Lipid peroxidation is a complex and natural deleterious process. The significant increase observed in levels of lipid peroxides in liver of CCL_4 intoxicated shows free radical induced liver damage [5,6]. In the present study there was significant increase in the serum activities of AST and ALT enzymes in the CCl_4 intoxicated group. These transaminases (AST and ALT) are well known diagnostic indicators of liver disease. Cholestasis may also contribute to increased levels of AST and ALT. Herbal mixtures T-50 and T-100 significantly ($p<0.01$) reduced the elevated levels on 8th day. Alkaline phosphatase (ALP) activity is related to the functioning of the hepatic cells. Increase in the serum level of ALP is due to its increased synthesis in presence of increasing biliary pressure [7]. Herbal mixtures T-50 and T-100 significantly reduced the levels of ALP on 8th ($P<0.01$). In the damaged liver the bilirubin metabolism was disturbed due to increased formation of bilirubin, abnormal uptake of bilirubin in the liver cells, defective conjugation and failure of normal amounts of bile to reach the duodenum i.e. development of condition called cholestasis. It is well established that CCl4 administration causes a significant decrease in serum total protein and albumin levels [8].The Total Bilirubin (TB) level was significantly ($p<0.01$) reduced on 8th day by herbal mixtures T-50 and T-100. The free radical generation causes the decrease in protein synthesis, so the Total protein level decrease by CCl_4 which significantly ($p<0.01$) restored up to the normal by herbal mixtures on 8th day. Silymarin significantly restored AST, ALT, ALP, TB and TP up to the normal level on 8th day ($p<0.01$). The T-25 herbal mixture treated group did not decrease the level of AST, ALT, ALP, TP and TB upon treatment. This shows that (25mg/kg) dose was not good enough to reverse or restore the Carbon tetrachloride induced hepatotoxicity. When the levels of AST, ALT, ALP, TB and TP of T-50 and T-100 herbal mixture treated groups was compared,

the levels of the enzymes was restored to normal level to similar extent. So the T-50 dose was selected as test dose for other models as it showed the significant response at minimal dose. When the serum levels of Silymarin treated group and T-50 herbal mixture treated group was considered, the levels of biomarkers in both the groups were restored to a similar level. From the current study in this model it can be said that herbal mixture dose (50mg/kg) showed the similar effects to that of Silymarin dose (100mg/kg) (Table No.3). The Ca++ homeostasis perturbation, inhibition of oxidative of NADPH and FADH substrate at the dehydrogenase co enzyme level [9] are also considered to be responsible for pathogenesis of GaIN induced hepatitis. The metabolites of β-D-galactosamine (GaIN), uridiophosphogalactosamine may deplete several uracil nucleotides such as UDP-galactose, UDP-glucose and UTP, causing reduction of mRNA and glycoprotein synthesis (i.e. reduction of ATP and glycogen synthesis), which leads to cellular membranes alteration [10]. Nevertheless there is increasing evidence that GaIN causes production of free hydroxyl radical leading to lipid peroxidation. Also the levels of SOD, CAT, GPx are also concomitantly reduced [11]. Ultimately the cellular damage and the inflammation caused by GaIN are similar to the histopathological features of viral hepatitis in humans. This phenomenon may lead to cellular damage and cellular inflammation resulting in histological and biochemical picture closely resembling viral hepatitis. The Ca^{++} homeostasis perturbation, inhibition of oxidative of NADPH and FADH substrate at the dehydrogenase co enzyme level are also considered to be responsible for pathogenesis of GaIN induced hepatitis. LPS is co administered with GaIN by certain investigators[12]. These leads to the release of TNF α from the macrophages and Kuffer cells. This cytokinin has been firmly implicated as an important causative mediator in the pathogenesis of alcoholic liver diseases and hepatitis. Nevertheless TNF α too has positive role as it is responsible for the normal proliferation of the hepatocytes, but in pathological condition it acts as apoptic agent [13]. On the initial 1 day treatment with D-galactosamine in all treated group, there was acute increase the serum levels of the ALT, ALP, TB due to the depletion of the of several uracil nucleotides which leads to cellular membranes alteration (Table No.4). Upon further treatment till day 8 with the herbal mixture (50mg/kg) i.e. T-50 the levels of ALT, ALP, TB were restored significantly ($p<0.01$) to the normal levels indicating the membrane stabilizing and regerative capacity of the herbal mixture. The Livfit (50mg/kg) also reversed the altered levels of the ALT, ALP, TB significantly ($p<0.01$) with the similar treatment pattern till day 8. When the serum levels of Livfit treated group and T-50 herbal mixture treated group was considered, the levels of biomarkers in both the groups were restored to a similar level. From the current study in this model it can be said that herbal mixture dose (50mg/kg) showed the similar effects to that of Livfit dose (100mg/kg) (Table No.5).

CONCLUSION

In models which cause hepatotoxicity by generation of free radicals like in case of carbon tetrachloride toxicity which causes the loss of cell membrane integrity or in case of the heavy metal toxicity like 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 viral hepatitis resembling model i.e. in case of D-galactosamine induced hepatotoxicity which cause the reduction in the ATP and glycogen synthesis which in turn cause the loss of structural integrity if cell membrane, the herbal mixture showed the similar effects to that of Livfit.

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