Protective effect of ethanolic extract of polyherbal formulation on carbon tetrachloride induced liver injury

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Abstract: *Protective effect of ethanolic extract of polyherbal formulation (PHF) of three medicinal plants was studied on carbon tetrachloride induced liver damage in rats. Treatment with 250mg / kg b.w. of ethanolic extract of PHF protected rats against carbon tetrachloride liver injury by significantly lowering 5'NT, GGT, GDH and SDH and bilirubin levels compared to control group of rats. Normalizing the effect of these parameters indicates strong hepatoprotective property of the PHF extract.*
Keywords: Hepatoprotective, carbon tetrachloride, polyherbal formulation.

INTRODUCTION

Liver disease leads to serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a major role in the management of various liver disorders (Sethuraman *et al.,* 2003). The experimental intoxication induced by carbon tetrachloride (CCl_4) is widely used for modeling liver injury in rats. Hepatotoxicity is connected with severe impairment of cell protection mechanisms. The location of liver injury is defined mainly by the biotransformation of CCl_4, which is cytochrome P450 dependent. Free radicals initiate the process of lipid peroxidation, which is generally caused by inhibition of enzyme activity. It is now generally accepted that the hepatotoxicity of CCl4 is the result of reductive dehalogenation, which is catalyzed by P450, and which form the highly reactive trichloromethyl free radical. This then readily interacts peroxy radical. Both trichloromethyl and its peroxy radicals are capable of binding to proteins and lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating lipid peroxidation and liver damage and by doing so plays a significant role in pathogenesis of disease (Sampathkumar *et al.,* 2005).

Plant derived natural products such as flavonoids, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including hepatoprotective activity. There has been growing interest in the analysis of certain flavonoids, triterpenoids, and steroids stimulated by intense research in to their benefits to human health. One of their main properties in this regard is their antioxidant activity (Defeudis *et al.,* 2003). This research is carried out to evaluate the hepatoprotective activity of ethanolic extract of polyherbal formulation against CCl_4-induced liver damage in rats.

MATERIALS AND METHODS

Collection of the plant materials

The plant materials used for the polyherbal formulation (PHF) preparation were *Asteracantha longifolia* Nees., *Cyprus rotundus* Linn. and *Bryophyllum pinnatum* Kurz. The plants were collected from Vallayar, Rajapalayam and Salem district of Tamilnadu, India respectively. They were identified and authenticated by Taxonomist Dr. V. Balasubramanian of Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. Voucher specimens were deposited at herbarium collection of the department of Botany.

Extraction and preparation of polyherbal formulation

The plant parts were washed, shade dried and powdered. In order to prepare the polyherbal formulation, about 25g (50%) of *Asteracantha longifolia*, 15g (30%) of *Cyperus rotundus* and 10g (20%) of *Bryophyllum pinnatum* plant powders were soaked overnight in 150ml of 95% ethanol. This suspension was filtered and the residue was resuspended in an equal volume of 95% ethanol for 48 hrs. and filtered again. The two filtrates were pooled and the solvent were evaporated in a rotary evaporator. This extract was dissolved in one liter of distilled water and this was administered orally to the rats at the rate of 1.0ml/day. The percentage composition of the plant parts used for the ethanolic extract of polyherbal formulation preparation is shown in table 1.

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Table 1. Composition of plant parts used for the preparation of polyherbal formulation (PHF)

S. No	Name of the Plant	Plant part used	Composition (%)
1	*Asteracantha longifolia* Nees.	Whole plant	50%
2	*Cyperus rotundus* Linn.	Bulbs	30%
3	*Bryophyllum pinnatum* Kurz.	Leaves	20%

Selection of animals

Healthy adult male wistar albino rats weighing about 150 to 200 g were procured from animal breeding centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The animals were housed in spacious cages. The animals were maintained for 12 hrs. in light and dark cycle at 28°C ± 2° C in a well ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India and provided with water *ad libitum*. All animal experiments were performed according to the ethical guidelines suggested by the institutional animal ethics committee (IAEC).

Experimental induction of hepatotoxicity

Hepatic damage was induced in experimental rats by intraperitoneal admistration of carbon tetrachloride at dose of 1.0ml per kg body weight in 1:1 volume/ volume of liquid paraffin, which served as a vehicle.

Experimental design of animals

The rats were divided into five groups of six animals each as given in table 2.

Table 2. Experimental design

Group	Experimental design
I	**Control rats**- received normal pelleted diet
II	**Toxic rats**- carbon tetrachloride was given (1.0ml/ kg b. w.) as single dose with 1:1 volume/ volume of liquid paraffin by intraperitoneal administration
III	**Standard drug treated rats** – Toxic rats were given Silymarin (25mg / kg / b. w.) by oral administration for 30 days at the rate of 1.0 ml / rat / day
IV	**Polyherbal formulation (PHF) treated rats**–Toxic rats were given (250 mg / kg b. w.) by oral administration for 30 days at a rate of 1.0 ml / rat / day
V.	**Protective group** - normal rats received polyherbal formulation extract (250 mg / kg body wt) by oral administration for 30 days at the rate of 1.0 ml / rat / day.

Collection of serum sample

After the experimental regimen the animals were sacrificed by cervical decapitation under mild chloroform anesthesia. Blood was collected and centrifuged for 10min. at 2500rpm. Serum was collected and then diluted in the ratio of 1:10 with saline. Aliquot of the diluted serum was used for the estimation of serum constituents and serum enzyme activities.

Collection of liver samples

Liver was removed immediately and washed with ice cold saline. 10% tissue homogenate was prepared by 0.1M tris HCl homogenizing buffer at pH 7.5. The homogenate was used for the assay of various biochemical parameters.

Chemicals

All the chemicals used in the present study were of analytical reagent grade.

Estimation of biochemical parameters

The serum and liver tissue homogenate was used to assayed the marker enzymes and serum constituents like 5'NT, GGT, GDH, SDH and bilirubin according to the method Capmbel (1962), Persign and Vanderslik (1976), Sadasivam and Manickam (2005), Slater and Bonner(1952), and Malloy and Evekyn, (1988) respectively.

Statistical analysis

The values were represented as the mean of six values ± S.D. The results were statistically analyzed using the statistical package (MINITAB, version 14). One way analysis of variance was employed for comparison among the six groups followed by Fisher's test. Statistical significance was set at $p<0.05$ (Danial, 2006).

RESULTS

The effect of ethanolic extract of polyherbal formulation on 5'nuleotidase (5'NT) and gamma glutamyl transferase (GGT) in serum and liver of control and experimental rats are shown in figure 1, 2 and table 3. From the figures and table it was evident that these enzymes were significantly increased ($p<0.05$) in serum and liver of CCl_4 induced hepatic damaged rats. After the treatment with polyherbal formulation, the values showed near normal range in group IV rats in serum and liver. The standard drug silymarin treated group (group III) also showed the normal activities. The group V rats, which were treated with polyherbal formulation alone, showed protective effect without any side effect.

Fig.1. Effect of PHF on 5'NT in serum of control and experimental rats

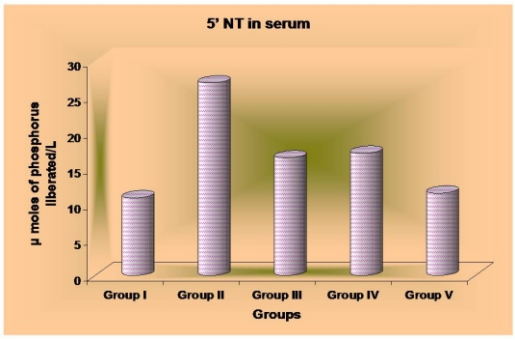


Fig.2. Effect of PHF on GGT in serum of control and experimental rats

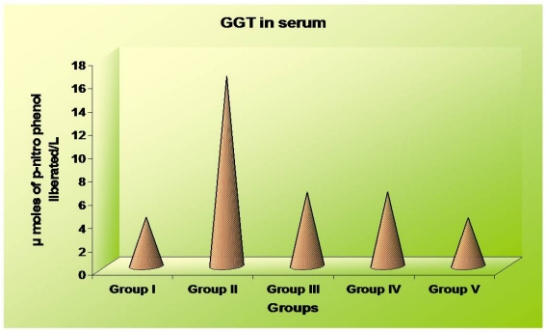


Table 3. Effect of PHF on LDH, 5'NT and GGT in liver of control and experimental in rats.

Groups	5'NT*	GGT¥
Group I	1.76±0.08	11.18±0.52
Group II	3.33±0.20a*	18.31±0.81a*
Group III	2.05±0.06b*	13.07±0.45b*
Group IV	2.10±0.14c*,c^{ns}	13.19±0.45c*,c^{ns}
Group V	1.67±0.06d^{ns}	11.05±0.20d^{ns}

Values are expressed as mean ± SD of six animals. Experimental design and statistical comparisons are as in table 1.

Units:

* µ moles of phosphorus liberated/L

¥ µ moles of p-nitro phenol liberated/L

Table 4. Effect of PHF on GDH and SDH enzymes in liver of control and experimental in rats

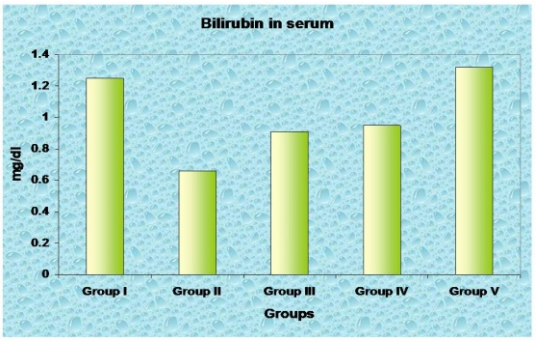
Values are expressed as Mean ± SD of six animals. Experimental design and statistical comparison are as in table 1.

Units: n moles of succinate formed /min/mg protein

Groups	GDH¥	SDH¥
Group I	7.07±0.37	3.52±0.12
Group II	15.45±0.78a*	0.99±0.05a*
Group III	9.01±0.41b*	2.92±0.06b*
Group IV	9.08±0.37c*,c^{ns}	2.95±0.07c*,c^{ns}
Group V	7.07±0.24d^{ns}	3.51±0.06d^{ns}

Table 4 and Figure 2 represent the levels of liver glutamate dehydrogenase (GDH), succinate dehydrogenase (SDH) and serum bilirubin. Levels of bilirubin and GDH were significantly ($p<0.05$) increased in toxic rats (Group II) where as the SDH level was significantly decreased in toxic group of rats. It is postulated that administration of CCl_4 could cause cell lysis, resulting in the release of cytoplasmic enzymes of the liver into blood circulation, leading to their increase in levels in serum and this property is often implicated to assess the extent of CCl_4 induced hepatocellular damage (Pradeep *et al.,* 2005)pg no 89. The PHF and silymarin administration (group III and IV) successfully altered the effect to normal level in the experimental rats.

Fig.3. Effect of PHF on bilirubin in serum of control and experimental rats



Discussion

5'NT the plasma membrane marker enzymes regulate many biochemical reactions in the body tissues. GGT level is known to be sensitive marker of hepatobiliary disorder and GGT is a membrane bound enzyme. Oxidative stress induced damage to the membrane of hepatocytes seems to contribute to the increased activity of GGT (Gupta *et al.,* 2005).

Our results coincides with that of Venukumar and Latha (2004) who showed the effect of *Coscinium fenestratum* on hepatotoxicity in rats and reported that the activities of 5'NT and GGT registered a significant elevation in CCl_4 treated rats which were significantly recovered towards an almost normal level in animals co-administrated with the *C.fenestratum*.

Bishayee *et al.* (1995) showed a reduction in the hepatic 5'NT activity by the adminstration of *D. carota* Linn (carrot) against carbon tetra chloride intoxication in mouse liver. Similar results were also observed in our studies.

Succinate dehyrogenase (SDH) is a mitochondrial enzyme tightly bound to the inner mitochondrial membrane and plays a important role in energy conversion. A significant fall in the succinate dehydrogenase activity could result in serious impairment of mitochondrial function and metabolic turnover. This may be due to the mitochondrial assembly. Active principle present in PHF may possibly play a role in retaining the impairment of mitochondrial function (Shukla *et al.,* 2005).

Hepatoprotective activity of *D.carota* Linn (carrot) against CCl4 intoxication with in mouse liver was studied by Bishayee *et al.,* (1995) and reported decrease in GDH level and increased SDH enzyme level on treatment with the extract. This study also coincides with the above studies.

Shukla *et al.,* (2006) showed the normal activity of SDH after the administration *Terminalia belerica* fruit extract against CCl_4 induced toxicity in rats. Similar result was observed in this studies.

Bilirubin, an endogenous organic anion binds reversibly to albumin and it is transported to the liver, and then conjucated with glucoronic acid and excreted in bile. Hepatobilary disease is indicated when conjugated fraction of bilirubin exceeds the upper limit of normal, even if the total serum bilirubin is normal or near normal (Raghavendren *et al*., 2004).

In conclusion, our findings clearly state that polyherbal formulation (PHF) extract, with its potent hepatoprotectant, seems to be highly promising agent in protecting hepatic tissue against carbon tetrachloride induced liver damage.

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