

RESEARCH ARTICLE

PHARMACOLOGY

HEPATOPROTECTIVE ACTIVITY OF A POLY HERBAL EXTRACT IN CARBON TETRA CHLORIDE INTOXICATED HEPATOTOXICITY IN MALE ALBINO RATS

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ABSTRACT

The Poly herbal ethanolic extract (PHEE) was evaluated for its anti hepatotoxic potential against carbon tetrachloride (CCL₄) induced hepatic damage in male albino rats. Ethanolic extract from the leaves of *Melia azadirachta*, seeds of *Piper longum* and whole plant of *Eclipta alba* at a dose level of 50 mg/kg body weight was administered orally daily once for 14 days. The substantially reduced levels of enzymatic anti-oxidants such as superoxide dismutase(SOD), catalase(CAT), glutathione peroxidase (GPx), glutathione-s-transferase(GST) and glutathione reductase (GR), due to carbon tetra chloride effect were restored to normal. Histopathological examination of liver sections of PHEE treated rats shows the restoration of normal tissues. Vitamin-E at a dose of 100 mg/kg taken as a standard reference also exhibited significant antioxidant activity against CCl₄ induced Hepatotoxicity. The results of this study strongly justified that PHEE has a potent hepatoprotective activity against carbon tetrachloride

KEY WORDS

Polyherbal ethanolic extract, Anti hepatotoxic potential, Carbon tetrachloride, Lipid peroxidation, Antioxidant enzymes.

1. INTRODUCTION

Liver is the largest gland in the body and is an extremely active organ¹⁶. Liver disease is still a worldwide health problem. Hepatotoxicity inflicted by foreign or natural chemicals has been recognized over a century before. Hepatotoxicity is mainly caused due to infections/autoimmune disorder, chemical agents (certain antibiotics, aflatoxin, carbon tetrachloride, etc.)¹ and excess consumption of alcohol.

In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders⁴. In recent years, plants containing flavonoids have gained much of interest in the research area, which is found to have antioxidant property, capable of defending free radical mediated pathological studies³.

There are many herbal formulations available in the market showing antioxidant activity. One such formulation chosen for the study the Polyherbal ethanolic extract (PHEE) consists of equal quantities of leaves of *Melia azadirachta*, seeds of *Piper longum* and the whole plant of *Eclipta alba*. These plants have traditional claim against Liver disorders and all of them are scientifically evaluated for their potency individually. The herb *Eclipta alba* is used as a tonic and deobstruent in hepatic and spleen enlargements and in skin diseases. *Piper longum* is reported to be useful in inflammation of the liver, arthritic conditions, lumbago, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, snake bite, night blindness [Unani]. *Melia azadirachta* is used in folk remedies for tumors, inflamed glands, as an anodyne, ascaricidal and deobstruent. Preliminary phytochemical analysis of the PHEE reveals the presence of flavonoids and glycosides¹³. In the present investigation, the

hepatoprotective activity of PHEE was studied against CCl_4 induced hepatic damage in rats taking Vitamin E as a standard reference.

2. Methodology:

2.1. Materials used:

Hepatotoxicity inducer like CCl_4 and other chemicals were obtained from Fisher, Aldrich and SRL companies.

2.2 Plant collection and extraction:

The plants *Melia azadirachta*, *Piper longum* and *Eclipta alba*, proposed for the study are the generous gift samples from Indian Medical Practitioners Co-operative Society (IMPCOPS) Adayar, Chennai, Tamilnadu, India. The leaves of *Melia azadirachta*, seeds of *Piper longum* and full plant of *Eclipta alba* were shade-dried and made into coarse powder in the grinding mill. 50gm of each plant powder were macerated with 400ml ethanol for 30 days with intermittent shaking. Then the solvent is filtered and evaporated to dryness¹⁵.

2.3 Preliminary phytochemical screening:

The Ethanolic extracts of *Melia azadirachta*, *Piper longum* and *Eclipta alba* were subjected to preliminary screening for their presence or absence of active phytochemical constituents.

2.4 Animals:

Normal healthy male albino wistar rats weighing from 150-200gms were used for the study. The animals were housed in a spacious hygienic cage. Food and water are given ad-libitum. The experimental protocol was subjected to the scrutiny of Institutional Animal Ethical Committee and was cleared before



starting .The animals were handled as per the guidelines of animal care.

2.5. Acute toxicity study:

The acute toxicity study was carried out as per OECD test guidelines 423(Fixed dose procedure) in Wistar albino rats. The Polyherbal ethanolic extract (PHEE) fall under class 4 (LD50 > 300-2000 mg/kg) and the lethal dose was found to be 400mg/kg body weight. One tenth of this dose was selected as the maximum therapeutic dose for evaluation. The toxicity study shows neither increase in the level of peroxide nor decreased level of cytoprotective enzymes. This indicates that the herbal extract has no toxic effect over normal liver architecture.

2.6 Hepato protective study:

The animals were divided into four groups of six rats in each. The Group I served as normal control received single daily dose of 5% tween 80 in water [5ml/kg;p.o] for seven days. Group II-IV received CCL₄ and 2ml/kg b.wt p.o once a day for seven days. The animals of Group III received a single dose of Vitamin – E (100mg/kg b.wt p.o) for one week which serves as a standard reference. The Polyherbal ethanolic extract (PHEE) of single daily dose 40mg/kg b.wt p.o was administered to Group IV animals for seven days⁸.

After the experimental period, all the animals were overnight fasted, anaesthetized and then sacrificed by cervical decapitation on day 8. Immediately after sacrifice, the liver was excised from the animals and washed in ice-cold saline. A 10% of the liver homogenate was prepared in Tris-HCL buffer (0.1M, pH.7.4). The homogenate was centrifuged and the supernatant was used for the assay of cytoprotective enzymes namely glutathione peroxidase (GPx)¹¹, glutathione-s-transferase (GST)⁷, glutathione reductase(GR)⁶, superoxide dismutase (SOD)^{5,9}, catalase (CAT) and Lipid peroxidation (LPO)¹². All the enzymatic assays were taken at particular intervals using Shimadzu spectrophotometer, UV-1601 model.

2.7 Histopathological studies:

The hepatoprotective activity was confirmed through histopathological studies on liver of rat. Small pieces of liver were collected in 10% formalin solution for histopathological examination. Slices of liver were cut and washed in ringer's solution, which are then soaked with filter paper for 15min. Then the liver slices were fixed in Carroy's fluid I (Ethanol: Chloroform: Glacial acetic acid–6:3:1) and processed for paraffin embedding following the standard Micro techniques. Section of liver was stained with aqueous hematoxylin and alcoholic eosin and was observed microscopically for histopathological changes¹⁰.

2.8. Statistical analysis:

Values are expressed in mean±S.E.M. for six animals in each group. P-value was calculated using ANOVA followed by Dunnett's test for multiple comparisons. Values of P < 0.05 were considered significant in all cases.

3. RESULTS

The phytochemical screening of ethanolic extracts of polyherbs were carried out and shows the presence of Alkaloid, Carbohydrate, Sterol, Protein, Flavonoids, Gum and Mucilage and Terpenes. In the present investigation, Carbon tetra chloride at a dose level of 2ml/kg body weight was given orally for 7 days. (Group II) was shown a significant reduction ($p < 0.001$) in the anti-oxidant enzyme (GST, GR, GPx, SOD, CAT) levels when compared to Control-0.3ml of Tween for 7 days (Group I). The PHEE (40mg/kg b wt) group treated for 7 days (Group IV) was shown significant increase ($p < 0.001$) in enzyme levels in liver homogenate when compared to Group II. Group III treated with Vit-E also shows significant variation ($p < 0.001$) in these parameters and serves as a standard control in the study. Results were shown in the Table I & Figure I.

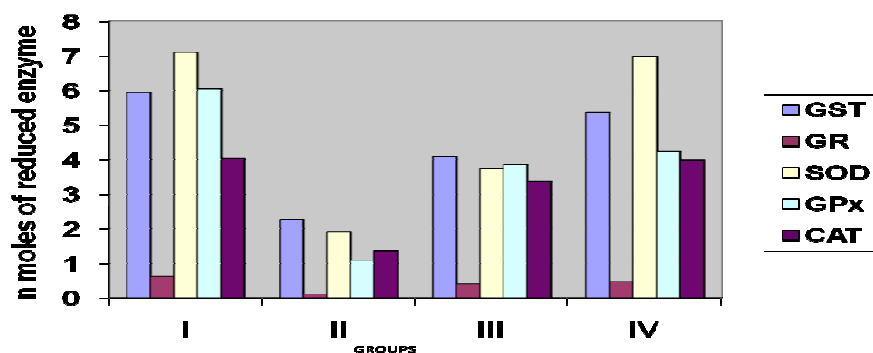
Table1
Effect of Polyherbal ethanolic extract (PHEE) on various Antioxidant Enzyme levels:

Parameters	Group I (control)	Group II (CCl ₄ 2ml/kg)	Group III (Vit-100mg/kg+CCl ₄)	Group IV (PHEE40mg/kg+ CCl ₄)
GST (moles of CDNB/mg)	5.94±2.012	2.26±1.008 ^a	4.08±1.010 ^b	5.36±1.240 ^{c*}
GR (moles of GSSH/mg)	0.62±1.004	0.10±1.002 ^a	0.40±1.004 ^b	0.46±1.012 ^c
SOD (U/mg)	7.09±0.194	1.90±0.060 ^a	3.73±0.075 ^b	6.98±0.273 ^c
GP_x (moles of GSH oxidized/mg)	6.04±2.04	1.08±2.120 ^a	3.84±1.080 ^b	4.23±1.002 ^c
CAT (U/mg)	4.02±1.05	1.36±1.003 ^a	3.36±1.005 ^b	3.98±1.020 ^c

Polyherbal ethanolic extract (PHEE) =Ethanolic extract of Eclipta alba, Melia azadarichta and Piper longum.
GST:Glutathione–S-Trasferase; GR:Glutathionereductase SOD:Superoxidedismutase; GP_x:Glutathione peroxidase ; CAT: Catalase.

Values are Mean ± SEM of 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n = 6). a-GroupII compared to GroupI, b-GroupIII compared to Group II, c-Group IV compared to GroupII. * - P<0.001.

Figure - I
Effect of polyherbal ethanolic extract(PHEE 40mg/kg bwt) on various enzyme levels against CCL₄ induced hepatotoxic damage in rats



GroupI- Control
 GroupII- CCl₄ Treated
 GroupIII- Vit-E+CCl₄ Treated
 GroupIV- PHEE+CCl₄ Treated

Lipid peroxidase in liver homogenate was significantly increased (p<0.001) in CCl₄ treated group when compared to normal. Treatment with the standard and test drug showed significant inhibition of LPO (p<0.001) in liver homogenate, when compared to CCl₄ treated group. Results were shown in Table II.

Table 2
Effect of Polyherbal ethanolic extract (PHEE) on CCl₄ induced Lipid Peroxidation:

SL.NO	GROUPS	% INHIBITION OF LIPID PEROXIDATION
1	I (Control)	-
2	II (CCL ₄ 2ml/kg)	-
3	III (Vit-E 100mg/kg+CCL ₄)	87%±4.28*
4	IV (PHEE 40mg/kg+CCL ₄)	93%±2.46*

PHE: ethanolic extract of Eclipta alba, Melia azadirachta and Piper longum.

Values are mean ± SEM of 6 replicates. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test (n=6).

* - p<0.001, when test and standard are compared against control.

4. DISCUSSION

CCl₄ induced lipid peroxidation is one of the main manifestations of oxidative cellular damages, which may be the critical step in Hepatotoxicity. CCl₄ mediated cell injury may lead to the release of free radicals in-vivo and these radicals initiate lipid peroxidation, a damaging process in all biological systems, since all cell membranes contain fatty acids which are substrates for the reaction. Hence, it can be deduced that Hepatotoxicity induction by CCl₄ may be chiefly mediated through lipid peroxidation (LPO). So we studied the effect of PHEE on LPO. The PHEE treated animals shown a decrease in lipid peroxide levels. Therefore it became convenient for us to suggest that the drug may have a beneficial effect against CCl₄ induced lipid peroxidation. From this juncture one may assume that Flavonoids present in the extract may be in part responsible for its protective effect against LPO, as reports suggested that flavonoids are potent inhibitors of lipid peroxidation¹⁴.

Enzymes for protecting against peroxide radicals, (catalase, glutathione peroxidase, glutathione-s-transferase, glutathione reductase and superoxide dismutase) have recently received much attention in connection with antioxidant property. Catalase, the enzyme which catalyses the disproportionation of hydrogen

peroxide and glutathione peroxidase, is thought to be first line of defense against oxidative damages. The liver catalase activity is depressed in all hepatotoxic animals in correlation with the impairment of free radical scavenger system in Hepatotoxicity. When the study was carried out to estimate the level of catalase in extract treated rats, we found that they brought their Catalase levels back to normal.

Glutathione peroxidase levels were also relatively low in hepatoma. The diminished enzyme activities along with excess radical production may lead to many of the observed deranged properties of hepatotoxic cells. The enzyme level was brought back to normal levels in drug treated animals².

Glutathione-s-transferase is an enzyme which catalyses the glutathione conjugation with electrophilic compounds bio-transformed from xenobiotics including aflatoxins. They can prevent initiation of hepatotoxic process by inactivating or detoxifying. The enzyme levels in liver were reversed back to near normal values in drug treated animals⁷.

Glutathione reductase level is also lowered in hepatotoxic animals. The results from treated animals exhibited no significant change in lipid peroxide and antioxidant levels when compared with the values obtained in control animals. Our investigation suggests that PHEE may have protective effect against per



oxidative damage and causes improvement in cytoprotective enzymes.

5. CONCLUSION

The oral administration of the ethanolic extract of poly herbal drug to such CCl₄ treated

rats, reduced the enhanced level of lipid peroxidation and attenuate reconstitution of the levels of SOD, CAT, GPx, GST and GR indicates the anti-peroxidative and anti oxidative efficiency of the Polyherbal drug.

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