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# ANTIHEPATOTOXIC ACTIVITY OF BIHERBAL EXTRACT OF DRIED FRUITS OF *PIPER LONGUM* AND DRIED STEM OF *TINOSPORA CORDIFOLIA* AGAINST $CCL_4$ INDUCED HEPATOTOXICITY

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## ABSTRACT

Medicinal plants assisted as a raised area for ancient ayurvedic system of medicine, in present scenario, herbal therapeutics gaining impulse in pharmacological claims and as molecular entities in the drug development. Herbal treatments offer respite from the high cost of expensive modern medicines as well as in being comparatively safe with less side effects. In the present research work the Biherbal extract of *Piper longum* and *Tinosporacordifolia*, the two medicinal plants used for the treatment of various pathological conditions in the traditional system of medicine were subjected to scientific evolution with the help of modern techniques and pharmacology. The Biherbal extract was found to be significantly effective in resisting induced hepatotoxicity due to $CCl_4$ exposure.

**KEYWORDS:** medicinal plants, Biherbal extract, *Piper longum*and *Tinosporacordifolia*, $CCl_4$.

## INTRODUCTION

Liver is one of the largest internal organ in human body and the chief site for intense metabolism and secretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction.[1]

Some of these major functions include carbohydrate, protein and fat metabolism, detoxification and secretion of bile. Therefore, the maintenance of a healthy liver is vital to overall health and wellbeing. Unfortunately, the liver is often abused by environmental toxins, poor eating habits, alcohol and prescription and over-the-counter drug use, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis and alcoholic liver disease.[2]

Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders. But there is not much drug available for the treatment of liver disorders. Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal models.[3,4]

Hepatoprotective effects of plant drugs and herbal formulations are studied against chemicals (alcohol, $CCl_4$, beta galactosamine, thioacetamide) and drugs (paracetamol, nimusalide, antitubercular drugs like isoniazid, rifampicin etc.) induced hepatotoxicity in rats and mice as they virtually mimic any form of naturally-occurring liver disease. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effect.[5]

Amongst the large number of herbal drugs existing in India *Piper longum* is a highly valuable medicinal plant belongs to family piperaceae and is one of the essential ingredients in the most of the compound preparations included in Ayurvedic literature.[6] *Piper longum* also known as long pepper; the word pepper being derived from Sanskrit. The whole plants as well as plant parts such as the fruit are used traditionally for pharmaceutical purposes.[7]

*Tinospora cordifolia* is an Ayurvedic plant which belongs to family menispermaceae has important medicinal values. It is widely known as Guduchi, Giloy or Amrita.[8] In Hindi, the plant is commonly known as Amrita which is a Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young. In Ayurveda, it is designated as Rasayana drug recommended to enhance general body resistance, promote longevity and as antistress and adaptogen, this is the fact that it is called "Amrita" signifies its use for revitalization and its importance in Ayurveda. This significant plant is also mentioned in important Pharmacopoeias. [9]

**Need for Biherbal Preparation:** A single herb may not be effective against all kind of severe liver diseases. Therefore, effective preparations have to be developed using medicinal plants, with appropriate pharmacological experiments and clinical trials. In this perspective, Biherbal extract made up of equal quantities of dried fruits of *Piper longum* and dried stem of *Tinospora cordifolia* was subjected to various assays in order to evaluate its antihepatotoxic activity.

## MATERIAL AND METHODS

### Part Investigated

Biherbal extract prepared from dried fruits of *Piper longum* and dried stem of *Tinospora cordifolia* (50:50) w/w.

### Collection of Plant materials

The dried fruits of *Piper longum* and dried stem of *Tinospora cordifolia* were collected from the market Kharibawli; chandnichauk, Delhi and verified by taxonomist at Department of Botany Jamia Hamdard University, New Delhi.

### Preparation of Biherbal Extract

The dried fruits of *P. longum* and shed dried stem of *T. cordifolia* crushed to coarse powder and extracted with ethanol using cold percolation method individually and concentrated on waterbath. To prepare the Biherbal extract equal quantity of ethonolic extract of *P. Longum* and *T. Cordifolia* were mixed into (50:50) w/w and dissolved in ethanol and concentrated on waterbath.

### Experimental animals

Albino male rats of Wistar strain (150 ± 10 g), 4–6-week-old, were obtained from Central Animal House of Hamdard University, New Delhi. They were housed in polypropylene cages in groups of 5 rats per cage and kept in a room maintained at 25±2 °C with a 12-h light/dark cycle. They were allowed to acclimatize for 1 week before the experiments and were given free access to standard laboratory feed (Amrut Laboratory, rat and mice feed, Navmaharashtra Chakan Oil Mills Ltd, Pune, India) and water *ad libitum*. Approval to perform the animal experiment was obtained from Institutional Animal Ethics Committee (IAEC) registered under the Committee for the Purpose of Control and Supervision of Experimental Animals (173/CPCSEA).

**Dosing and Grouping of Animals**

Rats were equally divided in to 4 groups with 5 animals in each group (Table-1). The time duration of experiment was 15 days.

**Table 1: Dosing and grouping of experimental animals.**

| | |
|---|---|
| Group 1 | **Control group:** Received only vehicle i.e. olive oil at the dose of 1.5 ml/kg of rat body weight orally. |
| Group 2 | **Toxic group:** Hepatotoxicity was induced in rats by an injection of $CCl_4$ at the dose of 2 ml/kg body weight, 1:1 with olive oil i.p. |
| Group 3 | **Standard group:** Received silymarin (silbyon-70) at the dose of 50 mg/kg Body weight was given orally after hepatotoxicity caused by $CCl_4$. |
| Group 4 | **Biherbal Extract group:** Received extract at the oral dose of 200 mg/kg of body weight with 24 h interval after the hepatic injury was induced by $CCl_4$ along with vehicle before four hour of first dose. |

**Blood Collection**

Each animal was anaesthetized with diethyl ether. Heart puncture was done with 5 ml disposable syringe and 2 ml blood was drawn very gently and slowly. The blood collected was shifted immediately to clean dried centrifugation tubes, allowed to clot and serum was separated by; centrifugation at 4000 rpm for 10 min. Serum was separated and then preserved in the cuvettes at -20°C in the freezer until analysis. Biochemical estimations were made the following day.

**Biochemical Assessment of Liver Function**

Biochemical parameters like AST and ALT were determined by (Reitman and Frankel, 1957)[10]; ALP was determined by (Kind and King, 1954)[11]; SOD (Dhindsa et al, 1981)[12]; GPx (Wheeler et al, 1990)[13]; GSH (Jollow et al, 1974)[14]; Total Protein (Lowery et al, 1951)[15]; Total Albumin (Dumas, 1981)[16]; Total Bilirubin (Rajendran et al,2009)[17] were determined by reported methods.

**Histopathological Study of Liver**

Liver sections taken immediately after dissection from the liver, and then fixed in 10% buffered formalin (Luna, 1986)[18], dehydrated in gradual ethanol (50 to 100%), cleared in xylene, and embedded in paraffin. Sections (4 to 5 µm thick) were prepared and then stained with Haemotoxylin and Eosin (H and E) dye for photomircoscopic observations like cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, infiltration of Kupffer cells and lymphocytes under power 40X and microphotographs were taken using an Olympus BX50 microscope system (Olympus, Japan).

**Statistical Analysis**

All values are expressed as mean ± S.E.M. the statistical comparisons were done by using analysis of variance (ANOVA) using prism graph pad software. Values P< 0.05 were considered as significant.

## RESULT AND DISCUSSION

Effect of Biherbal Extract of (*P. longum* & *T. cordifolia*) on Various Biochemical Parameters in $CCl_4$ induced hepatotoxicity in Albino Rats (Table 2-4)**:**

**Table 2: Effect of Biherbal Extract Supplementation on AST, ALT and ALP Levels.**

| Group | Treatment | Dose | AST (IU/L) | ALT (IU/L) | ALP (IU/L) |
|---|---|---|---|---|---|
| G1 | Control | ---------- | 48.28 ± 1.21 | 28.12 ± 1.21 | 145.62 ± 1.23 |
| G2 | Toxic group ($CCl_4$) | 2.0 ml/kg (p. o.) | 134.24 ± 2.05[a] | 71.96 ± 2.88[a] | 375.02 ± 1.73[a] |
| G3 | Standard group (silymarin) | 50 mg/kg (p. o.) | 55.46 ± 1.96[b] | 32.54 ± 3.01[b] | 158.14 ± 3.33[b] |
| G4 | Biherbal extract (*P. longum*&*T. cordifolia)* | 200mg/kg (p. o.) | 75.40 ± 2.18 [b] | 45.23 ± 1.98[b] | 170.40 ± 3.17[b] |

Values are expressed a mean ± S.E.M. (N=5) using ANOVA. $CCl_4$ group showed significant increase in AST, ALT, and ALP levels compared to the control group (a, P<0.05 $CCl_4$ vs. control group): Biherbal extract (*P. longum* & *T. cordifolia*) supplementation significantly decreased AST, ALT, and ALP levels in the $CCl_4$ + Biherbal extract extract (*P. longum* & *T. cordifolia*) group compared to the $CCl_4$-induced hepatotoxic group (b, P<0.05 $CCl_4$ +Biherbal extract (*P. longum* & *T. cordifolia*) vs. $CCl_4$ group).

**Table 3: Effect of Biherbal Extract Supplementation on SOD, GPx and GSH Level.**

| Group | Treatment | Dose | SOD (U/g) | GPx (U/g) | GSH (U/g) |
|---|---|---|---|---|---|
| G1 | Control | ---------- | 84.62 ± 1.25 | 42.62 ± 1.16 | 78.16 ± 1.14 |
| G2 | Toxic group | 2.0 ml/kg (p. o.) | 37.02 ± 2.51[a] | 17.02 ± 3.61 [a] | 29.12 ± 1.32[a] |
| G3 | Standard group (silymarin) | 50 mg/kg (p. o.) | 78.14 ± 3.22[b] | 39.15 ± 1.12 [b] | 70.53 ± 2.92[b] |
| G4 | Biherbal extract (*P. longum*&*T. cordifolia*) | 200mg/kg (p. o.) | 76.40 ± 1.69 [b] | 37.40 ± 2.56 [b] | 67.14 ± 2.64[b] |

Values are expressed as mean ± S.E.M. (n=5) ANOVA. $CCl_4$ hepatotoxic group showed significant decrease in SOD activity compared to the control group (a, P<0.05 $CCl_4$ vs. control group): Biherbal extract (*P. longum* & *T. cordifolia*) supplementation significantly increased SOD activity in the $CCl_4$ + Biherbal extract (*P. longum* & *T. cordifolia*) group

compared to the $CCl_4$-induced hepatotoxic group (b, P<0.05 $CCl_4$ + Biherbal extract (*P. longum* & *T. cordifolia*) vs. $CCl_4$ group).

Effect of Biherbal extract (*P. longum* & *T. cordifolia*) treatment on GPx activity. $CCl_4$ hepatotoxic group showed significant decrease in GPx activity as compared to the control group (a, P<0.05 $CCl_4$ vs. control group). Biherbal extract (*P. longum* & *T. cordifolia*) treatment significantly increased GPx activity in the $CCl_4$ + Biherbal extract groups compared to the $CCl_4$-induced hepatotoxic group (b, P<0.05 $CCl_4$ +Biherbal extract vs. $CCl_4$ group).

Effect of Biherbal extract (*P. longum* & *T. cordifolia*) treatment on GSH activity: $CCl_4$ hepatotoxic group showed significant decrease in GSH activity as compared to the control group (a, P<0.05 $CCl_4$ vs. control group). Biherbal extract treatment significantly increased GSH activity in $CCl_4$ + Biherbal extract group compared to the $CCl_4$-induced hepatotoxic group (b, P<0.05 $CCl_4$ +Biherbal extract vs. $CCl_4$ group).

**Table 4: Effect of Biherbal Extract Supplementation on Total Protein, Total Albumin, and Total Bilirubin Levels.**

| Group | Treatment | Dose | Total Protein (g/dl) | Total Albumin (g/dl) | Total Bilirubin (mg/dl) |
|---|---|---|---|---|---|
| G1 | Control | ---------- | 6.32 ± 1.23 | 4.12 ± .43 | 0.74 ± .34 |
| G2 | Toxic group | 2.0 ml/kg (p. o.) | 1.95 ± 0.36[a] | 1.09 ± .41[a] | 4.16 ± .53[a] |
| G3 | Standard group (Silymarin) | 50 mg/kg (p. o.) | 6.76 ± 1.06[b] | 4.04 ± 0.63[b] | 0.98 ± .21[b] |
| G4 | Biherbal extract (*P. longum*&*T. cordifolia*) | 200 mg/kg (p. o.) | 6.90 ± 0.49[b] | 4.23 ± 1.10[b] | 1.78 ± .26[b] |

Values are expressed as mean ± S.E.M. (n=5) using ANOVA. $CCl_4$ group showed significant decrease in total protein, total albumin, and increase in total bilirubin levels compared to the control group (a, P<0.05 $CCl_4$ vs. control group). Biherbal extract (*P. longum* & *T. cordifolia*) supplementation significantly increased total protein, total albumin, levels and decreased total bilirubin level in the $CCl_4$ +Biherbal extract (*P. longum* & *T. cordifolia*) group compared to the $CCl_4$-induced hepatotoxic group (b, P<0.05 $CCl_4$ + Biherbal extract vs. $CCl_4$ group).

**Histopathological Examination**

The histopathological examination of the liver section of the male albino Wistar rats treated with $CCl_4$ showed hepatocytic necrosis and evident vacuolation of hepatocytes. The rats treated with silymarin and Biherbal extract along with toxicant showed sign of protection against toxicants to considerable extent as evident from formation of normal hepatic cards

and absence of necrosis and vacuoles. Biherbal extract showed a remarkable recovery of hepatic cells, disappearance of necrosis, a mild vacuolation with almost normal central vein (CV) and portal triad (PT) indicating a potent antihepatotoxic activity and supporting the data obtained from the analysis of biochemical parameters (Fig. 1 to 4).

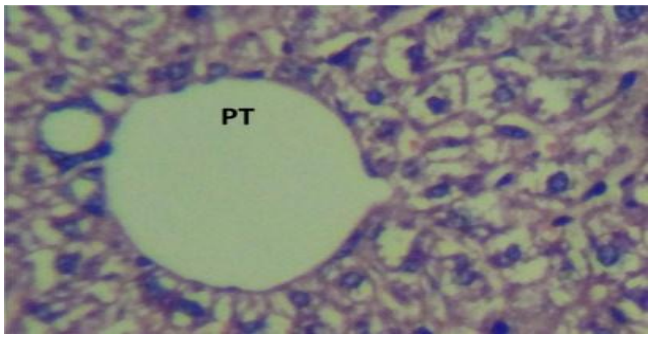


**Figure 1: Liver histopathology of normal animal at 40x magnification showing normal hepatic cells with distinct nucleus and sinusoidal architecture without any necrosis.**

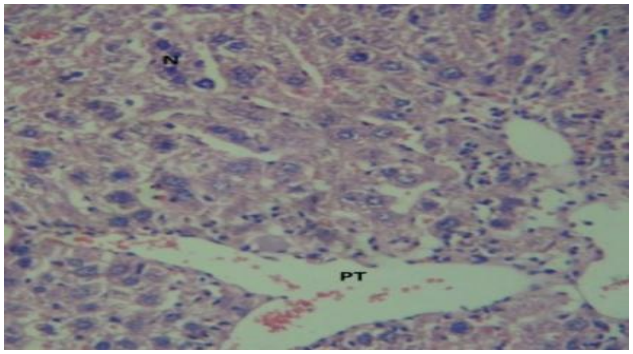


**Figure-2: liver histopathology of animal treated with $CCl_4$ at 40x magnification showing evident vacuolation of hepatocytes and focal necrosis in the centrizonal area PT portal triad and CV central vein hepatocytic necrosis (N).**

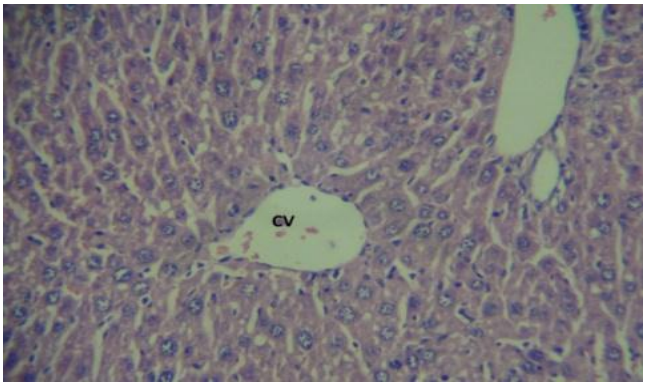


**Figure-3: Liver histopathology of animal treated with $CCl_4$ and Silymarin at 40 x magnifications showing mild sinusoidal dilation in the centrizonal area PT portal triad and CV central vein was clearly visible.**

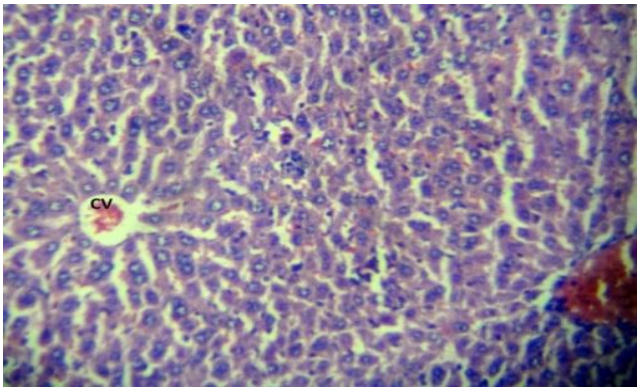


**Figure 4: Liver histopathology of animal treated with $CCl_4$ and Biherbal extract (*P. longum* and *T. cordifolia*) at 40x magnification showing mild hepatic necrosis and vacuolation with moderate degree of inflammation and CV is in normal condition.**

## CONCLUSION

Thus, from the above investigation we can say that Biherbal extract (*P. longum* and *T. cordifolia*) exhibited significant antihepatotoxic activity as well as antioxidant activity against $CCl_4$ induced hepatotoxicity. This significant antihepatotoxiceffect is probably mediated through antioxidant activity. These studies shows that the Biherbal extract of (*P. longum* and *T. cordifolia*) have major potential to develop in the form of an effective formula using modern methods for liver disorders and other physical ailments. Further studies are required to explain the exact mechanism of action in neutralising the toxic effects.

## ABBERIVIATIONS USED

ALT: Alanine Amino Transferase; AST: Aspartate Amino Transferase; ALP: Alkaline Phosphatase; SOD: Super Oxide Dismutase; GSH: Glutathione; GPx: Glutathione Peroxidase; TBL: Total Bilirubin level; IP: Intra-peritoneal; P.O.: Per Oral; PT: Portal Triad; CV: Central Vein; $CCl_4$: Carbon Tetra Chloride.

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