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Research Article

ANTIOXIDANT AND HEPATOPROTECTIVE EFFECT OF AQUEOUS AND ETHANOLIC EXTRACTS OF IMPORTANT MEDICINAL PLANT *PONGAMIA PINNATA* (FAMILY: LEGUMINOSEAE)

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ABSTRACT

Objectives: A lot of herbal plants and polyherbal formulations are used for the treatment of liver diseases. **Methods:** This present investigation was aimed to assess the hepatoprotective activity of aqueous and ethanol extract of *Pongamia pinnata* leaves against acetaminophen-induced liver damage in albino rats. Silymarin as a standard drug for comparing the activity. The activity was assessed by comparing the biochemical parameters in serum levels such as serum glutamate pyruvate transaminase, serum glutamate oxalate transaminase, total bilirubin, alkaline phosphatase of plant extracts treated group with acetaminophen treated animals. **Results:** Results showed, ethanolic extract treated group showed highly significant activity ($p<0.001$), whereas aqueous extract treated group has shown the significant ($p<0.01$) action but less compared with ethanolic extract. Plant extracts restores biochemical enzymes and brings down to normal as compared to standard drug silymarin. **Conclusion:** This results shows and confirms the significant protective activity against acetaminophen-induced hepatotoxicity.

Keywords: Hepatotoxicity, Antioxidant, Acetaminophen, *Pongamia pinnata*, Herbal plants.

INTRODUCTION

The liver is the most important organ where drugs are structurally altered; resulting biologically inactive or active metabolites and some of these are toxic. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorder. The classical systems of medicine such as Ayurveda, Siddha, Amchi, Unani and Tibetan use about 1,200 plants. It is estimated that about 7,500 plants are used in local health traditions in, mostly, rural and tribal villages of India. Of these, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to the mainstream population. The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, the fight against the disease, nutrient supply, energy provision and reproduction. Hepatic disease (liver disease) is a term that affects the cells, tissues, structures, or functions of the liver. Liver has a wide range of functions, including detoxification, protein synthesis, and production of biochemical necessary for digestion and synthesis as well as breakdown of small and complex molecules, many of which are necessary for normal vital functions. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, etc., chronic alcohol consumption, and microbes is well-studied.

- *P. pinnata*
- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Fabales
- Family: Leguminoseae
- Genus: *Pongamia*
- Species: *Pinnata*

There is various plants from different family have been used for the hepatoprotective activity. In that, Amaranthaceae family plants are *Aerva lanata* [1], *Alternanthera sessilis* [2], *Amaranthus spinosus* [3], Asteraceae family plants are *Achilea millefolium* [4], *Chrysanthemum balsamita* [5], *Calendula officinalis* [5], *Eclipta alba* [6], *Eclipta prostrate* [7], *Echinacea pallid* [5], *Taraxacum officinale* [8]. Zingiberaceae family plants are *Curcuma xanthorrhiza* [9], *Zingiber officinale* [10], Asclepiadaceae family plants are *Calotropis procera* [11], *Decalepis hamiltonii* [12], *Pergularia daemia* [13], *Sarcostemma brevistigma* [14], Compositae family plants are *Epaltes divaricata* [15], *Pluchea indica* [16], *Tridax procumbens* [17]. Euphorbiaceae family plants are *Emblica officinalis* [18], *Phyllanthus emblica* [19], *Phyllanthus urinaria* [20], *Phyllanthus niruri* [20], *Phyllanthus amarus* [21], Rubiaceae family plants are *Hedyotis corymbosa* [22], *Rubia cordifolia* [23], Meliaceae family plant is *Aphanamixis polystachya* [24], Betulaceae family plant is *Alnus japonica* [25], *Corylus avellana* [5], Araliaceae family plant is *Acathopana senticosus* [26] Aegiceratataceae family plant is *Aegiceras corniculatum* [27] Liliaceae family plant is *Aloe barbadensis* [28], Ranunculaceae family plant is *Aquilegia vulgaris* [29], Berberidaceae family plant is *Berberis aristata* [30], Nyctaginaceae family plant *is Boerhavia diffusa* [31], Chenopodiaceae family plant is *Beta vulgaris* [32], Theaceae family plant is *Camellia oleifera* [33], Apiaceae family plant is *Daucus carota* [34], Fumariceae family plant is *Fumaria indica* [35], Rutaceae family plant is *Glycosmis arborea* [36], Ganodermataceae family plant is *Ganoderma lucidum* (fungi) [37], Clusiaceae family plant is *Hypericum Perforatum* [38], Labiatea family plant is *Hyssopus officinalis* [5], Lygodiaceae family plant is *Lygodium flexuosum* [39], Moringaceae family is *Moringa oleifera* [40], Cucurbitaceae family plantis *Mamordica subangulata* [41], Oenotheraceae family plant is *Oenothera Biennis* [5], Polygalacea family plant is *Polygala arvensis* [42], Fabaceae family plant is *Pterocarpus santalinus* [43], Phyllanthaceae family plant is *Phyllanthus maderaspatensis* [44], Polygonaceae family plant is *Rumex patientia* [45], Apocynanaceae family plant is *Rhazya stricta* [46], Loganiaceae family plant is *Strychnos potatorum* [47], Gentianaceae family plant is *Swertia chirata* [48], Lamiaceae family is plant *S. miltiorrhiza* polysaccharides [49], Combretacea family plant is *Termnalia arjuna* [50], Aizoaceae family plant is *Trianthema portulacastrum* [51], Vitaceae family plant is *Vitis vinifera* [52], Verbenaceae family plant is *Vitex trifolia* [53], Scrophulariaceae family plant is *Veronica officinalis* [54], Solanaceae family plant is *Withania frutescens* [55].

METHODS

Chemicals

Analytical grade acetaminophen, Silymarin, and other chemicals were purchased from Himedia laboratories private limited,

Mumbai. *P. pinnata* plant was collected from, Vellore district, Tamil Nadu, India.

Preparation of plant extracts

The leaves of *P. pinnata* were collected, and shade dried for 5-8 days. The shade dried leaves were subjected to pulverization to get coarse powder which was then used for extraction with water and ethanol. 100 g of dry powder was loosely packed in the thimble of soxhlet apparatus and extracted with 80% ethanol at 60°C for 24 hrs. The extract was air dried at 25-30°C and weighed. For oral administration, extracts were dissolved in distilled water.

Experimental design for hepatoprotective activity of *P. pinnata*

Adult male Wister albino rats maintained at the college weighing 150-170 g were used for the hepatoprotective studies. Animals were divided into 6 groups, each comprising 6 rats as:

Group I (Normal): Orally received distilled water for 7 days

Group II (Induced): Orally received acetaminophen (2 g/kg body weight) dissolved in distilled water for 7 days

Group III (Standard): Orally received Silymarin (20 mg/kg body weight) dissolved in distilled water for 7 days

Group IV (Treatment): Orally received acetaminophen (2 g/kg body weight) along with aqueous leaf extracts of *P. pinnata* (300 mg/kg body weight) dissolved in distilled water for 7 days

Group V (Treatment): Orally received acetaminophen (2 g/kg/body weight) along with ethanol leaf extracts of *P. pinnata* (300 mg/kg body weight) dissolved in distilled water for 7 days.

Assessment of hepatoprotective activity

Collection of blood and biochemical analysis

On the 8th day, all the animals were sacrificed by mild ether anesthesia. Blood samples were collected in a glass tube from a retro-orbital puncture to obtain hemolysis for 30 minutes at 37°C. The clear serum obtained after centrifugation was used for the estimation of alanine aminotransferase (ALT), Aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GT), serum bilirubin and serum protein.

Antioxidant activity of *P. pinnata*

Liver homogenate preparation

Liver homogenates were obtained by using a tissue homogenator, Ultraturrax T-25 polytronat 4°C. The homogenates (1:10 w/v) were prepared by using a 100 mMol KCl buffer (pH7.0) containing 0.3 mM ethylenediaminetetraacetic acid. All homogenates were centrifuged at 6000 rpm for 45 minutes at 4°C, and the supernatant was used for biochemical analysis. The tissue homogenate was the following antioxidant levels were analyzed superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [56-58].

Statistical analysis

The difference of biochemical parameters were measured using the statistical method of, Analysis of Variance (ANOVA). ANOVA refers to the examination of differences among the samples.

RESULTS AND DISCUSSION

Biochemical characterization

The present studies were performed to assess the hepatoprotective activity of various solvent derived leaf extracts of *P. pinnata* in rats against acetaminophen as hepatotoxin which cause liver damage. Altering liver microsomal membranes of hepatocytes and cell damage results in release of enzymes AST, GT, and ALT. Estimation of levels of AST, ALT and γ-GT in serum is used to assess hepatic function in rats are shown in [Figs. 2 and 3]. The hepatic enzymes AST, ALT and GT in serum was significantly ($p<0.001$). The toxic effect of acetaminophen was controlled in the animals treated with aqueous and ethanol extracts of *P. pinnata* (300 mg/kg) was shown in Fig. 1. Among the 6 groups, ethanolic extracts were effectively controlled the liver damage induced by the acetaminophen by way of restoration of the levels of liver function.

In Group II animals, acetaminophen treatment significantly increased the serum liver enzyme levels, *viz*., AST, ALT, and GT. The activity of AST (94.98±0.65 U/L, ALT (29.98±0.65 IU/L] and GT (299.68±0.15 IU/L) was significantly higher ($p<0.05$) in acetaminophen treated group while comparison with normal control (AST 65.15±0.14; ALT 23.15±0.14; GT 166.15±0.24 IU/L) indicating a marked hepatocellular injury (Table 1). The activity of standard drug (Group III) in levels of enzymes AST (69.42±0.31 IU/L, ALT (24.42±0.31 U/L and GT (186.12±0.21 IU/L) are significantly higher ($p<0.01$) than the extracts of *P. pinnata* treated animals i.e. Group IV and Group V. The animals in Group V shows significantly low release of enzymes than Group IV indicating that ethanol extracts are efficient drug to control hepatotoxicity.

Protein and bilirubin analysis

A significant increases ($p<0.05$) in serum total protein and bilirubin levels was observed in animals treated with Acetaminophen compared to normal. Pretreatment with extracts of *P. pinnata* decreases the above parameters significantly ($p<0.05$) as compared to Acetaminophen treated Group II animals. The standard drug silymarin pretreatment (Group III) produced significant decrease ($p<0.05$) in the serum bilirubin and total protein when compared to acetaminophen treated Group II animals.

Fig. 1*: Pongamia pinnata* leaves

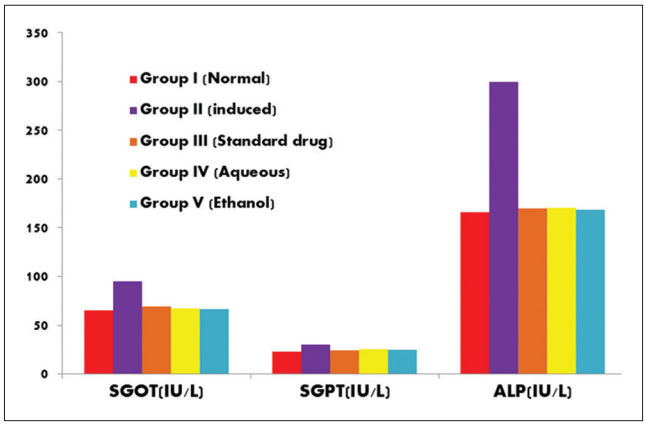

Fig. 2: Level of serum Alkaline phosphatase, serum glutamate oxaloacetic transaminase and serum glutamic pyruvic transaminase in different group of rats

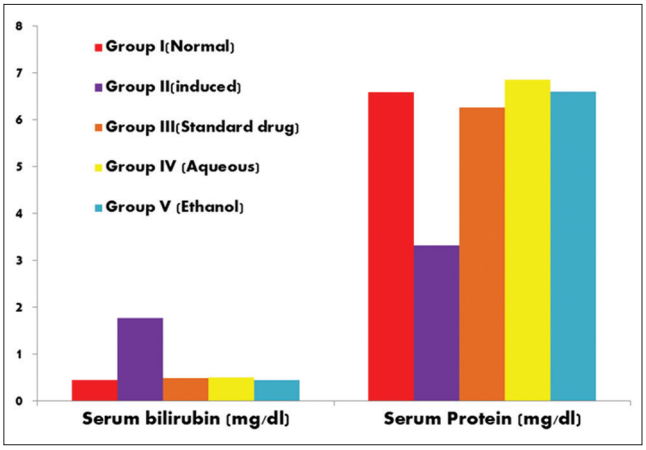

Fig. 3: Level of serum bilirubin and protein different group of rats

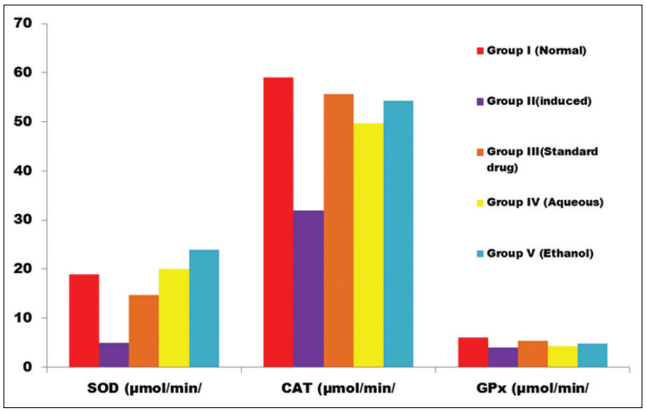

Fig. 4: Level of serum superoxide dismutase, catalase and glutathione peroxidase in different group of rats

Antioxidant activity

The effect of *P. pinnata* on SOD, CAT and GPx in antioxidant activity is shown in Fig 4. It shows that SOD, CAT and GPx activity were significantly decreases ($p<0.01$) in silymarin treated animals when compared with those animals in normal control and Acetaminophen treated group. On the other hand, the Groups IV and V with received aqueous and ethanol leaf extracts of *P. pinnata*, the values of enzymatic parameters were significantly ($p<0.001$) controlled than normal and silymarin treated animals.

Liver damage was induced by acetaminophen in an animal model is used for the screening of hepatoprotective activity. Liver damage causes and releases the enzymes ALT, AST, and GT, Total protein and bilirubin in the serum due to the membrane and cellular damages of the liver. Acetaminophen is a hepatotoxin induces the liver damage by affecting the metabolic functions. These liver enzyme level concentration was increased in serum due to the toxic effect of Acetaminophen. This was a decrease in serum levels due to the protective effect of *P. pinnata* leaf extract on liver cells followed by restoration of membrane permeability [15]. Phenol compounds are responsible for hepatoprotective and antioxidant activity of *P. pinnata* leaf extracts [7]. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals [26-28].

CONCLUSION

Our results of this study reported that aqueous and ethanol extract of *P. pinnata* leaves was an effective treatment for the control of hepatotoxicity induced by acetaminophen toxin. The degree of protection was measured by using biochemical parameters like serum transaminases (ALT and AST), alkaline phosphatase, total protein and bilirubin and antioxidant characters. The ethanolic extracts showed the most significant hepatoprotective activity comparable with standard drug silymarin. Other extracts namely aqueous also exhibited potent activity. From this investigation, phenolic compounds of plant leaves may be responsible for the most hepatoprotective activity. Our results demonstrated that the plant derived drugs is the best alternative drug for the synthetic or chemical drug.

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