IJPSR (2014), Volume 5, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 March 2014; received in revised form, 25 May 2014; accepted, 11 July 2014; published 01 October 2014

# HEPATOPROTECTIVE POTENTIAL OF AQUEOUS AND ETHANOLIC STEM-BARK EXTRACTS OF *PONGAMIA PINNATA* AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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**Keywords:**

Hepatoprotective, Paracetamol, *Pongamia pinnata*

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**ABSTRACT: Introduction:** *Pongamia pinnata* is commonly known as karanja. In Ayurvedic and Unani system of medicine, it has been used as anti-inflammatory, antiplasmodial, anti-nociceptive, anti-hyperglycaemic, anti-lipid oxidative, anti-diarrheal, anti-ulcer, anti-hyperammonemic, antioxidant, *etc*. Moreover, it has been used traditionally in the treatment of liver disorders. However, there are no scientific bases or reports in the modern literature regarding its usefulness as a hepatoprotective agent. **Aim:** To evaluate the hepatoprotective activity of the *Pongamia pinnata* bark extracts. **Methods:** The extracts were subjected to preliminary phyto-chemical investigation. The *in-vivo* hepatoprotective activity of both the ethanolic and aqueous extracts was assessed using paracetamol-induced liver damage in albino rats. The efficacy of protection was measured by evaluation of biochemical parameters, such as SGOT (serum glutamate oxalate transaminase), SGPT (serum glutamate pyruvate transaminase), ALP (alkaline phosphatase) and total bilirubin levels, as well as *in-vivo* estimation of GSH (glutathione) from liver tissue. **Results:** Phytochemical studies revealed the presence of phenolics and flavonoids, which were further estimated quantitatively. A significant hepatoprotective effect of both the extracts was observed against hepatic damage induced by paracetamol. The results also demonstrated that the activity of SGPT, SGOT, ALP, and total bilirubin was reduced in extract treated rats as compared to intoxicated rats. **Conclusions:** The hepatoprotective property of the bark extracts might be due to the presence of phenolics and flavonoids.

**INTRODUCTION:** Paracetamol is a well-known antipyretic and analgesic drug which inhibits prostaglandin synthesis by inhibiting COX (cyclooxygenase).

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(10).4275-80

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(10).4275-80

At a higher dose, it causes hepatic necrosis. The reason behind paracetamol-induced hepatotoxicity is the generation of oxidative stress by its metabolite NAPQI (N-acetyl-p-benoquinamine), formed through cytochrome P450 pathway in liver [1].

*Pongamia pinnata* is commonly known as karanja. Various parts of the plant have been used as a crude drug for the treatment of skin diseases, tumors, piles, itches, rheumatic joints wounds, diarrhea, ulcers *etc*. [2].

In Ayurvedic and Unani system of medicine, it has been used as anti-inflammatory, antiplasmodial, anti-nociceptive, anti-hyper-glycaemic, anti-lipid oxidative, anti-diarrheal, anti-ulcer, anti-hyper-ammonemic, antioxidant, *etc*. [3] *Pongamia pinnata* has been reported to be rich in phenolics [4] and flavonoids [5]. Polyphenolics are strong natural antioxidants [6]. Moreover, it has been used traditionally in the treatment of liver disorders. However, there are no scientific reports in modern literature regarding its hepatoprotective activity. Thus, the present study was conducted to evaluate the hepatoprotective activity of the *Pongamia pinnata* bark.

## MATERIALS AND METHODS:

**Procurement of Plant Extracts:** The ethanolic and aqueous extract of *Pongamia pinnata* bark was procured from Unico Pharmaceuticals, Ludhiana under the batch no. HC/PG/001/11 and HC/PG/S/ 001/11, respectively.

**Procurement of Animals:** Male albino rats (220-250 gm) were procured from NIPER, Mohali. The animals were fed regularly with diet and water *ad libitum*. The protocol was approved by the Institutional Animals Ethics Committee (954/ac/06/CPCSEA/ 11/1).

**Drugs and Chemicals:** Paracetamol was procured from Frankfinns laboratories, Ludhiana. Silymarin was procured from Hangzhou Oriental Pharma-Tech Co. Ltd., China. The standard kits for ALP (alkaline phosphatase), bilirubin, SGOT (serum glutamate oxalate transaminase), and SGPT (serum glutamate pyruvate transaminase) were purchased from Erba diagnostics Manheim Gmbh.

**Preliminary Phytochemical Screening of Plant Extracts:** The ethanolic and aqueous extracts of *Pongamia pinnata* bark were screened for the presence of various phytoconstituents such as phenols, flavonoids, tannins, saponins, carbohydrates, alkaloids, resins, *etc*. [7, 8]

**Estimation of Total Phenolic and Total Flavonoid content:** The total phenolic content was determined by Folin-Ciocalteu method using tannic acid as an internal standard and was expressed as tannic acid equivalents in milligrams per gram of sample [9, 10]. The flavonoid content was determined by the method of Huo *et al.*, [11] using quercetin dehydrate as internal standard and was expressed as micrograms of quercetin equivalent per mg of sample.

**Evaluation of Hepatoprotective Activity:** Animals were divided into seven groups (n=5). The group I (negative control) and II (positive control) received distilled water (0.5ml) for a week. The group III received silymarin (100 mg/kg p.o.) once a day for a week. The group's IV and V received 400mg/kg and 800 mg/kg of ethanolic extract of *P. pinnata* bark, respectively once a day for a week. The groups VI and VII received 400mg/kg and 800 mg/kg of aqueous extract of *P. pinnata* bark, respectively once a day for a week. On the fifth day, after the administration of the respective treatments, animals of all the groups, except group I, were administered with paracetamol 2 g/kg orally. On the seventh day, two hours after the respective treatment, the blood samples were collected for the analysis of biochemical markers. The animals were sacrificed under ether anesthesia, and liver from all the animals was collected, washed and weighed, and further used for the estimation of GSH levels [12].

1. **Biochemical Parameters (Liver Function Test):** The blood samples were collected by retro-orbital bleeding, centrifuged at 6000 g for 10 min, and serum was separated. The serum was further used for the analysis of biochemical markers SGPT, SGOT, ALP, total bilirubin levels [12].

2. **GSH Level:** The liver sample was homogenized in 10 ml of cold phosphate buffer and centrifuged at 3000 gyrations for 15 min. The GSH level in liver tissue was evaluated using the method of Naskar *et al*. [13] Briefly, the reaction mixture contained 0.1 ml of supernatant, 2.0 ml of 0.3 M phosphate buffer (pH-8.4), 0.4 ml of double distilled water, 0.5 ml of 50% w/v of TCA (trichloroacetic acid) and 0.5 ml of DTNB {dithiobis-(2-nitrobenzoic acid)}. The reaction mixture was incubated for 10 min. and the absorbance was measured at 412 nm.

## RESULTS:

**Preliminary Phytochemical Screening of Plant Extracts:** The phytochemical investigation of both

the extracts revealed the presence of various bioactive phytoconstituents such as alkaloids, saponins, phytosterols, resins, phenols, and flavonoids.

**Estimation of Total Phenolic and Total Flavonoid Content:** The bark of *P. pinnata* was found to contain highest phenolic content in the aqueous extract, *i.e.* 41.875 ± 0.29 µg/mg equivalent of tannic acid than ethanolic extract, *i.e.* 29.37 ± 0.29 µg/mg equivalent of tannic acid. Similarly, the flavonoid content was also found to be highest in the aqueous extract, *i.e.* 37.69 ± 0.023 µg/mg equivalent of quercetin than in the ethanolic extract, *i.e.* 28.46 ± 0.023 µg/mg equivalent of quercetin.

**Evaluation of Hepatoprotective Activity:** In the positive control group, the weight of the liver in rats was significantly increased as compared to the negative control group **Table 1**.

However, pretreatment with silymarin reduced the liver weight to be close to that of the normal. Similarly, ethanolic and aqueous extracts of *P. pinnata* bark also produce a significant reduction in liver weight when compared to the positive control group.

**TABLE 1: EFFECT OF *P. PINNATA* ON LIVER WEIGHT AGAINST PARACETAMOL-INDUCED HEPATO-TOXICITY**

| Groups | Liver weight (gm) | % change |
|---|---|---|
| Control | 6.57 ± 0.14** | 32.40 |
| Paracetamol | 9.72 ± 0.14 | - |
| Silymarin | 7.64 ± 0.14** | 21.3 |
| ETPP400 | 9.18 ± 0.1* | 5.55 |
| ETPP800 | 8.84 ± 0.05** | 9.05 |
| AQPP400 | 8.44 ± 0.10** | 13.16 |
| AQPP800 | 7.32 ± 0.20** | 24.69 |

Values are expressed as Mean ± SEM (n=5); Data were analysed by one-way ANOVA followed by Dunnett's test; ***P*<0.01 and **P*<0.05 *vs*. Group II; ETPP400= ethanolic extract of *P. pinnata* (400 mg/kg); ETPP800= ethanolic extract of *P. pinnata* (800 mg/kg); AQPP400= aqueous extract of *P. pinnata* (400 mg/kg); AQPP800= aqueous extract of *P. pinnata* (800 mg/kg).

**Biochemical Parameters (Liver Function Test):** A significant elevation in the level of liver biomarkers enzymes was observed in the blood serum of rats treated with paracetamol alone as compared to the normal rats **Table 2**. Pretreatment with silymarin, ethanolic, and aqueous extracts of the plant significantly reduced the elevated levels of liver biomarker enzymes in the serum of animals and thus reversed the hepatic damage.

**TABLE 2: EFFECT OF *P. PINNATA* ON LIVER ENZYME BIOMARKERS IN SERUM AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY**

| Groups | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/L) | Total Bilirubin (mg/ml) | Direct Bilirubin (mg/ml) |
|---|---|---|---|---|---|
| Control | 155.8±1.74** | 83.4±1.80** | 139.6±1.99** | 0.65±0.01** | 0.25±0.01** |
| Paracetamol | 328.8±2.01 | 285±1.70 | 348.6±1.60 | 2.83±0.02 | 0.74±0.01 |
| Silymarin | 172.6±0.67** | 117.2±0.96** | 173.2±1.20** | 1.2±0.04** | 0.29±0.02** |
| ETPP400 | 322.6±1.03* | 276±1.88* | 342.0±2.02* | 2.73±0.01* | 0.648±0.01* |
| ETPP800 | 316.0±1.92** | 264.2±1.93** | 335.8±1.65** | 2.54±0.01** | 0.592±0.02** |
| AQPP400 | 214±1.87** | 157.2±1.53** | 234.4±1.47** | 1.61±0.06** | 0.42±0.04** |
| AQPP800 | 193.8±1.11** | 137.4±1.86** | 207.8±1.77** | 1.35±0.02** | 0.38±0.01** |

Values are expressed as Mean±SEM (n=5); Data were analyzed by one-way ANOVA followed by Dunnett's test; ***P*<0.01 and **P*<0.05 *vs*. Group II; SGOT= serum glutamate oxalate transaminase; SGPT= serum glutamate pyruvate transaminase; ALP= alkaline phosphatase; ETPP400= ethanolic extract of *P. pinnata* (400 mg/kg); ETPP800= ethanolic extract of *P. pinnata* (800 mg/kg); AQPP400= aqueous extract of *P. pinnata* (400 mg/kg); AQPP800= aqueous extract of *P. pinnata* (800 mg/kg).

**SH Level:** Paracetamol intoxication significantly reduced the glutathione concentration in the positive control of animals. The animals which were pretreated with silymarin, ETPP800 (ethanolic extract of *P. pinnata*, 800 mg/kg), AQPP400 (aqueous extract of *P. Pinnata*, 400 mg/kg), and AQPP800 (aqueous extract of *P. Pinnata*, 800 mg/kg) showed a significant rise in the enzyme level, however, no significant elevation was observed in ETPP400 (ethanolic extract of *P. pinnata*, 800 mg/kg) pretreated group **Table 3**.

**TABLE 3: EFFECT OF *P. PINNATA* ON HEPATIC GLUTATHIONE LEVEL AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY**

| Groups | Tissue GSH absorbance | % increase |
|---|---|---|
| Control | 0.722 ± 0.007** | - |
| Paracetamol | 0.284 ± 0.009 | - |
| Silymarin | 0.408 ± 0.012** | 43.6 |
| ETPP400 | 0.292 ± 0.009 | 2.74 |
| ETPP800 | 0.328 ± 0.001* | 15.47 |
| AQPP400 | 0.326 ± 0.008* | 14.62 |
| AQPP800 | 0.340 ± 0.006** | 19.54 |

Values are expressed as Mean ± SEM (n=5); Data were analysed by one-way ANOVA followed by Dunnett's test; **$P$<0.01 and *$P$<0.05 *vs*. Group II; GSH= glutathione; ETPP400= ethanolic extract of *P. pinnata* (400 mg/kg); ETPP800= ethanolic extract of *P. pinnata* (800 mg/kg); AQPP400= aqueous extract of *P. pinnata* (400 mg/kg); AQPP800= aqueous extract of *P. pinnata* (800 mg/kg).

**DISCUSSION:** The present work explored the ability of ethanolic extracts of *Pongamia pinnata* bark to exhibit protection against hepatotoxicity induced by paracetamol (an over the counter drug). The liver is a major metabolic organ affected by various chemicals and toxins and liver injuries induced by various hepatotoxins has been recognized as a major toxicological problem for years [14].

Paracetamol is one of the most common drugs used for the treatment of minor to moderate pain in humans. At 300 mg/kg or higher dose, paracetamol leads to severe acute liver necrosis. Firstly, it gets metabolized in the liver to non-reactive metabolites by sulphation and glucuronidation reactions. Further, these primary metabolites get converted into NAPQI, which is known to be hepatotoxic **Fig. 1**.

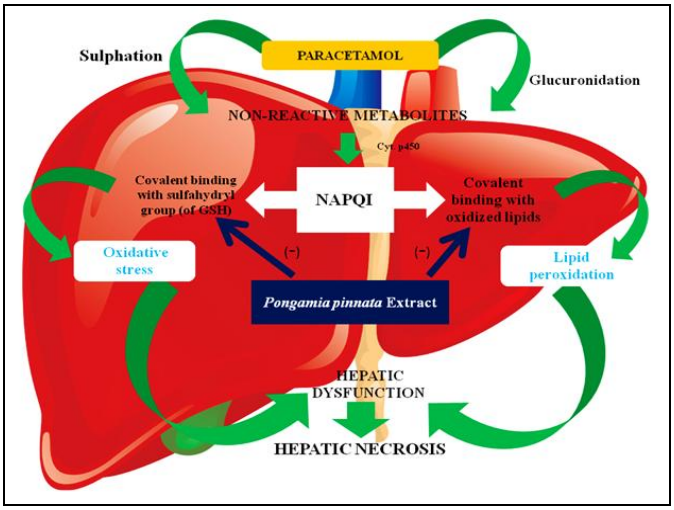


**FIG. 1: MECHANISM OF PARACETAMOL-INDUCED HEPATOTOXICITY**

It covalently binds to oxidized lipids and sulphydryl groups in the liver tissue, leading to severe damage of cell membrane [15]. That is why; paracetamol-induced liver damage is a commonly used the experimental model to evaluate the potency of hepatoprotective agents.

Silymarin is a polyphenolic antioxidant component obtained from *Silybum marianum*. It is used traditionally as an immunostimulant, hepato-protectant, and dietary supplement. Silymarin also has chemopreventive and antineoplastic agent [16]. Its hepatoprotective activity is due to the presence of flavonolignans principal [17]. It possesses strong free radical scavenging activity, inhibits lipid peroxidation, and promotes regeneration of damaged hepatocytes. Also, silymarin inhibits the 5-lipoxygenase pathway and also possesses membrane stabilizing properties. All these factors together contribute to its hepatoprotective activity [18].

One of the important morphological parameters which represent liver intoxication is the change in liver weight of the rats. As shown in the results, paracetamol significantly increases the liver weight of rats. This increase may be due to the accumulation of lipids. The silymarin, as well as both the extracts, inhibited the increase in liver weight, showing their protective effect against liver damage. The liver injury caused by paracetamol overdose leads to the alteration in the transport function of hepatocytes. As a result of which leakage of cellular membrane takes place and the serum enzyme levels are increased [19]. Hence, the biochemical estimation of liver enzymes in the blood of animals is used routinely for the determination of hepatic damage, *e.g.* SGPT, SGOT, ALP, total bilirubin, *etc*.

In this context, a sharp increase in the liver enzymes, in positive control animals, represents paracetamol-induced liver damage. Pretreatment of animals with ethanolic and aqueous extracts resulted in a significant reduction of paracetamol-induced liver marker enzymes in serum, almost comparable to the silymarin. One of the most important intracellular antioxidant enzymes which maintain the cellular proteins and lipids in their functional state is GSH [20]. At lower concentration, NAPQI is detoxified in the liver by GSH to form paracetamol–GSH conjugate [21].

As the concentration of NAPQI increases, GSH, being depleted, can no longer prevent hepatic proteins from getting oxidized by NAPQI [12]. The GSH levels were found to get depleted in the positive control group as compared to the normal control, which is due to the consumption of enzymes during detoxification of reactive oxygen metabolite, *i.e.* NAPQI. Treatment with Silymarin, ETPP800, AQPP400, and AQPP800 significantly elevated the GSH levels.

**CONCLUSION:** It may be concluded that the hepatoprotective effect of *Pongamia pinnata* aqueous and ethanolic extract is because of prevention of the depletion in the tissue GSH levels. Upon literature review, it was found that the bark of *Pongamia pinnata* contains flavonoid and phenolic contents in both the extracts. Therefore, there is a possibility that bark extracts may possess antioxidant property, which may be involved in the hepatoprotective activity. Furthermore, it is necessary to carry-out further studies to rule out if treatment with both the extracts can inhibit oxidation of paracetamol to highly reactive NAPQI.

There is also a need for further isolation of the components present in the plant extracts which are responsible for the hepatoprotective effect to explore the molecular mechanism by which these maintains the integrity of the liver.

**ACKNOWLEDGEMENT:** The authors are thankful to Dr. Monica Gulati, Sr. Dean, Lovely Faculty of Applied Medical Sciences, Lovely Professional University (INDIA) for providing helpful critique and suggestions.

**CONFLICT OF INTEREST:** Nil

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**How to cite this article:**
Kaur H, Kaur M, Singh A and Kumar B: Hepatoprotective potential of aqueous and ethanolic stem-bark extracts of *Pongamia pinnata* against paracetamol induced hepatotoxicity in rats. Int J Pharm Sci & Res 2014; 5(10): 4275-80. doi: 10.13040/IJPSR.0975-8232.5(10). 4275-80.