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Investigation of the Hepatoprotective Properties of *L. coromandelica* Bark Extract Against Paracetamol Induced Hepatotoxicity in Rats

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Abstract

Similar to viral hepatitis in humans, paracetamol causes liver damage. Recent developments indicate that traditional plants have been used to treat hepatotoxicity. The current study has been performed to investigate the effectiveness of *Lannea coromandelica* (LC) bark in reducing the hepatotoxicity caused by paracetamol in rats due to its application in folk medicine. Rats (150–200 g) were split up into 5 groups (n=6). Paracetamol (600 mg/kg body weight) was given once daily for a week, causing acute hepatotoxicity, while the plant extract under investigation was taken orally at 250 and 500 mg/kg body weight for the duration of the experiment. As is customary for hepatoprotective medications, silymarin (100 mg/kg b.w.) was administered orally. Hepatoprotection was ascertained by estimating biochemical indicators such as ALT, AST, ALP, total protein, albumin and globulin level.Treatment with *LC* extracts significantly attenuated the increased plasma levels of ALT ($p<0.05$), AST ($p<0.05$) and ALP ($p<0.001$) level whereas insignificantly improved total protein, albumin and globulin level. The extract insignificantly progressed the body weight and significantly increased the liver weight ($p<0.05$) as compared to the disease control animals. The estimated biomarkers provide evidence that the *Lannea coromandelica* bark extract has shown hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Key words: *Lannea coromandelica*, hepatoprotective activity, paracetamol induced hepatotoxicity, Liver enzymes.

Introduction

The liver is an essential organ that performs a number of vital tasks, including the metabolism, secretion, storage, plasma protein synthesis, hormone production and has regenerative capabilities. It is highly capable of detoxicating poisonous compounds, managing drug metabolism and excretion of waste metabolites from the body (Anthea *et al.,* 1993; Saleem *et al.,* 2010).Numerous xenobiotics have been shown to have hepatotoxic potential (Ajith, Hema and Aswathy, 2007). Several cases of cirrhosis, hepatitis, and suicide attempts have been linked to paracetamol-induced hepatotoxicity (Avila *et al.,* 2011). A sudden or prolonged overdose of the analgesic medication paracetamol can cause serious liver damage (Lee, 2004). Approximately 160 phytoconstituents from 101 plants have been shown to exhibit hepatoprotective properties (Chatterjee, 2000). Thus, the global market has placed emphasis on the development of hepatoprotective medications derived from plants (Kumar, 2012). Many indigenous medicinal plant species in Bangladesh have a long history of use and have significant phytotherapeutic potential (Karunakar, 2009) and therefore, research

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on medicinal plants is essential to hunt for new and promising medications (Mia and Ghani, 1990).

Lannea coromandelica L. often referred to as the "Indian Ash tree" or "Jiga" in Bengali, is a medium-sized deciduous tree which belongs to the Anacardiaceae family and is primarily found in Bangladesh, India, and Africa. It grows up to 25 meters tall, with a rough, grey to dark brown bark surface that exfoliates in tiny, irregular flakes and is fibrous. Its young parts are stellate-rusty tomentose, and its exudation is sticky and red (Jackson, 1994; The Plant List,2010 and Jain, 1991).It has phenolics, glycosides, alkaloids, flavonoids, and carbohydrates. Polyphenols, which include tannins like gallic and ellagic acids, make up the majority of themtogether with flavonoids such as isoquercetin, kaempferol, and quercetin. Furthermore, it has some sterols in it (Sankara Subramanian and Nair, 1971; Reddy, Joy and Kumar, 2011). According to reports, *L. coromandelica* has anti-inflammatory properties (Saravanan *et al.,* 2010), anti-microbial, wound healing (Sathish *et al.,* 2010), hypotensive and aphrodisiac character (Singh, and Singh, 1996), ulcerative stomatitis, dyspepsia, general debility, gout, cholera, diarrhoea, dysentery (Jain and Tarafder, 1970), sore eyes, leprosy, sprains, bruises (Kirtikar and Basu, 2000), elephantiasis (Shah, Yadav and Badri, 1983). Furthermore, the plant gum is utilized in sprains, asthma and as a cordial to women during lactation (Kunte and Shastri, 2007; Anonymous, 1987). It is also used in the treatment of coma caused by narcotics, general debility, and impotency (Jain, 1991).

The present work aims to investigate the hepatoprotective potential of the ethanolic extract of *L. coromandelica* bark against acetaminophen-induced liver damage in rat model.

Materials and Methods

Plant collection and Extraction: The bark of the plant *Lannea coromandelica* (LC) was gathered from the Savar region, and the Department of Botany at Jahangirnagar University in Savar, Dhaka, recognized and verified it. After being completely cleaned with water, the materials were divided into smaller pieces, allowed to dry at 35 to 40°C for a week, and then ground into an extractable powder. Then ethanol was used to extract the powder in a Soxhlet system. Following solvent washing, the powder's contents were gathered in a container and dried using a rotary evaporator at lower pressure to produce a viscous product. When a solid mass was finally achieved, it was stored in a petridis in the refrigerator.

Chemicals and Reagents: Acetaminophen (AAP) was obtained from Gonoshaysthaya Pharmaceutical Ltd., Assay kits for ALT, AST, ALP and total bilirubin were obtained from HUMAN GmbH (Germany). All additional compounds and reagents used in this work were made using distilled water and were of analytical quality.

Experimental animals: Both sexes of Sprague Dawley rats weighing 150–200g were gathered for the experiment from the animal research lab, Department of Pharmacy at Jahangirnagar University, Savar, Dhaka. The animals were kept under conventional circumstances, which included a temperature of 27.0±1.0°, a relative humidity of 55–65%, and a 12-hour light/12-hour dark cycle. They also had unrestricted access to food and drink. Before the studies began, the animals were given a week to get used to the conditions in the lab. The institutional animal ethics committee gave its approval to all animal experimentation protocols.

Toxicity studies: Swiss Albino mice of both sexes weighing 20–25 g were used for acute toxicity tests of the extract. It was discovered that the extract was safe up to 4000 mg/kg p.o. Consequently, dosages of 250 mg/kg and 500 mg/kg b.w. were chosen for further study (Kim *et al.,* 2006)

Experimental design for the assessment of liver functions: The rats were separated into five groups of six individuals (n = 6) and kept in polypropylene cages at room temperature (27±2ºC). The study design was as given below:

Group I: received water (10 mL/kg p.o.) once daily for 7 days, and served as normal control

Group II: received water (10 mL/kg p.o.) and paracetamol 600 mg/kg, p.o. once daily for 7 days and served as negative control.

Group III: received standard drug silymarin (25 mg/kg p.o.) and paracetamol 600 mg/kg, p.o. once daily for 7 days, serving as STD (positive control).

Group IV and V: received *Lannea coromandelica* (LC) bark extract (250 and 500 mg/kg respectively) and paracetamol 600 mg/kg p.o. once daily for 7 days (Bhattacharya *et al.*, 2003).

Rats were put to sleep with ketamine (500 mg/kg I.P.). Following sacrifice, each set of rats' blood samples were taken, centrifugation was used to separate the serum and estimation of biomarkers was conducted (Lowry *et al.*, 1951; Yoshiyuki *et al.*, 1981; Kalyani *et al.*, 2010).

Statistical Analysis: SPSS 16.0 for Windows was used to do statistical analysis for animal studies using one-way ANOVA after Dunnet's post hoc test. The data was displayed as Mean±SEM (n=6). A comparison was made between the outcomes of test extract and the vehicle control group. The following p-values were deemed statistically significant, highly significant, and very highly significant, respectively: $p<0.05$, $p<0.01$ and $p<0.001$.

Results and Discussion

The present study demonstrated, the LC extract decreased the ALT and AST level insignificantly at 250 mg/kg whereas significantly ($p<0.05$) at 500 mg/kg dose (figure 1 & 2). It also significantly lowered the ALP level at 250 mg/kg ($p<0.01$) and 500 mg/kg dose ($p<0.001$) as compared to the corresponding disease control group (figure 3).

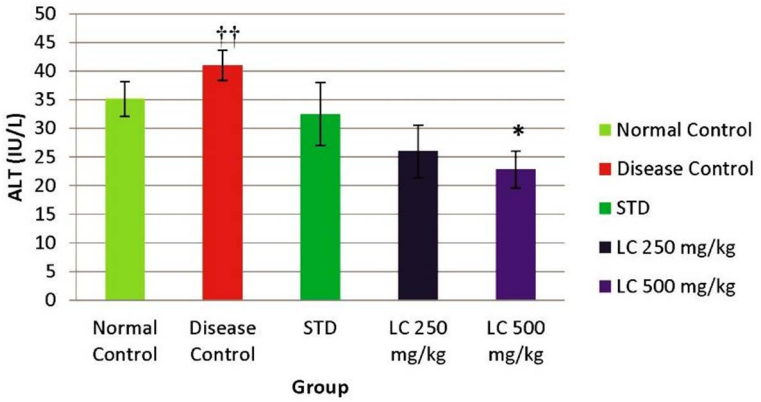

Figure 1. Effect of *L.coromandelica* bark on plasma ALT level in hepatotoxic rats

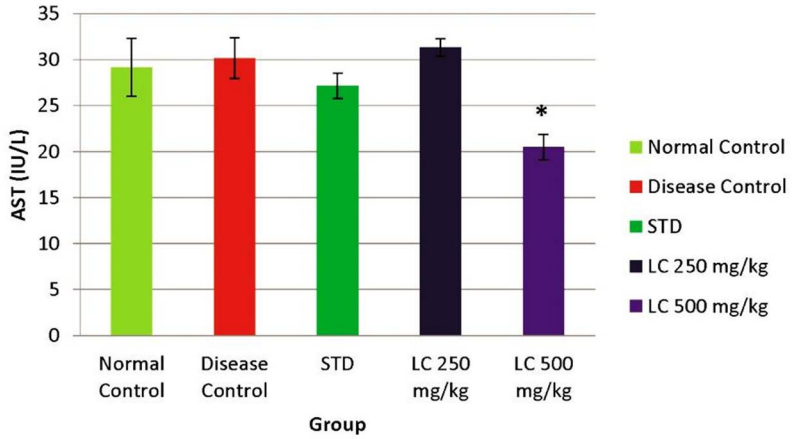

Figure 2. Effect of *L.coromandelica* bark on AST level in paracetamol-induced hepatotoxic rats

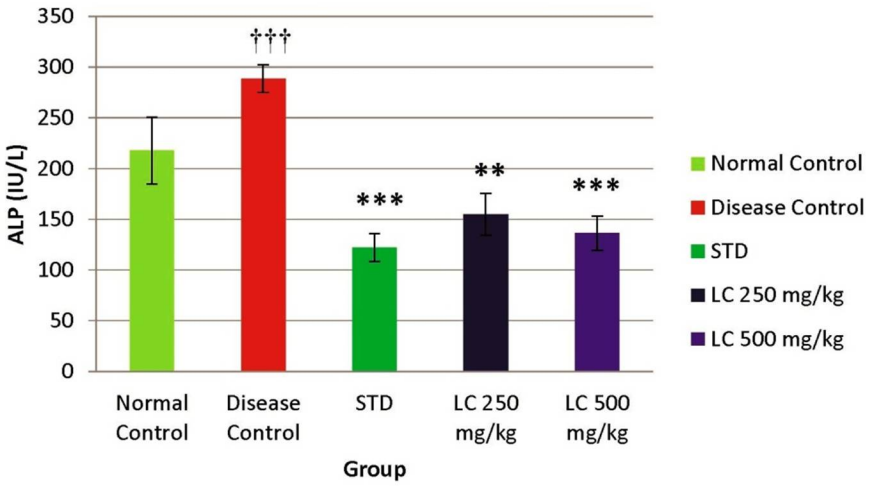

Figure 3. Effect of *L.coromandelica* bark on ALP level in paracetamol-induced hepatotoxic rats

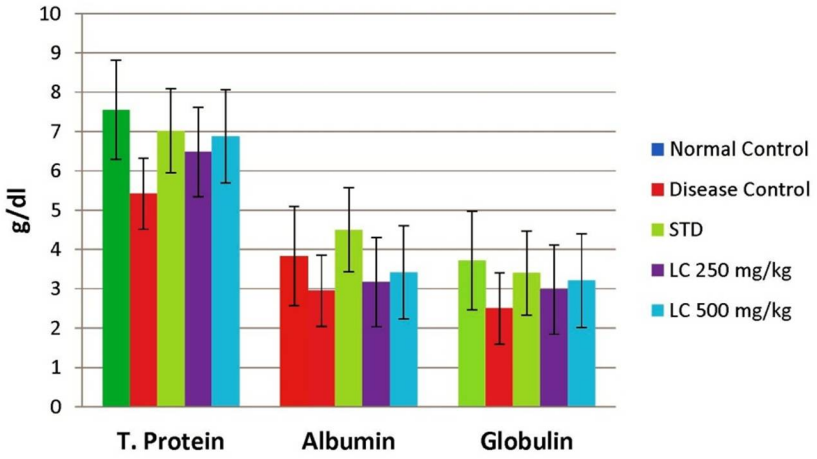

Figure 4. Effect of *L.coromandelica* bark on total protein, albumin and globulin level

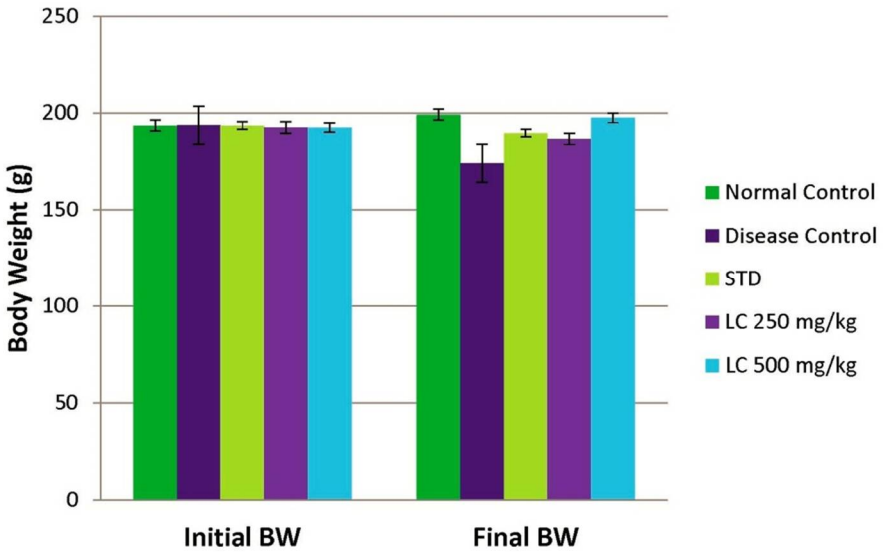

Figure 5. Effect of *L. coromandelica* bark on the body weight in hepatotoxic rats

The results indicated that after 7 days treatment with the test extract, it could not increase the total protein, albumin and globulin level significantly at any dose (250 mg/kg & 500 mg/kg b.w.) (figure 4). After the study period, treatment with *L. coromandelica* extract in hepatoxic rats for 7 days improved the body weight as well as the weight of injured liver of rats as compared to the corresponding disease control animals (figure 5 & 6) which was insignificant.

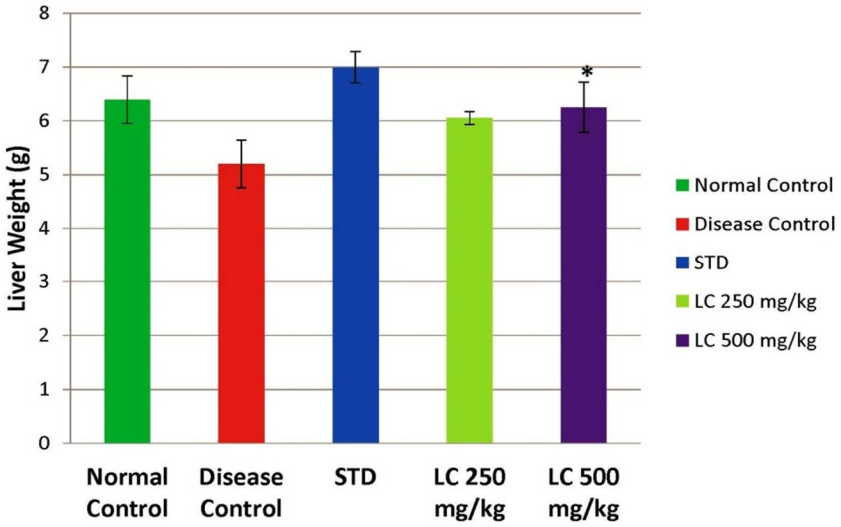

Figure 6. Effect of *L. coromandelica* bark on the liver weight in paracetamol-induced hepatotoxicity in rats

Acetaminophen (AAP), a commonly used analgesic, can result in hepatic necrosis when taken in excess because it forms NAPQI, which lowers GSH levels in the liver. More NAPQI will bind covalently to cellular macromolecules at large dosages (Black, 1980; Pacifici, Back and Orme, 1988; Jollow *et al.*, 1973; Potter and Hinson, 1986). Since enzymes have tissue selectivity and catalytic activity, they are discharged into the bloodstream along with other macromolecules that leak from injured tissues (Hearse, 1979). Furthermore it has been discovered that examining these enzyme activity in the blood is crucial for determining the extent of liver damage (Plaa and Zimmerman, 1997).

The elevated ALT and AST levels in the liver are a sign of cellular damage and a breakdown in the functional integrity of the cell membrane (Drotman and Lowhorn, 1978). The pathogenic change in biliary flow is reflected in the elevated ALP in liver disease, which is caused by increased manufacture of the enzyme by cells lining the canaliculi, typically either intra- or extrahepatic (Plaa and Hewitt, 1989).

This investigation showed that the LC extract had a strong effect on lowering the levels of ALT, AST, and ALP. LC extract appeared to provide protection against liver damage and preserve the functional integrity of hepatic cells by lowering the elevated levels of serum ALT, AST, and ALP.

The liver produces the majority of the proteins in plasma, with immunoglobulins being the main exception. Reduced production of different proteins has been linked to severe liver damage, which lowers serum levels of albumin, globulin, and/or total protein (Alper, 1974; Killingsworth, 1981). Other test results may be abnormal due to decreased protein synthesis. For instance, prolonged prothrombin or activated partial thromboplastin durations may arise from the depletion of coagulation factors, all of which are globulins (Badylak and Van Vleet, 1981). Additionally, elevated protein loss through urine or feces as a result of gastrointestinal or renal disorders will lower serum protein levels (Alper, 1974).

The findings show that both the 250 mg/kg and 500 mg/kg doses resulted in a small rise in protein levels. In this study, it was discovered that giving rats

LC for seven days increased both their body weight and liver weight, suggesting that LC extract can repair liver damage brought on by acetaminophen.

Conclusion

Based on the above experimental outcomes, the *Lannea coromandelica* bark extract has significant potential to protect the hepatic damages induced by paracetamol. Additional research is recommended to elucidate the potential hepatoprotective mechanisms with the isolation of bioactive molecules responsible.

Conflict of interest

Authors declared no conflicts of interest.

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