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RESEARCH ARTICLE

Assessment of Anti-Hepatotoxic Effect of *Bahuinia tomentosa* Linn against Paracetamol induced Hepatocellular Damage In Albino Mice

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ABSTRACT:

In today's modern world the chemical induced hepatotoxicity is one huge threat to human life, even the drugs which have easy accessibility and availability are also produces side effects, when they are used irrationally, so the need for antidote from herbal industry is a common factor. *Bauhinia tomentosa* Linn belongs to fabaceae, considered as one such potential agent which constitutes wide range of chemical compounds which has therapeutic as well as antidote effect. In this study *Bahuinia tomentosa* Linn was extracted with ethyl alcohol and the prepared ethanolic extract was evaluated for its hepato protective effect against Acetaminophen induced hepato toxicity in albino mice. The biochemical estimation, histo pathological studies are served as index for the assessment of hepatoprotective activity. Modification in body and liver weight, proteins, levels of biomarkers, antioxidant enzymes along with histopatological variations of extract treated groups were compared with standard hepatoprotective drug silymarin. Marked hepatoprotective activity was noticed in extract treated groups in dose dependent manner. The study results revealed the antihepatato toxic effect of *Bauhinia tomentosa* Linn and recommended as an excellent natural source of drug in the treatment of acetaminophen induced hepatotoxicity.

KEYWORDS: *Bahuinia tomentosa*, Hepatoprotective, Antihepatotoxic effect, Paracetamol toxicity, Histopathology.

INTRODUCTION:

Hepatotoxicity is a major pharmacological defectcaused by the drugs has been an important reason for the withdrawal and banning of many drugs in the market. Liverunique organ play a major role in the metabolism and biotransformation of xenobiotics and food substances that enter the body[1]. A significant amount of deaths occur due to diseases aggravated by the toxic action of therapeutic substances prescribed by allopathic system on the liver. This situation warrants us to find out remedies for hepatotoxicity.

Herbal remedies are safe and effective in restoration process with no or less side effects. The vast diversity of plant kingdom encourages for herbal research and the medicinal properties of many plants are yet to be explored. Efficacy testing of the traditional / new herbal products by experimental screening method is an important tool to establish standard therapeutic profile. However, there should be adequate data from *in vivo* and *in vitro* studies to validate the claimed therapeutic potential of testing substance. A reliable model to find out hepatoprotective efficacy of herbal preparations is paracetamol induced hepatotoxicity evaluation in albino mice as per earlier literatures[2-5].

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Paracetamol or Acetaminophen, over-the-counter drug commonly used for the relief of fever and pains, impair liver at lethal dose. Paracetamol is considered a safe drug with analgesic and antipyretic activity until it overdosed[6]. The hepatotoxicity associated with paracetamol is mainly due to excessive accumulation of its toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which oxidizes liver tissue macromolecules such as lipid or -SH group of protein causing oxidative stress and hepatic necrosis[7]. The extensive use of paracetamol/ Toxic dose cause fatal hepatic damage like liver fibrosis and cirrhosis which may leads to death[8,9]. Paracetamol doses exceeding 150 mg/kg are considered hepatotoxic.Acetaminophen had well documented toxicity profile. So, Paracetamol induced hepato toxicity in mice is considered as one of the reliable *in-vivo* model to project the hepatoprotive activity of any targeted crude extract or synthesized or derived drug moieties.

In Indian system of medicine reported that *Bauhinia* species used to prevent/cure diseases and alleviate many disorders successfully. *Bauhinia tomentosa* Linn commonly called as yellow bell orchid tree belongs to the Fabaceae family[10]. The medicinal properties of this plant species may be due to the presence of highly rich therapeutic phytochemicals under the classes of tannins, flavonoids, terpenoids and steroids. Many research studies have been done on *Bauhinia* species including paracetamol induced hepatotoxicity. *Bauhinia tomentosa* Linn used for diverse ailments including antibacterial, antioxidant, antifungal, anti cancer, anti inflammatory, hypo glycemic and anti lipidemic[11-17]. Hence, in order to contribute further to the knowledge of Indian traditional medicine, and its rich history, the present study is involved in assessment of hepatoprotective effect of the traditionally well-known Bauhinia species, *Bauhinia tomentosa* Linn. in Paracetamol induced hepatotoxicity model.

MATERIALS AND METHODS:

The leaves of *Bauhinia tomentosa* L. were collected locally from Tamilnadu, India. Identification of the plant specimen was done by Dr.G.V.S.Murthy, Scientist and Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore and a voucher specimen (BSI/SRC/5/28/2015-16/Tech/1934) was deposited for future reference. The collected leaves were gently washed with tap water to remove dirt and dried under shade for a period of 2 weeks. Then, they were pulverized into coarse powder by using mortar and pestle.

Chemicals and Reagents:

Paracetamol was obtained from Sigma Aldrich Pvt. Ltd. Silymarin and ethanol was obtained from Hi-media Pvt. Ltd.

Pretreatmentof plant material:

The air dried powdered material (100g) was extracted with petroleum ether for seven days to remove fatty material.

Preparation of Plant Extract:

After the completion of pre treatment, the marc was dried and subjected to soxhletion with absolute ethanol (99%) until the colour faded. The obtained extract was concentrated, dried and utilised for experiment[18].

Experimental animals:

Healthy Swiss albino mice of either sex (female for acute toxicity and male for the main study) with the age of 4-5 weeks and having a weight range of 28-36 g inbred in the animal house of the JKK Nattaraja College of Pharmacy, Kumarapalayam (Ref.No:JKKNCP/B.C./ 0114F16) were used for the experiment. The mice were housed in polypropylene cages (6 mice per cage) under standard environmental conditions and 12 h-12 h light-dark cycle. The animals were allowed free access to tap water and laboratory pellet and acclimatized to laboratory conditions for 1 week before the experiment.

Acute toxicity studies:

Doses were selected and determined according to the acute toxicity test reported previously. The dose of 2 g/kg was well tolerated without any signs of toxicity and mortality. So we presumed that LD50 was beyond the dose of 2g/kg bw. Two different graded doses 200 mg/kg and 400 mg/kg bw, were selected for test of hepatoprotective activity[19].

Experimental Design For Hepatoprotective Activity:

Table 1. Treatment groupings of the experimental subjects employed in the study

Groups	Treatment
I	Receives (Distilled water) as control for 14 days
II	Receives a daily dose of Paracetamol (3g/ Kg of body weight, p.o) for 14 days (p.o)
III	Receives a daily dose of Paracetamol (3g/ Kg of body weight) and after one hour a daily dosage of Standard Silymarin (100mg/kg) of body wieght for 14 days (p.o)
IV	Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of EEBT 200mg / Kg of body weight for 14 days (p.o)
V	Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of EEBT 400mg / Kg of body weight for 14 days (p.o).

At the end of the 14th day treatment, the blood samples were withdrawn from the retro orbital sinus after fasting for 16 hours. After the withdrawal blood, all animals were sacrificed and their livers were isolated and washed with ice cold normal saline followed by with 0.1 M Tris-HCl buffer (pH 7.4) and stored. The blood samples were subjected in to various experimental procedures to find its hepatoprotective activity based on earlier literatures[20-23].

Estimation of enzyme levels in serum:
The serum was extracted from blood samples by centrifugation process (10000 rpm in 10 minutes) and subjected into evaluation of biochemical parameters like SGOT, SGPT and ALP according to previous literature procedures[24-25].

Estimation of Bilirubin in serum:
The Bilirubin level was estimated in serum based on previous literatures[26].

Preparation of tissue homogenate:
The liver homogenate (10%) was prepared by homogenizing the stored livers with 0.1 M Tris-HCl buffers (pH 7.4). The homogenate was centrifuged at 10000rpm for 10 min at 5˚C. The supernatant was collected.

Estimation of antioxidant enzymes:
The bio chemical parameters like superoxide dismutase SOD, Catalase and glutathione peroxidase as estimated by standard methods[27,28].

Estimation of lipid peroxides:
The Lipid peroxidase (LPO) was estimated based on the standard procedures[29].

HistophatologicalStudy:
By paraffin slicing techniques, the Liver tissue sections of thickness of 3 to 5mm were implanted in paraffin blocks after staining procedure using Hematoxylin and 0.5% Eosin stains followed by washing process wihxylol. The stained microscopic slides were mounted and examined under the light microscope and well photographed[30].

Statistical Analysis: Results were expressed as Mean ± S.E.M. The statistical difference between the groups was calculated in terms of one-way analysis of variance (ANOVA) followed by Dunnett's test. The statistical significance criterion was P<0.05 (95% level). P<0.05 is considered as significant.

RESULTS:

Hepatoprotective activity:

Body Weight:
A gradual elevation of body weight and marked reduction of relative liver weight showed in Table. 2. The high dose and low dose EEBT treated groups were compared to the standard drug silymarinand the results are significant ($^{*}P< 0.001$ to $^{**}P < 0.01$).

Table 2: Effect of EEBT on Body Weight in Paracetamol-induced mice.

Group	Initial Body Weight	Final Body Weight	Liver Weight
I	23.67±0.236	37.333±1.429	0.721±0.236
II	33.83±0.543	16.166±5.179*	1.522±0.524
III	28.00±0.837	16.166±7.231	1.033±0.463
IV	29.50±0.671	16.000±7.197	0.857±0.385**
V	31.83±0.910	22.333±7.214	1.188±0.398*

ns- no significant $^{*}P< 0.001$, $^{**}P < 0.01$, $^{***}P < 0.05$ calculate by comparing treated group with control group.

Total, Direct bilirubin and Total Protein Levels:
The levels of total and unconjugated bilirubin along with protein levels were showed in Table. 3. A significant reduction of Bilirubin was observed in Group III, IV and V pointing to hepato protective activity. A slight elevation in the level of total proteins was observed in Group IV and V compared to Group III ($^{*}P< 0.001$).

Table 3: Effect of EEBT on activities of Total, Direct bilirubin and Total Proteinin Paracetamol-induced mice.

Group	Total Bilirubin	DirectBilirubin	Total Protein
I	0.958±0.456	0.897±0.237	8.90±0.153
II	0.660±0.299	$0.970±0.012^{ns}$	8.60±0.404
III	0.503±0.232	$0.347±0.064^{ns}$	8.20±1.02
IV	0.440±0.207	0.240±0.021*	9.87±1.81
V	0.328±0.206	0.243±0.086*	9.03±0.463

ns- no significant $^{*}P< 0.001$, $^{**}P < 0.01$, $^{***}P < 0.05$ calculate by comparing treated group with control group.

Liver Function test:
The values of serum liver biomarkers were shown in Table 4. In cirrhosis or necrosis condition there is a increased amounts of bio markers like SGOT, SGPT, ALP in the blood. Group V showed significant protection from liver damage by registering a lower level of biomarkers suggesting the hepatoprotective nature of EEBT. A marked elevation LDH was noted. SOD and Catalyse are key enzymes in free radical protection, increases significantly in the liver tissue of group II suggesting that products of free radical reactions are involved in pathogenesis. A significant decrease in Group IV and V shows that the hepatoprotective activity of *Bauhinia tomentosa* Linn. is comparable to Group IIIand the results are significant ($^{*}P< 0.001$ to $^{**}P < 0.01$).

Table 4: Effect of EEBT on activities of serum marker enzymes and LDH content in Paracetamol-induced mice expressed in (U/L).

GROUP	SGOT	SGPT	ALP	LDH
I	334.5±45.87	61.17±19.92	299.37±54.57	2426±325.6
II	349.7±89.96	42.93±19.61	336.3±27.89	1808±325.6
III	271.3±44.92*	34.93±4.313**	356.3±35.41	3966±682.9*
IV	186.9±60.03**	52.30±22.27	325.9±29.27*	1636±65.86**
V	160.4±3.467**	31.67±2.00**	255.8±79.96**	4158±698.5*

ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with control group.

Table 5: Effect of EEBT on activities of Non enzymatic and enzymatic Antioxidant in Paracetamol-induced mice expressed in (U/L).

GROUP	GPX	LPO	SOD	CATALASE
I	0.102±0.047	0.028±0.013	0.107±0.047	0.114±0.053
II	0.346±0.155	0.049±0.020	0.346±0.155	0.393±0.160
III	0.044±0.019	0.032±0.015	0.044±0.019**	0.174±0.071*
IV	0.036±0.017**	0.038±0.017	0.036±0.017**	0.214±0.088**
V	0.040±0.019**	0.035±0.016*	0.147±0.068*	0.166±0.068**

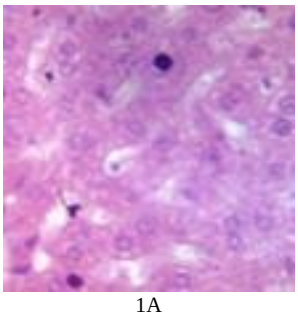
ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with control group.
Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's t test.

Antioxidant enzymes:

The quantity of anti oxidant enzymes, LPO and GPX were shown in Table 5. The level of glutathione peroxidase was reduced in Group IV and V but not as well as the standard drug. On the contrary, LPO levels were increased in Group IV and V reveal protect antihepato toxic activity like standard drug, Silymarin (*P< 0.001 to**P < 0.01).

Histopathological Examinations:

Histopathological findings revealed that the administration of paracetamol resulted in necrosis of hepatocytes as well as deposition of fats in the tissues when compared with controls, but the severity was reduced in those groups of animals pretreated with 100 mg/kg of silymarin, 400 mg/kg and 200 mg/kg of the ethanolic extract of *Bauhinia tomentosa* Linn. This is a significant find in the evaluation of hepatoprotective activity of *Bauhinia tomentosa* Linn.



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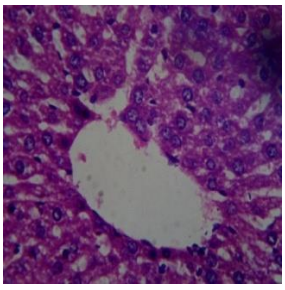

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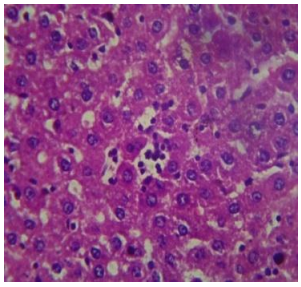

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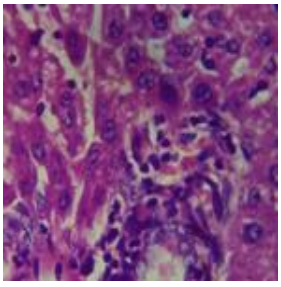

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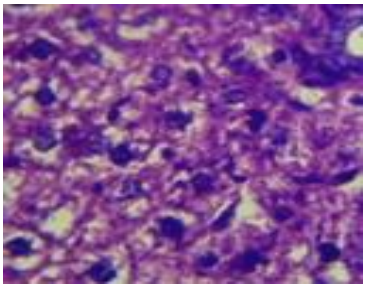

5A

Figure 1: Histological appearance of liver sections in anti hepato toxicity evaluation (H&E stain (40X)): 1A- Control, 2A- Group II - Paracetamol treated group, 3A- Group III- Paracetamol and Silymarin treated group, 4A- Group IV- Paracetamol and Low dose EEBT treated group, Group V- Paracetamol and High dose EEBT treated group

DISCUSSION:

Most of the drugs are safe at therapeutic doses but they produce lethal damage to liver if it is overdosed or in long term usage. Liver is sole target organ for all xenobiotics results in liver necrosis/cirrohsis. Medicinal plants described in traditional medicinal practices are possessed various pharmacological activities including hepatoprotective activity[31,32]. The hepatoprotective activity of medicinal plants are all the way through complicate multiple pathway mechanisms includes enhancement of antioxidant defense mechanisms (superoxide dismutase, catalase and glutathione peroxidase activity), reduced lipid peroxidation, reversed hepatic fibrosis via enhancement of the expression of matrix metalloproteinase and removal of collagen deposits, with attenuation of hepatic stellate cells activation[33,34].

The records of traditional medicine portrayed the diverse biological properties of *Bauhinia tomentosa* Linn which initiated to evaluate its anti hepato toxic activity against paracetamol induced hepatoxicity in albino mice. Paracetamol, a familiar non steroidal analgesic and antipyretic drug widely used to treat cold, fever and pain

cause liver cirrohsis and necrosis at lethal dose[35,36]. Hepatotoxicity with paracetamol is due to its highly reactive metabolite, NAPQI. Increase in NAPQI quantity leads to glutathione depletion, which finally causes an alteration in homeostasis, an increase in the permeability of the cell membrane with a consequent cellular swelling, karyolysis, and vacuolization of hepatocytes and an elevation of liver enzymes[37,38]. Evaulation of liver weight, levels of serum enzymes, antioxidant enzymes and histological studies were done to analyse hepatoprotective properties of this plant[39]. In the study, the lower dose and a high dose of ethanolic extract of *Bauhinia tomentosa* Linn EEBT was compared with the standard hepato protective drug, Silymarin.

The abnormality in the size of liver, levels of serum enzymes, range of total protein and bilirubin are occurs during hepatic damage caused by lethal dose of acetaminophen[40].

The water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass during the liver injury[41]. A marked reduction in liver weight was noted in EEBT and silymarin treated groups.

The abnormal increase in the levels of serum bilirubin was noted in hepatobiliary disease condition[42]. Decreased serum bilirubin level in Group IV and V indicated the anti hepato toxic effect of the *Bauhinia tomentosa* Linn.

The serum protein synthesis was depleted due to decreased number of hepatocytes in cirrohsis condition. Decreased levels of protein illustrate the hepatopathy[43]. Silymarin and EEBT refurbish the hepatocytes and increased the total protein level in Group III, IV and V.

If liver cell membrane is damaged, the liver enzymes like SGOT, SGPT, LDH, GPX and ALP were leaked and entered into the blood circulation. The enzyme activities will increase in the bloodstream and could be indicator for the hepatic damage. Estimation of serum liver enzymes is a parameter for detecting the liver cell necrosis[44]. Silymarin and EEBT reverse the leakaging process by membrane stabilizing activity. The marked reduction of Serum enzymes in Group IV and V revealed the hepato protective nature of *Bauhinia tomentosa* Linn.

In the enzymatic antioxidant defense system, SOD and CAT are main enzymes. The depletion of intracellular antioxidant defenses, leading to an imbalance in the redox status of the hepatic cells and estimation of the level of antioxidant enzymes is also index for liver function assessment[45,46]. In non enzymatic defence system, GPx and LPO are markers to asses hepato toxicity[47]. The GPx level was elevated and LPO activity was reduced in the extract treated groups IV and V compared with Group III and the results were significant.

The biochemical findings were confirmed by histological examinations of liver tissue. Histological sections of liver showed that centrilobular necrosis, the pathogenomonic feature of hepatotoxicity, which appeared in paracetamol-intoxicated mice, was remarkably reduced in siymarin and EEBT treated groups. The lesions evoked by paracetamol was considerably decreased by EEBT indicating its possible anti hepatotoxic action. So, histopathological findings were illustrated the hepatoprotective nature of *Bauhinia tomentosa* Linn which supports the earlier biochemical results.

Above biochemical and histological findings are evident for hepatoprotective nature of *Bauhinia tomentosa* Linn.

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