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BIOCHEMICAL STUDIES ON HEPATO AND NEPHROPROTECTIVE EFFECT OF BUTTERFLY TREE (*BAUHINIA PURPUREA* LINN.) AGAINST ACETAMINOPHEN INDUCED TOXICITY

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ABSTRACT
The present study was carried out to evaluate the hepato and nephroprotective activity of ethanolic extract of stem bark of *Bauhinia purpurea* against paracetamol induced toxicity in rats. 100, 200 and 400 mg/kg, Oral doses of ethanolic extract of stem bark of *Bauhinia purpurea* was administered to group of animals for 14 days. Silymarin (25 mg/kg) served as a standard and paracetamol suspension at a dose of 750 mg/kg, Body weight, was used to induce liver and kidney damage. Parameters of study were glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), bilirubin, triglycerides and total protein as liver function tests, blood urea nitrogen (BUN), creatinine and urea as kidney function tests. Biochemical studies showed increase in the levels of serum GOT, GPT, ALP, total bilirubin, triglycerides, BUN, creatinine and urea and reduction in the levels of total protein in paracetamol induced groups. These values are retrieved significantly ($p< 0.05$) in a dose dependant manner by treatment with ethanolic extracts of *Bauhinia purpurea* stem bark at three different doses. The overall result suggests that the ethanolic extract of stem bark of *Bauhinia purpurea* possesses hepato and nephroprotective activity against paracetamol induced toxicity.

Keywords: Acetaminophen, Hepatotoxicity, Nephrotoxicity, *Bauhinia purpurea*.

INTRODUCTION

Most human beings and indeed many other animals are exposed to drugs soon or later, in their lives. Over dosage with drugs is now one of the commonest means of committing suicide[1]. Many xenobiotics are capable of causing some degree of liver injury. The liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism, its portal location within the circulation, and its anatomic and physiologic structure. The kidney is highly susceptible to toxicants for two reasons. A high volume of blood flows through it and it filters large amounts of toxins which can concentrate in the kidney tubules[2]. Acetaminophen (Paracetamol) is an effective analgesic - antipyretic drug which is often used to treat pain and fever. Acetaminophen is available without prescription in many parts of the world. The most serious adverse effect of acute overdose of acetaminophen is dose-dependent, potentially fatal hepatic necrosis which may be associated with renal tubular necrosis[3]. Previous evidence suggests that oxidative stress with increased generation of reactive oxygen species, depletion of reduced glutathione (GSH) and lipid peroxidation play a crucial role in the development of acetaminophen-induced hepatic and renal damage[4]. Although there is ample evidence to indicate that N-acetyl Cysteine (NAC) treatment can prevent or reverse acetaminophen- induced hepatocellular damage in humans if administered within 12 hours after overdose, its therapeutic application is not without adverse effects. For some clinical cases, administration of NAC reversed liver damage, but not kidney damage[5]. Studies are going on throughout the world for the search of protective molecules that would provide maximum protection of the liver, kidney as well as other organs and practically very little or no side effects would be exerted during their function in the body. A number of herbs are traditionally used in different countries during drug or toxin induced hepatic and renal disorders[6].

The plant *Bauhinia purpurea* L. (Leguminosae) is a medium-sized deciduous tree, sparingly grown in India. Traditionally this plant is used in the treatment of dropsy, pain, rheumatism, convulsion, delirium and septicemia etc[7]. *Bauhinia purpurea* contain major class of secondary metabolites are glycosides, flavonoids, saponins, triterpenoids, phenolic compounds, oxepins, fatty acids and phytosterols[8]. Flavonoids generally have been shown to protect against various forms of disorders such as coronary heart diseases, liver and kidney disorders[9]. Previous studies indicated the antioxidant activity of ethanolic extract of *Bauhinia purpurea* leaves[7]. Hence, the present study is undertaken to investigate whether the ethanolic extract of stem bark of *Bauhinia purpurea* possess hepato and nephroprotective activity against acetaminophen induced hepato and nephrotoxicity in rats.

MATERIALS AND METHODS

Collection and identification of the plant materials

The stem bark of *Bauhinia purpurea* L. were collected from Paneer, Deralakatte, Karnataka, India during June-July 2011 and its botanical identity was confirmed by Dr. Noeline J. Pinto Head of Botany Department St. Agnes College, Mangalore. A voucher specimen (Voucher no.109) is deposited for future reference.

Preparation of extract

The collected stem bark of *Bauhinia purpurea* L. was dried under shade and powdered with a mechanical grinder to obtain a coarse powder. About 200 gm of powder was taken in the soxhlet extractor and extracted with ethanol (60-80ºC) for 72 hrs. The solvent was filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator and the residue (9.7% w/w yield) was stored in dessicator. Stem bark extract due to

its sticky constituency was suspended in 0.4% sodium CMC for oral administration.

Preliminary phytochemical screening

The phytochemical examination of the *Bauhinia purpurea* L. extract was performed by the standard methods[10, 11].

Drugs and Chemicals

Paracetamol was obtained as gift sample from Strides Arcolab Ltd, Bangalore and Silymarin was obtained as gift sample from Micro labs, Bangalore. All other reagents used for the experiments were of high analytical grade.

Experimental animals

Laboratory bred adult albino wistar rats of either sex weighing between 150-200 g were selected for the study. The animals were maintained under standard laboratory condition at 25±2°C, relative humidity 50±15°C and normal 12:12 hour's light/dark cycle. The animals were given standard rat pellets and tap water *ad libitum*, but they were deprived of food 36 h before the experiments. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. The experiment protocol has been approved by the Institutional Animal Ethics Committee (KSHEMA/AEC/11/2011). All procedures involving laboratory animal use were in accordance to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity test (LD_{50})

The acute toxic study was used to determine a safe dose for the ethanolic extract of stem bark of *Bauhinia purpurea* L. Eighteen wistar albino rats (9 males and 9 females) were assigned equally each into 3 groups labelled as vehicle (CMC, 0.25% w/v, 5 ml/kg), 1 and 2 g/kg of *Bauhinia purpurea* L. stem bark extract preparation, respectively. The animals were fasted overnight (water but not food) prior to dosing. Food was withheld for a further 3 to 4 h after dosing. The animals were observed for 30 min, 2, 4, 8, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms. Mortality, if any was observed over a period of 2 weeks. The acute toxicity LD_{50} was calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all.

Experimental design

Animals were divided into six groups of six animals each.

Group I served as vehicle control and treated with normal saline (1 ml/kg, orally) for 14 days.

Group II served as acetaminophen treated control, which was received normal saline (1 ml/kg, orally) for 14 days.

Group III pretreated with silymarin (25mg/kg, orally) for 14 days.

Group IV pretreated with ethanolic extract of stem bark of *Bauhinia purpurea* L. (100 mg/kg, orally) for 14 days.

Group V pretreated with ethanolic extract of stem bark of *Bauhinia purpurea* L. (200 mg/kg, orally) for 14 days.

Group VI pretreated with ethanolic extract of stem bark of *Bauhinia purpurea* L. (400 mg/kg, orally) for 14 days.

Liver and kidney damage was induced in these rats with acetaminophen suspension, administered at the dose of 750 mg/kg, orally on day 14 in all groups except the rats in group I. After 48 h of acetaminophen treatment blood was collected from all groups of rats by puncturing the retro-orbital plexus. Serum was separated by centrifugation (Research Centrifuge Remi R-24) at 5000 rpm at 37ºC for 10 min and analyzed for various biochemical parameters[6] by using semi- auto analyzer (SEAC, Mispa Plus).

Assessment of liver and kidney functions

To assess liver function, activities of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin, triglycerides and total protein were measured, for renal function assessment, levels of blood urea nitrogen (BUN), serum creatinine and serum urea were measured using commercially available kits (AGAPPE Diagnostics LTD, Kerala).

Statistical Analysis

The results of biochemical estimation were expressed as mean ± SEM. The total variation present in the data was analyzed by one way analysis of variance (ANOVA) followed by Post hoc Dunnett's test by using the Graphpad prism software (version 5.01). $P < 0.05$ is considered significant.

RESULTS

Preliminary phytochemical analysis

The preliminary phytochemical analysis of ethanolic extract of *Bauhinia purpurea* L. stem bark indicated the presence of carbohydrates, flavonoids, glycosides, triterpenoids, saponins, steroids and tannins.

Acute toxicity study

Acute toxicity study revealed the non-toxic nature of the extracts. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period. Since no mortality was observed at 2000 mg/kg, therefore $1/20^{th}$ (100 mg/kg), $1/10^{th}$ (200 mg/kg) and $1/5^{th}$ (400 mg/kg) of LD_{50} were selected for the study.

Hepato and nephroprotective activity of stem bark of *Bauhinia purpurea* against acetaminophen induced liver and kidney damage

Administration of acetaminophen at the dose of 750 mg/kg b.w to rats caused significant liver and kidney damage, as evidenced by the altered serum biochemical parameters. Pretreatment of rats with ethanolic extract of stem bark of *Bauhinia purpurea* L. exhibited marked protection against acetaminophen induced hepato and nephrotoxicity (shown in Table). The effects produced by ethanolic extract of stem bark of *Bauhinia purpurea* L. were comparable with that produced by the standard, silymarin.

Table 1: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* L. (EEBBP) on liver and kidney biochemical parameters against paracetamol induced toxicity

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TB (mg/dL)	TG (mg/dL)	TP (mg/dL)	BUN (mg/dL)	SC (mg/dL)	SU (mg/dL)
Normal	56.9±2.66	45±3.91	24.9±1.64	1.53±0.05	49.1±1.87	6.60±0.52	19.7±2.32	0.97±0.04	38.1±5.55
Paracetamol (750 mg/kg)	125±4.91[a]	94.8±3.49[a]	60.4±5.11[a]	5.47±0.42[a]	84.1±0.82[a]	4.14±0.31[a]	48.4±4.33[a]	2.12±0.09[a]	94±6.79[a]
Silymarin (25 mg/kg)	77±5.05[d]	60.4±5.11[d]	31.5±2.07[d]	2.03±0.16[d]	62.2±1.81[d]	6.45±0.25[c]	27.8±2.01[d]	1.62±0.07[d]	54.9±5.91[d]
EEBBP (100mg/kg) + Paracetamol	112±2.97[b]	80.6±1.79[b]	43.4±3.95[c]	3.72±0.31[d]	75.5±2.09[c]	5.62±0.44[b]	36.4±3.68[b]	1.96±0.06	70±5.91[b]
EEBBP (200mg/kg) + Paracetamol	99.4±2.41[d]	75.8±1.86[c]	37.9±2.98[d]	3.13±0.23[d]	67.6±1.05[d]	5.81±0.49[b]	33.2±2.37[c]	1.79±0.05[c]	68.4±6.14[b]
EEBBP (400mg/kg) + Paracetamol	82.6±1.70[d]	69.5±1.40[d]	31.8±2.70[d]	2.60±0.17[d]	63.0±1.35[d]	6.44±0.23[c]	29.3±2.16[d]	1.67±0.05[d]	57.8±4.97[d]

Values are mean ± SEM (n=6 animals). One way of ANOVA followed by DUNNET'S multiple comparison test. [a] $p<0.001$ when compared with vehicle treated control group,

[b] $p<0.05$, $p<0.01$, [d] $p<0.001$ when compared with paracetamol treated control group.

SGOT- serum glutamate oxaloacetate transaminase, SGPT- serum glutamate pyruvate transaminase, ALP- Alkaline phosphatase, TB- Total Bilirubin, TG- Triglycerides, TP- Total Protein, BUN- Blood urea nitrogen, SC- Serum Creatinine, SU- Serum Urea

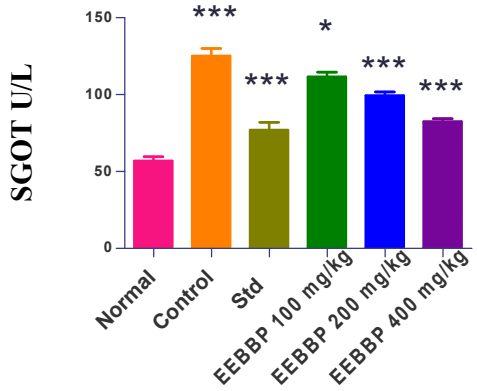

Figure 1: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* on SGOT level in paracetamol induced hepatotoxicity

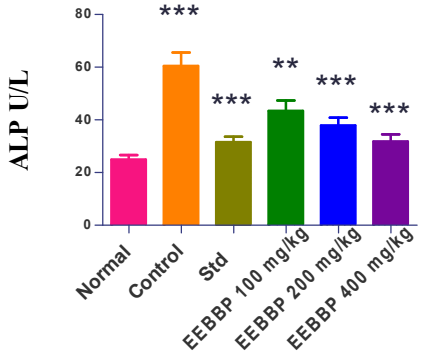

Figure 3: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* on ALP level in paracetamol induced hepatotoxicity

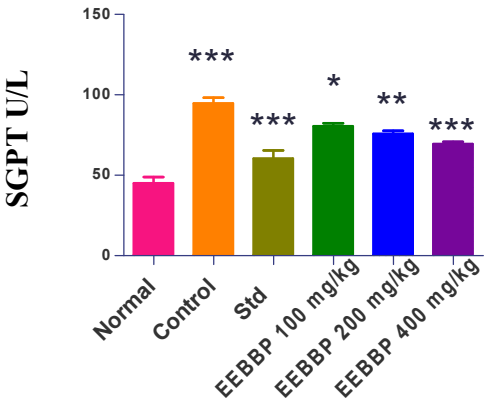

Figure 2: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* on SGPT level in paracetamol induced hepatotoxicity

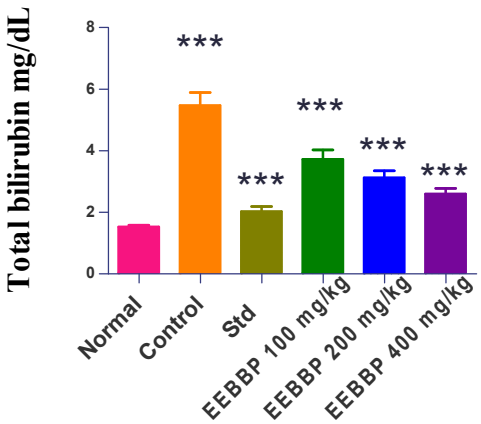

Figure 4: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* on Total Bilirubin level in paracetamol induced hepatotoxicity

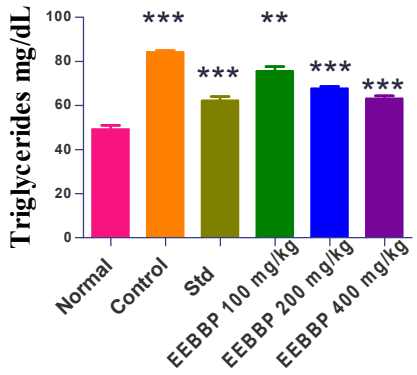

Figure 5: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* on Triglycerides level in paracetamol induced hepatotoxicity

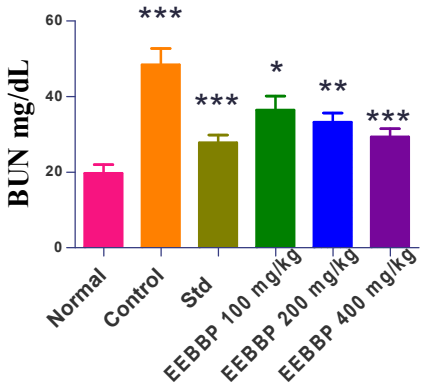

Figure 7: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* on BUN level in paracetamol induced nephrotoxicity

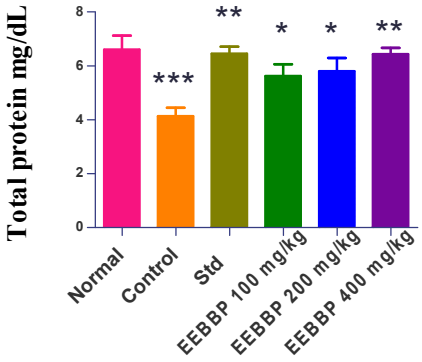

Figure 6: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* on Total protein level in paracetamol induced hepatotoxicity

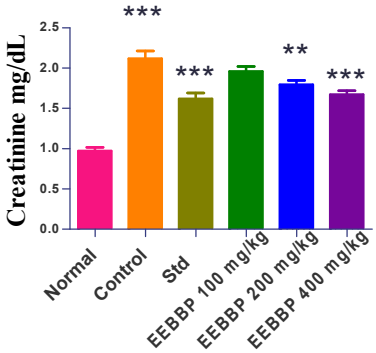

Figure 8: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* on Creatinine level in paracetamol induced nephrotoxicity

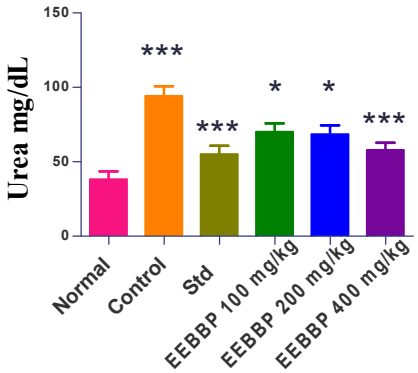

Figure 9: Effect of ethanolic extract of stem bark of *Bauhinia purpurea* on Urea level in paracetamol induced nephrotoxicity

DISCUSSION

Acetaminophen (Paracetamol) is a widely used analgesic and antipyretic agent which is safe at therapeutic doses, overdoses can cause severe centrilobular necrosis and renal proximal tubular necrosis in humans and laboratory animals[5]. Acetaminophen follows three pathways for metabolism; conjugation with sulfate, glucuronide and metabolism by cytochrome p450 oxidase enzyme system. 90% of ingested dose is metabolized through glucuronidation and sulfation pathway and 5% through cytochrome p450 oxidase enzyme system. Metabolism by cytochrome p450 enzyme system produces a metabolite, N-acetyl-p-benzoquinone imine (NAPQI) which is toxic to liver and kidney. In therapeutic dose, this is rendered ineffective by reduced glutathione, an antioxidant compound in the liver and NAPQI-reduced glutathione is excreted by kidney. In acetaminophen overdose, sulfation and glucuronidation pathways become saturated. The amount and rate of formation of NAPQI is greatly increased, depleting body's reduced glutathione

stores and outstripping its capability to make new glutathione. NAPQI then binds covalently with cells causing their death, resulting in liver and kidney dysfunction[12].

In the assessment of liver damage by acetaminophen the determination of enzyme levels such as SGOT, SGPT is largely used. Necrosis or membrane damage causes the releases of enzymes into circulation and hence it can be measured in the serum. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Serum ALP, bilirubin and total protein levels on other hand are related to the function of hepatic cell[13]. High level of ALP in the blood serum is related to the increase synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increases biliary pressure. Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes. Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of the serum proteins except for γ- globulins. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. Paracetamol seems to cause impairment in lipoprotein metabolism and also alteration in cholesterol metabolism. Elevation of triglycerides level during paracetamol intoxication could be due to increased availability of free fatty acids, decreased hepatic release of lipoprotein and increased esterification of free fatty acids[14].

Kidney excretes blood urea nitrogen found in the liver proteins which are derived from diet or tissue source. Elevated BUN usually indicates glomerular damages. Its level can also be affected by hepatotoxicity resulted from toxicants. Creatinine is a metabolite of creatine excreted in the urine via glomerular filtration and the tubule. Elevation of creatinine in the blood thus can be an indication of impaired kidney function. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance. Elevation of urea and creatinine levels in the serum was taken as the index of nephrotoxicity[15, 16].

In the present study, results obtained showed that acute dose acetaminophen hepato and nephrotoxicities were reliably established with 750 mg/kg/day oral acetaminophen suspension, as evidenced by significant ($p< 0.001$) elevation of activities of SGOT, SGPT, ALP, bilirubin, triglycerides, BUN, creatinine and urea in serum (Group II) against their respective normal values (Group I). On the other hand, total serum protein level was lowered significantly ($p< 0.001$). Pretreatment with ethanolic extract of stem bark of *Bauhinia purpurea* L. shows significant ($p< 0.05$) protection in a dose dependant manner against acetaminophen induced toxicity by reversing the biochemical parameters.

The protection offered by the extracts could have been due to the presence of any of the active principles contained in the extracts. Phytochemical screening has shown *Bauhinia purpurea* L. to contain high concentrations of glycosides, flavonoids, saponins and tannins. Flavonoids are capable of modulating the activity of enzymes and affect the behaviour of many cell systems and exerting beneficial effects on body[17]. Flavonoids generally have been shown to protect against various forms of disorders such as coronary heart diseases, liver and kidney disorders. This is thought to be as a result of the induction of detoxifying enzymes such as epoxide hydroxylase, glutathione S transferase, UDP-glucuronosyltransferases, etc Although the molecular mechanism through which the induction of these detoxification enzymes is not well known, it has been suggested that these phytochemicals interact with various intracellular signaling cascades[9]. So the observed hepato and nephroprotective activity of *Bauhinia purpurea* may be due to the presence of flavonoids.

CONCLUSION

In conclusion, the overall result suggests that the ethanolic extracts of stem bark of *Bauhinia purpurea* L. possesses hepato and nephroprotective activity and improves biochemical changes associated with acute dose acetaminophen toxicity. This may be due to presence of flavonoids. Although, the specific active principles were not isolated and their exact mechanisms of actions were not investigated in the present study, these could constitute an area of future studies.

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