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Studies on hepatoprotective and nephroprotective activities on ethanolic extracts of bauhinia *tomentosa*

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ABSTRACT

In this current investigation the hepatoprotective and nephroprotective activity of ethanol extract of Bauhinia *tomentosa* on Paracetamol induced nephrotoxicity in male Wistar rats. In this model of nephrotoxicity, 30 adult male wistar rats (150-200gms) were evenly divided into 5 groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4 and 5 were the treatments groups which were simultaneously treated with standard, 200 and 400 mg/kg extract respectively, after each dose of Paracetamol (200 mg/kg, i.p. for 3 days) from 4 to 14 days. On 11th day, blood samples for biochemical parameters, while the rats kidneys for histology were obtained under inhaled diether anaesthesia. Paracetamol treatment caused hepato and nephrotoxicity as evidenced by marked elevation in blood urea, uric acid and creatinine, bilirubin. Co-administration of extract with Paracetanmol decreased rise in blood urea, uric acid and creatinine, bilirubin. Apart from these, histopathological changes also showed the protective nature of extract against Paracetamol induced necrotic and hepatic damage of renal and hepato tissues. It was observed that the ethanol extract of nephroprotective and hepatoprotective activities by histopathological and biochemical observation against Paracetamol induced nephrotoxicity and hepatotoxicity in rats. In the near future could constitute a lead to discovery of a novel drug for treatment of drug induced nephrotoxicity and hepato toxicity.

Keywords: Hepatoprotective and nephroprotective activity, Ethanolic axtracts, Wistar rats.

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INTRODUCTION

On top of this play an important part in the maintenance of our endocrine and acid-base balance, blood pressure, erythropoiesis (creation of new red blood cells) etc., a real multi-tasking unit inside our body, which comes in pairs (a dual core processor by mother nature). Therefore it becomes critical when kidney functions decline, induced by diseases which seem to have no direct relation to renal pathophysiology.

Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication, on the kidney. They are commonly associated with renal dysfunction although the actual incidence of drug-induced renal failure has not been reported, since incidence is complicated by the complexity of the causes of ARF in seriously ill patients.

The incidence of nephrotoxicity from aminoglycosides has increased from 2 to 3% in 1969 to 20% in the past decade. Despite nephrotoxicity and ototoxicity, the aminoglycosides are continuously being used in clinical practice because of their bactericidal efficacy, synergism with ß-lactam agents, low cost, limited bacterial resistance, and a post-antibiotic effect.

Nephrotoxicity has been recognized as noteworthy changes of aminoglycoside anti-infection agents for a long time. Amid the previous 6 to 8 years, this issue has pulled in the consideration and enthusiasm of various specialists, bringing about the age of a substantial assemblage of exploratory information that has enormously extended our understanding of the pathogenesis of this disorder [2].

The human beings are exposed to environmental, occupational and xenobiotics challenges due to modern life style. Huge free radicals are created amid the introduction to such unpleasant difficulties. What's more the procedure of metabolism and excretion of xenobiotics may likewise produce free radicals. These free radicals tie covalently with the tissue macromolecules prompting the cell necrosis. [3]

Paracetamol is a safe and effective analgesic and antipyretic. [4] It is broadly accessible as a solitary part solution and furthermore as a segment of a plenty of blend over-the-counter and professionally prescribed pharmaceuticals.More than 28 billion doses of Paracetamol-containing products were dispensed in 2005 [5]. With more than 89 million prescriptions, hydrocodone/Paracetamol was the most commonly dispensed medication in 2003[6]. Serious toxicity results in hepatic injury, which may progress to fulminant hepatic failure (FHF) and death[7-18]. In children, Paracetamol is much less frequently the cause of acute liver failure.

EFFECTS OF CLINICAL DOSES IN ANIMALS AND HUMANS

Several case reports have attempted to define patients at increased risk of acetaminophen- induced nephrotoxicity. In spite of the fact that the information is restricted, it is sensible to expect that patients in danger might be like those in danger for hepatotoxicity: patients with drained glutathione because of causes, for example, starvation, fasting, or alcohol abuse. In addition, a medication that induces the CYP-450 enzyme system may worsen toxicity and outcomes by increasing the formation of these intermediates [19]. Adolescents and young adults may be more prone to renal insufficiency in the setting of APAP overdose; however, the reason for this finding is unclear [20]. These case reports have also provided valuable information regarding the clinical course of APAP-induced nephropathy. The onset of renal insufficiency tends to range between 1 and 8 days, although most cases were reported between 2 and 5 days post-ingestion. [21].

MATERIALS AND METHODS

Collection of plant material

The *Bauhinia tomentosa* used for the present studies was collected from Chitoor district of Andhra pradesh. The plant was identified, confirmed and authenticated.. The dried material was then pounded independently into coarse powder by a mechanichal processor. The resulting powder was then used for extraction.

Preparation of Ethanolic Extract

The powdered drug was dried and packed well in Soxhlet apparatus and extracted with 1500 ml of alcohol for seven days. The extract has been concentrated and dried by means of Rotary evaporator. It was kept in dessicator until used

Qualitative phytochemical screening [23]

The dried material was then pounded independently into coarse powder by a mechanical processor

Detection of carbohydrates

Small quantity of the extract was dissolved in distilled water and filtered. The filterate was subjected to i.Molisch's test ii.Fehling's test iii.Barfoed's test

Molisch's test

To the filterate few ml of alcoholic α-napthol was added and 2ml of concentrated acid was supplementary slowly through the slides of the test tube. No purple colored ring was formed at junction of the two layers, which indicates absesnce of carbohydrates.

Fehling's test

Little part of the concentrate was treated with fehling's solution I and II and afterwards warmed on bath. No brick red colored precipitate was formed, which indicates absence of carbohydrates.

Barfoed's test

Little quantity of the extract was subjected to barfoed's reagent. No red precipitate formed, which indicates absence of carbohydrates.

Test of starch

A little quantity of powdered drug was treated with diluted iodine solution. No blue color was observed, which indicates absence of starch.

Test for proteins & amino acids

A quantity of extract was liquefy in drops of water and was subjected to million's, biuret and ninhydrin test.

Million's test

Extract has been subject to million's reagent. No white puffy has been formed, shows the absence of proteins and free aminoacids.

Biuret test

To the extract equal volume of 5%w/v NaOH and four drops of 1%w/v $CuSO_4$ solution were added. No pink or purple color was formed indicating the absence of proteins.

Ninhydrin test

The extract was treated with ninhydrin reagent. No purple color was produced, indicating the absence of proteins.

Identification of phenolic compounds

The decoction were added distilled water and filtered. The filtrates were treated with following reagent.

Ferric chloride test

The filtrate added with 5% of $feCl_3$ solution. No black precipitate was found in the decoction of the plant, shows lack of tannins trace and phenolic compounds.

Test with Lead acetate Solution

Few ml of filtrate were treated with lead acetate solution. No puffy precipitate was produced in the decoction of plant.

Gelatin test

To the filtrate of decoction, add 1ml of 1% solution of gelatin. No white precipitate was seen, which indicates absence of tannin in plant.

Test for phytosterols

Small quantities of decoction were dissolved in 5ml of chloroform separately. Then these chloroform layer subjected to,

a. Salkowski test
b. Libermann – Burchards test

Test for fixed oils and fats a) Spot test

A small quantity of extract was pressed between two filter papers. Oil stain was observed, show presence of fixed oils.

Saponification

Few drops of 0.5N alcoholic potassium hydroxide was added to extract along with a few drops of phenolphthalein. The mixture was heated on a water bath for about 1 – 2 hours. Formation of soap or a partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for alkaloids

Small amount of extract was stirred with a few ml of dil HCl and filtered. The filtrate was tested

with various alkaloidal reagents such as Mayer's, Dragendroff's, Wagner's and Hager's reagent.

Mayer's test

To the small amount of filtrate add few drops of Mayer's reagent. A white color precipitate was formed, indicating the presence of alkaloids.

Dragendroff's test: (potassium bismuth iodide)

To the small amount of filtrate add few drops of Dragendroff's reagent. An orange red color precipitate was formed, indicating the presence of alkaloids.

Wagner's test

To the small amount of filtrate add few drops of Wagner's reagent. A brown color precipitate was formed, indicating the presence of alkaloids.

Hager's test: (picric acid)

To the small amount of filtrate add few drops of Hager's reagent. An yellow crystalline precipitate was formed, indicating the presence of alkaloids.

Test for glycosides

A small amount of the extract was hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolysate was subjected to

a) Legal's test
b) Balget's test
c) Borntrager's test
d) Modified borntrager's test.

Legal's test

To the hydrolysate 1ml pyridine few drops of sodium nitroprusside solution was added and then made alkaline with NaOH solution. Pink color was obtained showing the presence of glycosides.

Balget's test

To a solution of extract sodium picrate solution was added. Yellowish orange color was obtained showing, the presence of glycosides.

Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Pink color was observed in ammoniacal layer, confirms the presence of glycosides.

Test for flavonoids

The extract was dissolved in ethanol and then subjected to the following tests.

Ferric chloride test

To a small quantity of Ethanolic solution of extract few drops of neutral ferric chloride was added. Blackish red color was observed, showing the presence of flavanoids.

Shinoida's test

To the alcoholic solution a small piece of magnesium ribbon was added along with conc HCl. Magenta color was formed, showing the presence of flavanoids.

Fluorescence test

Alcoholic solution was seen under ultra violet light. Green color fluorescence was observed, indicating the presence of flavanoids.

Detection of coumarins

Extract were dissolved in alcohol and exposed to UV light, shows green fluorescence. To small quantity of extract were dissolved in alcohol and add ferric chloride solution, shows green color, indicating the presence of coumarins.

Experimental Animals

Albino rats (Wistar strain) of either sex weighing between 150-200 g were procured from the Sai nath Agencies .zed for seven days under laboratory conditions. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC). (ApprovalIAEC/869/11-12) studies were performed in accordance with the CPCSEA guidelines.

RESULT AND DISCUSSION

Biochemical parameters

In Acetaminophen treated group of creatures the convergence of serum urea and creatinine were significantly expanded than the typical animals (group 1) which demonstrates extreme nephrotoxicity. Treating (group 4 & 5) with ethanol extract of showed significant decrease ($p<0.001$) in concentration of urea and creatinine contrast to

Acetaminophen treated group 2. Nevertheless the concentration of uric acid not so much considerably increased in the Acetaminophen treated groups (group 2) than control group (group1). Treatment with methanol extract of significantly ($p<0.05$) decreases the uric acid levels in group 4 & 5 ($p<0.01$) compared to Acetaminophen treated group (group 2).

Table 1: Effect of 500 mg/kg/day Oral Acetaminophen and Bauhinia tomentosa leaves oral on serum creatinine; blood urea and serum uric acid in treated rats for 14 days

Group	Drug treatment	Serum creatinine (mg/dl)	Blood urea (mg/dl)	Uric acid (mg/dl)
1	2% tween 80, p.o.,	0.29±0.01	21.59±3.73	2.35±0.12
2	500 mg/kg p.o, Acetaminophen	0.96±0.04***	118.76±5.981***	8.72±0.21
3	500 mg/kg p.o, Acetaminophen+200 mg/kg	0.82±0.01***	59.86±5.1** *	6.95±0.17***
4	500 mg/kg p.o, Acetaminophen+400 mg/kg	0.52±0.01** *	47.76±3.2** *	6.15±0.24* **
5	500 mg/kg p.o, Acetaminophen+Silymarin 25 mg/kg	0.43±0.01** *	44.26±4.20** *	5.35±0.11* **

N=6 animals in a group; Values are expressed as Mean ± SEM;

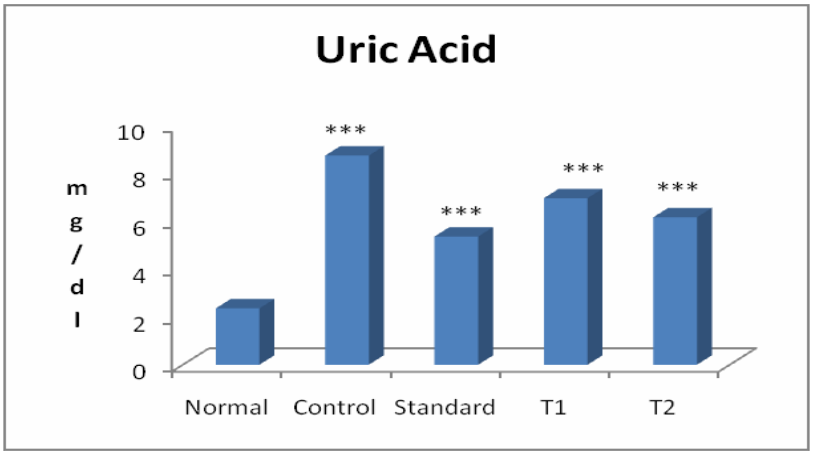

Figure 1. Consequence of extract on Uric acid

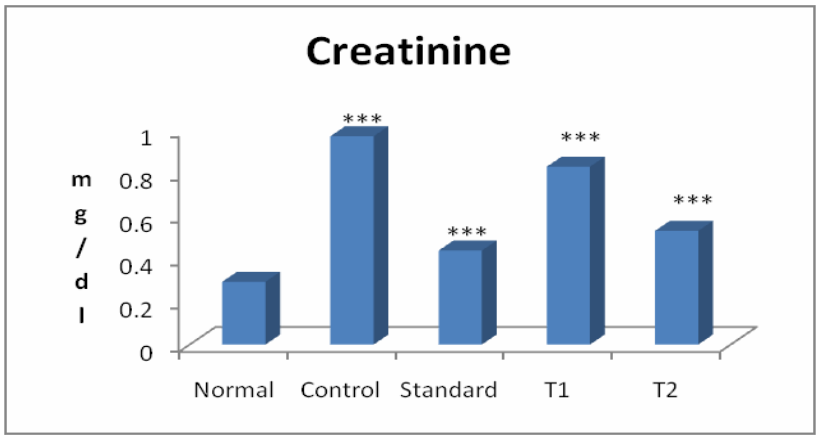

Figure 2. Consequence of extract on Creatinine

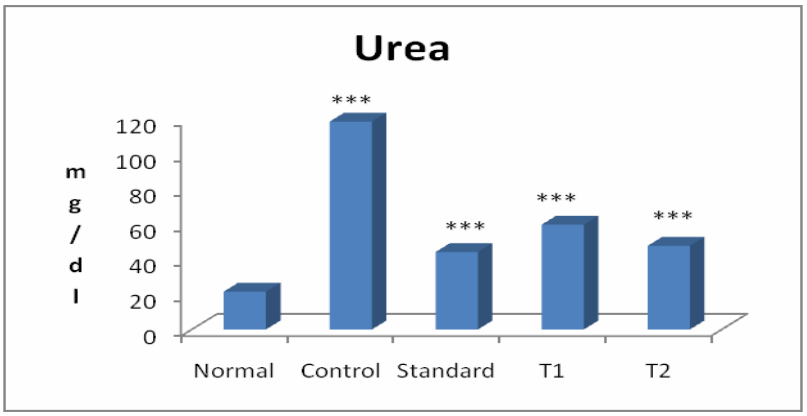

Figure 2.Consequence of extract on Urea

Kidney weight

In Acetaminophen treated group of animals weight of kidneys were considerably increased compared to normal animals (group1) and treating (group 4 & 5) with ethanol extract showed significant decrease ($p<0.001$) in kidney weight.

Table 2: Effect of 500 mg/kg/day Oral Acetaminophen and Bauhinia tomentosa leaves oral on *kidney weight in treated rats for 14 days*

Group	Drug treatment	Kidney weight (gm)
1	2% tween 80, p.o.,	0.467±0.0136
2	500 mg/kg p.o, Acetaminophen	0.812±0.0138***
3	500 mg/kg p.o, Acetaminophen+200 mg/kg	0.66±0.0146***
4	500 mg/kg p.o, Acetaminophen+400 mg/kg	0.587±0.0099***
5	500 mg/kg p.o, Acetaminophen+Silymarin 25 mg/kg	0.536±0.0078***

N=6 animals in a group; Values are expressed as Mean ± SEM;

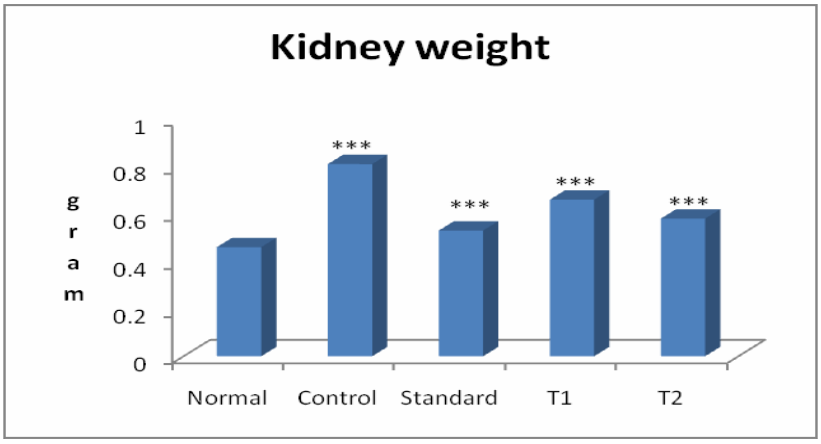

Figure 4: Effect of extract on various on Kidney weight

Rats treated with Actaminophen developed a significant hepatic damage observed as elevated levels of enzymes like SGPT, SGOT and ALP when evaluate to standard control. Behavior with Silymarin, ethanolic extract had showed good protection against Actaminophen induced toxicity to liver. Test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals which are evident in table no.5.3.

Table 3: Effect of 500 mg/kg/day Oral Acetaminophen and Bauhinia tomentosa leaves oral on SGOT, SGPT, ALP in treated rats for 14 days

Group	Treatment	Dose	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)
A	Normal control	2% tween 80 p.o	31.8±1.37	40.87±1.49	28.78±1.62
B	Acetaminophen Control	500 mg/kg, p.o.	105.87±1.69***	128.91±3.33***	86.02±2.68***
C	Standard	25 mg/kg, p.o+Acetaminophen	51.26±0.91***	50.64±1.35**	47.02±1.95***
D	*ESC*	200mg/kg, p.o+Acetaminophen	72.17±2.02***	76.88±1.41***	59.86±1.42***
E	*ESC*	400mg/kg, p.o+Acetaminophen	60.4 9±1.36***	53.07±1.94***	50.47±1.58***

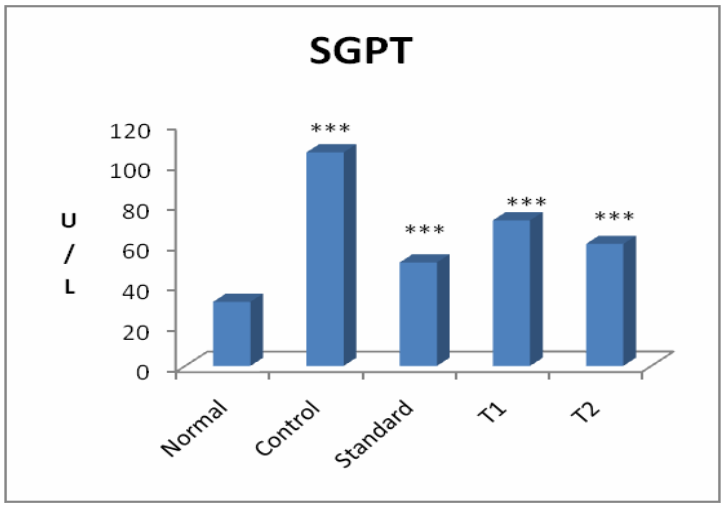

Figure 5: Consequence of extract on biochemical parameter SGPT

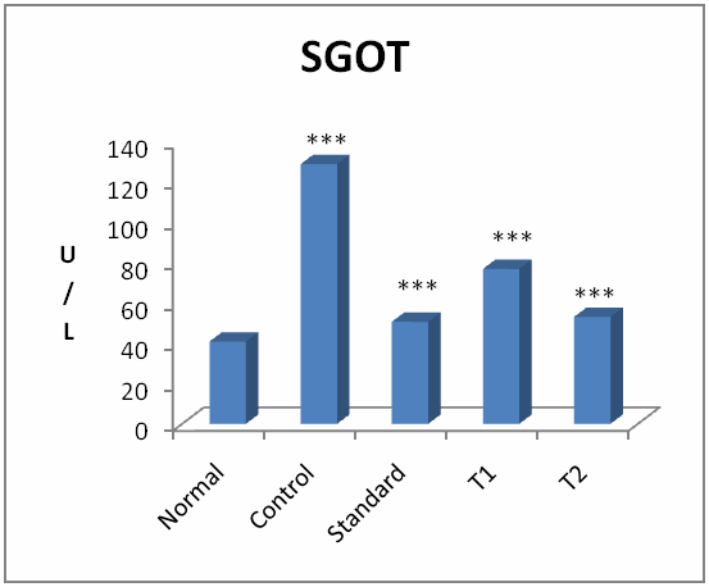

Figure 6: C**onsequence of extract on biochemical parameter SGOT**

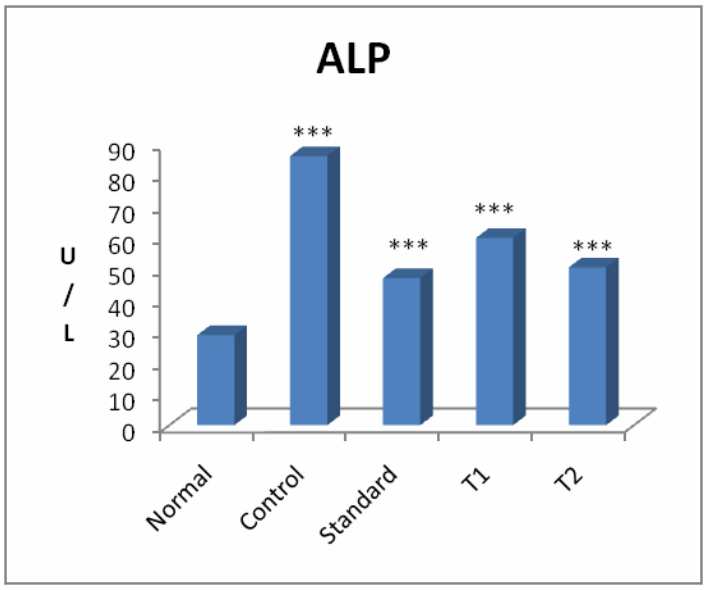

Figure 7: O**utcome of extract on an assortment of biochemical parameter ALP**

Bilirubin and total bilirubin

Elevation of direct and total bilirubin levels after administration of ethanol indicat its hepatotoxicity. Pretreatment with Silymarin, methanolic extract signficantly reduced levels of direct and total bilirubin levels when compared to toxic control group indri citing Hepatoprotective effect of ethanolic extract of Bauhinia tomentosa leaves which can be seen in table no.5.4.

Table 4: Effect of ethanolic extract of Bauhinia tomentosa leaves on direct bilirubin & Total bilirubin levels in Acetaminophen induced hepatotoxic rats.

Group	Treatment	Dose	Direct bilirubin levels(mg/dl)	Total bilirubin levels (mg/dl)
A	Normal control	2% tween 80 p.o	0.14±0.01	0.21±0.01
B	Acetaminophen Control	500 mg/kg, p.o.	0.81±0.01***	1.18±0.04***
C	Standard	25 mg/kg, p.o+Acetaminophen	0.33±0.01***	0.55±0.01***
D	*ESC*	200mg/kg, p.o+Acetaminophen	0.51±0.01***	0.81±0.02***
E	*ESC*	400mg/kg, p.o+Acetaminophen	0.41±0.01***	0.6±0.01***

Values are mean ± SEM (n=6) one way ANOVA

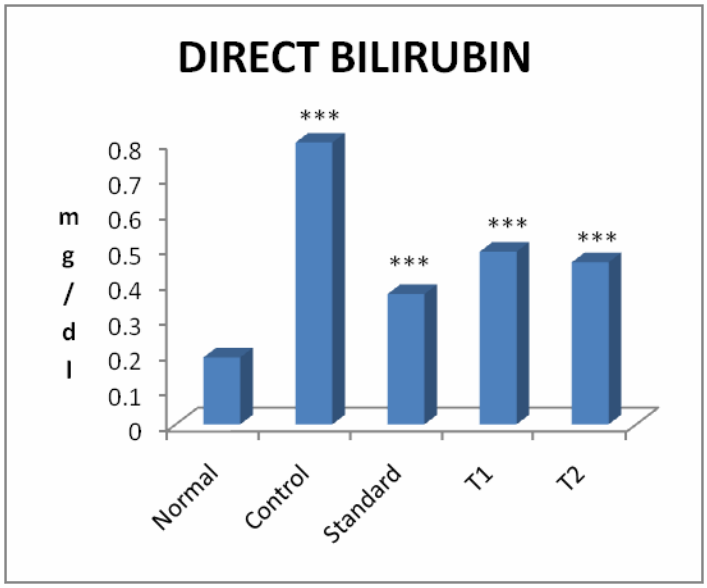

Figure 8: Consequence of extract on biochemical parameters Direct Bilirubin

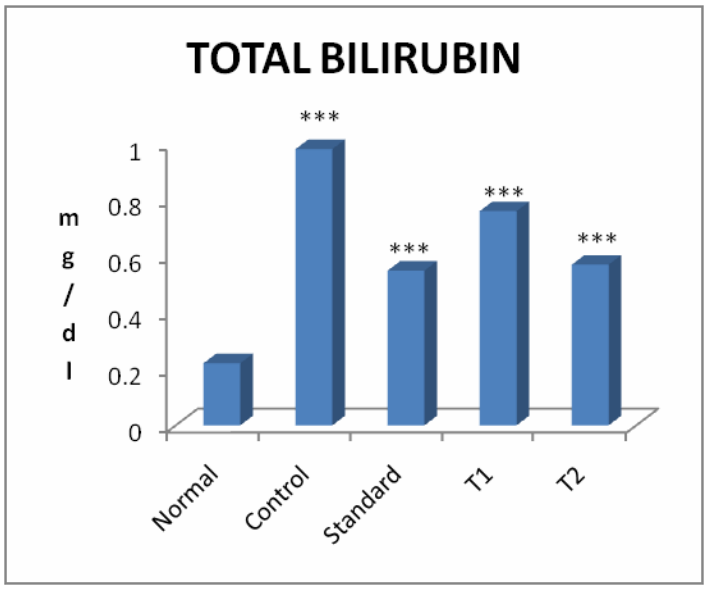

Figure 9: Consequence of extract on biochemical parameter Total Bilirubin

Microscopy

Section studied shows renal parenchyma with intact normal architecture. The glomerular and tubular changes appear unremarkable. Some of the blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium.

500 mg/kg p.o, Acetaminophen 4 days daily (Group- II).

Microscopy

Section examined indicates renal parenchyma with in place design. There are seen diffuse glomerular congestion periodic tubular throws bolt), central hydropic degeneration of the tubular epithelial cells and peritubular congestion. A portion of the tubules indicate halfway desquamation of the epithelial cells. Likewise observed are blood vessel congestion and scattered mononuclear provocative cell penetrations inside the interstitium. Peritubular congestion, epithelial desquamation, Blood vessel congestion.

500 mg/kg p.o, Acetaminophen+200 mg/kg daily for 14 days. (Group-III)

Microscopy

There are seen focal glomerular congestion (Figure.4 (c)), few the tubular epithelial cells show hydropic degeneration and peritubular congestion.

Also seen are few scattered mononuclear inflammatory cell infiltrations within the interstitium glomerular congestion, Peritubular congestion,Focal hydrophic degeneration of tubular epithelial cells. Diffuse glomerular congestion, Tubular casts,

500 mg/kg p.o, Acetaminophen+400 mg/kg daily for 14 days. (Group – IV)

Microscopy

Section examined demonstrates renal parenchyma with in place engineering. The glomerular and tubular changes seem unremarkable. A portion of the blood vessels are enlarged and congested, inside the interstitium. Likewise observed are few scattered mononuclear incendiary penetration is seen inside the interstitium. Essential Highlights: Some blood vessels indicate congestion.

The histopathological evaluation of ethanol toxicity in all the groups was examined and shown in figure no.5.14. The depiction is as follows, Section of rodent liver treated with vehicle control assemble demonstrates liver parenchyma with in place engineering which is the typical appearance.

Section of liver in toxicant control group shows partially effaced architecture. A few of the hepatocytes show apoptotic changes, perivenular mononuclear inflammatory infiltration.

Section of liver in test tranquilize treated groups (200 and 400mg/kg) indicates in place engineering, couple of regenerative hepatocytes, sinusoidal blockage and scattered mononuclear incendiary cells which is like silymarin treated gathering.

CONCLUSION

In the current study, the extract of Bauhinia tomentosa significantly reduced the toxicant elevated levels of above mentioned serum markers and increase in the levels of protein. Hence, at this point it is concluded that the extract of Bauhinia tomentosa offers nephroprotection. Acetaminophen induced hepatotoxicity was significantly prevented by pretreatment with ethanolic extract of Bauhinia tomentosa. Reduction in elevated biochemical parameter levels like serum SGPT, SGOT, ALP, direct and total bilirubin, after treatment with methanolic extract of Bauhinia tomentosa inveterate the hepatoprotective action of extract under study. In liver injury models in rats restoration of hepatic cells with minor fatty changes and absence of necrosis after treatment with extract was observed, indicating satisfactory hepatoprotection. Based on improvement in serum marker enzyme levels, functional parameters and histopathological findings it was portraits that ethanolic extract of Bauhinia tomentosa has got note worthy hepatoprotective activity.

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