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Evaluation of Hepatoprotective Activity of Ethanol Leaf and Bark Extract of *Bauhinia acuminata*

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Keywords: *Bauhinia acuminata* leaf and bark extract, paracetamol, Tamoxifen, ALT, AST, ALP,Total bilirubin, direct bilirubin.

ABSTRACT

The present study was designed to evaluate the hepatoprotective activity of ethanol extract of leaf and bark of *Bauhinia acuminata* against hepatic damage induced by paracetamol (PCM) and tamoxifen (TAM) in albino rats. In this study we investigate both protective and curative effect of the plant. In PCM model the study is intended to evaluate the protective effect of the plant extract. Rats of either sex were pretreated with silymarin (100mg/kg, 7 days, orally) or low and high dose of *Bauhinia acuminata* leaf and bark extract (200 and 400mg/kg, 7 days, orally) and subsequently subjected to PCM administration (2g/kg, *p.o.*) as a single dose on the fifth day. The protective effect of prophylactic treatment was analyzed by quantification of biomarkers like AST, ALT, ALP and bilirubin (total and direct) and histopathological observation. The activities of all marker enzymes registered a significant elevation in PCM treated rats which were significantly recovered towards an almost normal level in animals co-administered with extract. It was concluded that high and low dose of *Bauhinia acuminata* leaf and bark extract demonstrated reduced serum biomarkers activity significantly which was supported by histopathological study. In TAM model the study is intended to evaluate the curative effect of the plant extract. Rats of either sex were treated with silymarin (100mg/kg, orally) or with low and high dose of *Bauhinia acuminata* leaf and bark extract (200 and 400mg/kg, orally) along with TAM for 18 consecutive days. The influence of treatment was analyzed by quantification of biomarkers like ALT, AST, ALP and bilirubin (total and indirect) and histopathological observation. The activities of all markers showed a significant elevation in PCM treated rats which were significantly recovered towards an almost normal level in animals co-administered with extract. It was concluded that high and low dose of *Bauhinia acuminata* leaf and bark extract demonstrated reduced serum biomarkers activity significantly which was supported by histopathological study.

INTRODUCTION:

Liver is the largest and most vital organ in our body, responsible for the metabolism of endogenous and exogenous agents.[1] The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for the overall health and well being.[2]

Liver damage may be caused by xenobiotics, alcohol consumption, malnutrition, infection, anaemia and medications. Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent. Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions.[3]

Managements of hepatic disorders are continuously challenged due to the unavailability of effective drugs that target, arrest, or reverse disease progression, their adverse effects, and their costs, especially in the developing world. Plant-derived compounds from indigenous cultural practices are an effective alternative for the sources of new hepatoprotective remedies. Scientific findings have shown that phytochemicals with antioxidant, anti-inflammatory, and antiviral properties are effective hepatoprotective agents.[4]

Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine which is predominantly based on plant materials.[5] Therefore, several herbal medicines are experimented for their possible antioxidant and hepatoprotective effects against various chemical induced liver damages in animals.[6]

Many herbs such as *Silybum marianum, Tridax procumbens and Andrographis paniculata* , have been reported to possess hepatoprotective activity.[7]

Bauhinia acuminata L. (Common Name- Dwarf White Bauhinia, Family- Fabaceae) is a species of flowering shrub native to tropical southeastern Asia. The bark, flower and root of the *B. acuminata* are used for various skin diseases, worms, tumours and diabetes. The leaf of *B. acuminata* is used to treat bladder stone, venereal diseases, leprosy, asthma and digestive diseases.[8] The term Bauhinia is derived from the new Latin word 'Bauhin'. The synonym of

Bauhinia is 'dwarf white orchid tree. This is a genus of more than 200 species of flowering plants in the subfamily Caesalpinioideae of the large flowering plant family Fabaceae, with a pan tropical distribution. The genus was named after the Bauhin brothers, Swiss-French botanists: Jean Bauhin and and Gaspard Bauhin. Many species are widely planted in the tropics as ornamental, particularly in northern India, Vietnam and southeastern China.[9] *Bauhinia acuminata* Linn. or its local name "Dwarf White Orchid", "TapakKuda" or "Safed Kachnar" is a recent medicinal plant discovered which has been employed by the folks in treating of different types of ailment.[10] Therefore, the present study was to attempt to investigate its hepatoprotective activity in albino wistar rat.

MATERIALS AND METHODS

PLANT MATERIAL AND EXTRACTION[5]

The plant was collected from Kannur, Kerala and washed under running tap water.

The leaves of *Bauhinia acuminata* were dried in shade, powdered and extracted with ethanol (55^0c to 65^0c) in a Soxhlet apparatus. Before and after extraction the marc was completely dried and weighed. The extract was evaporated under reduced pressure by a rotary vacuum evaporator until all the solvent had been removed to give an extract with a yield of 23.3%. The crude extract such obtained is tested for its hepatoprotective activity.

The shade dried stem bark of *Bauhinia acuminata* were powdered (4Kg) and exhaustively extracted by Soxhlet extractor, using 95 % ethanol as solvent. The ethanol was distilled off and the concentrate was evaporated on a waterbath to a sticky mass.

ANIMALS:

Experimental male wistar rats weighing 15-250g were housed at 25° ± 5°C, relative humidity 50 ± 5% in a well-ventilated animal house under 12:12 h light dark cycle. All the rats were provided with commercially available standard pellet diet, water ad libitum.

APPARATUS AND CHEMICALS USED:

1.Analytical balance (Shimazu, Japan)

2.Auto analyser (Robonik, Mumbai)

3.Centrifuge (Remi, India)

4.Paracetamol (National chemicals, Vadodara, Chennai)

5.Sucrose (Merk specialties Pvt limited, Mumbai)

6.Micropippettes (Unitron Bio-medicals, Bangalore)

7.SGPT kits (Robonik India Pvt limited,Mumbai)

8.SGOT kit (Robonik India Pvt limited)

9.ALP kits (Robonik India Pvt limited,Mumbai)

10.Bilirubin (total and direct) kits (Robonik India Pvt limited,Mumbai)

11.Tamoxifen

12.Ethanol

RESULTS:

EFFECT OF LEAF EXTRACT OF BAUHINIA ACUMINATA ON PCM INDUCED HEPATOTOXICITY

a *EFFECT ON SERUM ENZYMES LEVEL*

The experimental procedure for this specific model witnessed prophylactic treatment with the group like toxic control (PCM) demonstrated extremely significant (P <0.001) increase in marker enzymes such as AST, ALT, ALP, total bilirubin and direct bilirubin when compared with vehicle control. Further results documented that standard drug (silymarin) demonstrated extremely significant (p<0.001) decrease in biomarkers when compared with toxic control (PCM). The low dose and high dose of *Bauhinia acuminata* leaf extract demonstrated moderately significant (p<0.01) decrease in biomarkers such as AST, ALT, ALP and bilirubin (total and direct) when compared to toxic control groups (PCM).

All values are mean ±SEM,n=6,**p<0.01, ***p<0.001 when compared to vehicle control.$^{\#\#}$p<0.001, $^{\#\#\#}$P<0.001 compared to PCM control.

BALE-200(*Bauhinia acuminata* leaf extract 200mg/kg), BALE-400(*Bauhinia acuminata* leaf extract 400mg/kg), Silymarin 100mg/kg.

Table 1: Effect of silymarin and BALE on serum ALT, AST, ALP, Bilirubin (total and direct) in PCM induced liver injury in rats

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Vehicle control	128.39±0.836	66.32 ± 2.74	156.85±3.129	0.55± 0.029	0.165 ± 0.01
PCM control	387.86±1.60***	394.68± 1.571***	411.99± 4.93***	2.755±0.115***	0.715± 0.02***
Silymarin(100 mg/kg)	246.54± 0.744###	228.81±1.056###	315.39± 1.35###	0.861±0.06###	0.443± 0.02###
BALE-200	370.62±4.216##	346.7± 13.68##	393.098±1.85##	2.21±0.04##	0.583± 0.02###
BALE-400	369.78± 4.918##	342.82± 13.72##	393.602± 2.38##	1.87±0.015###	0.56±0.02###

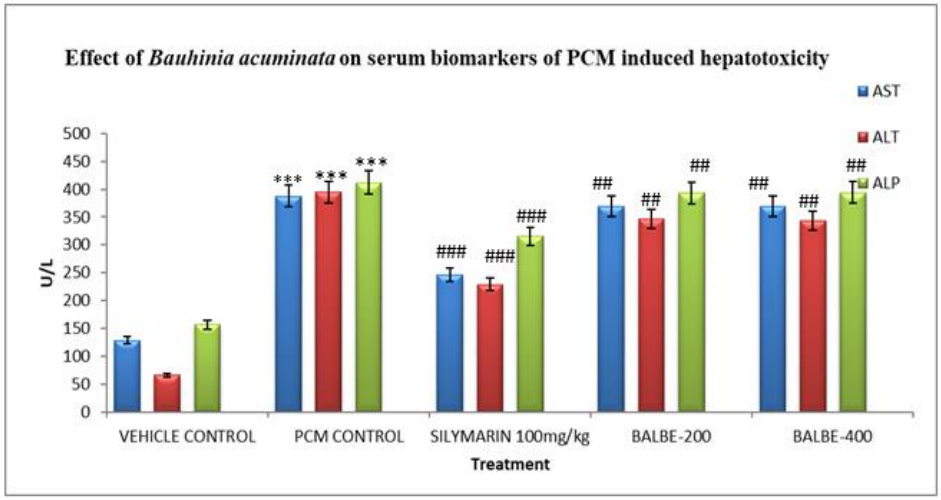

Figure:1 Effect of BALE on serum AST, ALT, ALP on PCM induced hepatotoxicity

All values are mean ±SEM, n=6, **p<0.01, ***p<0.001 when compared to vehicle control. ##p<0.001, ###P<0.001 compared to PCM control

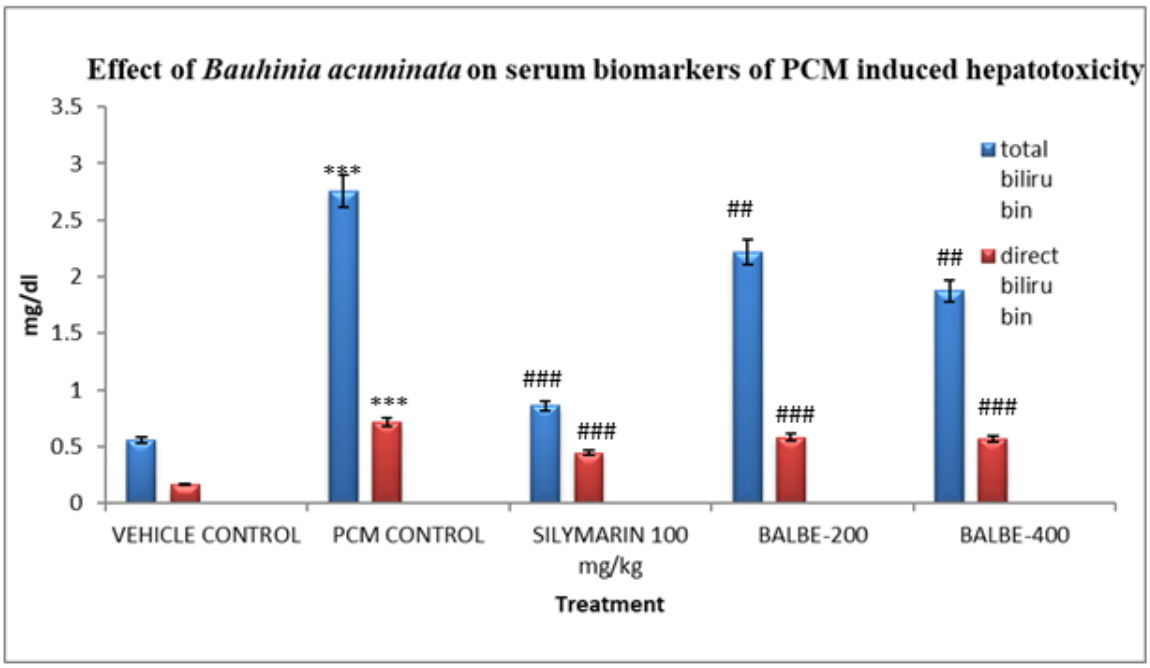

Figure:2; Effect of BALE on Total and Direct Bilirubin of PCM induced hepatotoxicity

All values are mean ±SEM,n=6, $^{**}p<0.01$, $^{***}p<0.001$ when compared to vehicle control. $^{\#\#}p<0.001$, $^{\#\#\#}P<0.001$ compared to PCM control.

b. HISTOPATHOLOGY

Histopathological observations performed in this study demonstrated that the vehicle control group showed normal lobular architecture and normal hepatic cells. The section of PCM intoxicated liver exhibited massive necrosis of the hepatocytes and presence of haemorrhage. BALE (200 mg/kg) +PCM (2g/kg) shows moderate hepatocyte damage with less hemorrhage. BALE (400 mg/kg) +PCM(2g/kg) showed effective inhibition of PCM induced hepatic damage.

c. STATISTICAL ANALYSIS

Results are expressed as Mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P<0.05$ was considered significant.

The mean values ± SEM will be calculated for each parameter.

Figure:3; Hematoxylin and eosin (H&E) stained section of liver in PCM induced liver toxicity. Photographed at magnification 400X.

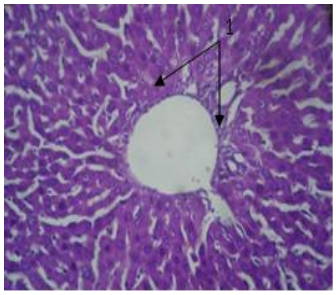

Figure3-a: Normal control. Normal

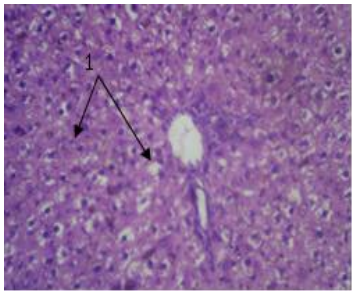

Figure3-b: Toxic control. Moderate

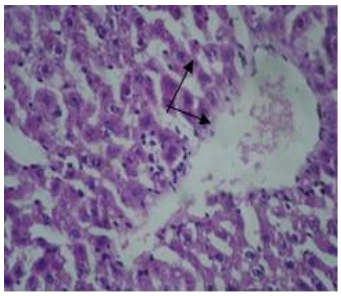

Figure3-c: Standard drug. Mild to texture of liver tissue to severe tissue degranulation moderate liver tissue degranulation

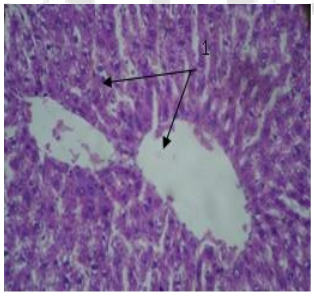

Figure-3d: BALE-200. Moderate tissue degranulation

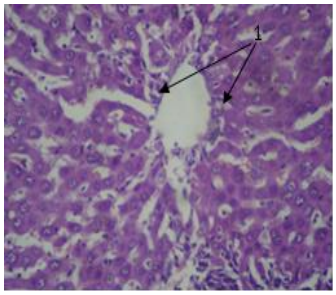

Figure3e: BALE-400. Mild to moderate tissue degranulation

Figure:3; Hematoxylin and eosin (H&E) stained section of liver in PCM induced liver toxicity. Photographed at magnification 400X.

EFFECT OF BARK EXTRACT OF BAUHINIA ACUMINATA ON PCM INDUCED HEPATOTOXICITY

a. ***Effect on serum enzymes level***

The experimental procedure for this specific model witnessed prophylactic treatment with the group like toxic control (PCM) demonstrated extremely significant (P <0.001) increase in marker enzymes such as AST, ALT, ALP, total bilirubin and direct bilirubin when compared with vehicle control. Further results documented that standard drug (silymarin) demonstrated extremely significant (p<0.001) decrease in biomarkers when compared with toxic control (PCM). The low dose and high dose of *Bauhinia acuminata* bark extract demonstrated moderately significant(p<0.01) decrease in biomarkers such as AST, ALT, ALP and bilirubin (total and direct) when compared to toxic control groups (PCM).

b. HISTOPATHOLOGY

Histopathological observations performed in this study demonstrated that the vehicle control group showed normal lobular architecture and normal hepatic cells. The section of PCM intoxicated liver exhibited massive necrosis of the hepatocytes and presence of haemorrhage. BABE (200 mg/kg) +PCM (2g/kg) shows moderate hepatocyte damage with less hemorrhage. BABE (400 mg/kg) +PCM(2g/kg) showed effective inhibition of PCM induced hepatic damage.

c. STATISTICAL ANALYSIS

Results are expressed as Mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. P<0.05 was considered significant.

The mean values ± SEM will be calculated for each parameter.

Table 2: Effect of silymarin and BABE on serum ALT, AST, ALP, Bilirubin (total and direct) in PCM induced liver injury in rats

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Vehicle control	128.39±0.836	66.32 ± 2.74	156.85±3.129	0.55± 0.029	0.165 ± 0.01
PCM control	387.86±1.60***	394.68± 1.571***	411.99± 4.93***	2.755±0.115***	0.715± 0.02***
Silymarin(100 mg/kg)	246.54± 0.744###	228.81±1.056###	315.39± 1.35###	0.861±0.06###	0.443± 0.02###
BABE-200	363.21±4.216##	339.31± 13.68##	386.81±1.85##	1.98±0.04##	0.501± 0.02###
BABE-400	362.72± 4.918##	337.48± 13.72##	389.65± 2.38##	1.75±0.015###	0.485±0.02###

All values are mean ±SEM,n=6,**p<0.01, ***p<0.001 when compared to vehicle control.,##p<0.001, ###P<0.001 compared to PCM control.

BABE-200(*Bauhinia acuminata* bark extract 200mg/kg), BABE-400(*Bauhinia acuminata* bark extract 400mg/kg), Silymarin 100mg/kg.

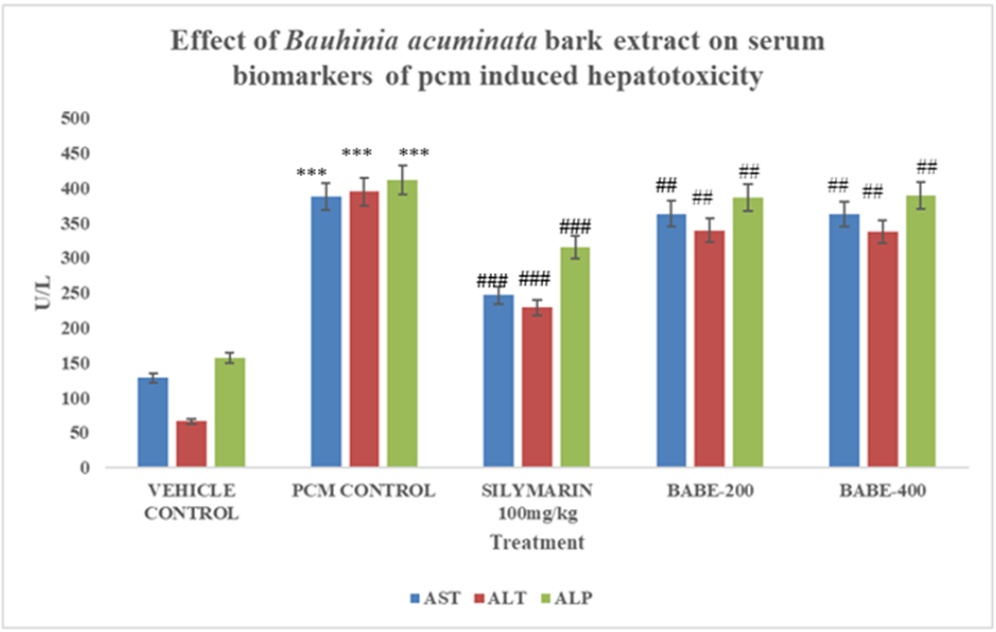

Figure:4 Effect of BABE on serum AST, ALT, ALP on PCM induced hepatotoxicity

All values are mean ±SEM, n=6, $^{**}p<0.01$, $^{***}p<0.001$ when compared to vehicle control. $^{\#\#}p<0.001$, $^{\#\#\#}P<0.001$ compared to PCM control

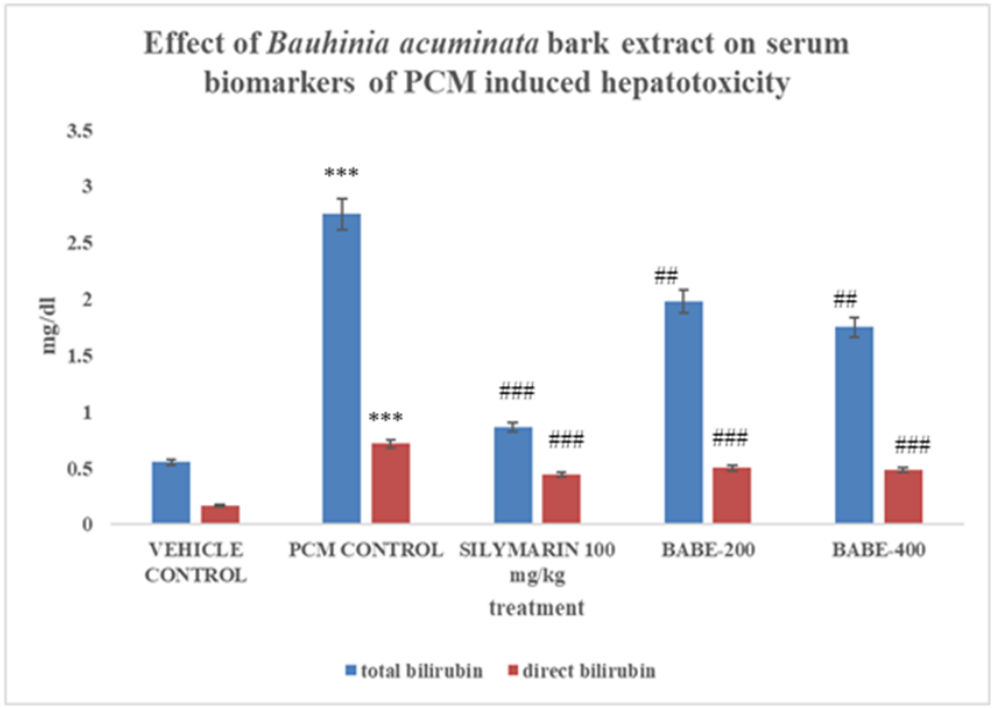

Figure:5; Effect of BALE on Total and Direct Bilirubin of PCM induced hepatotoxicity

Figure 6: Hematoxylin and eosin (H&E) stained section of liver in PCM induced liver toxicity. Photographed at magnification 400X

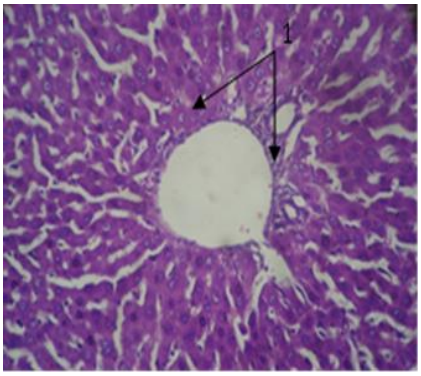

Figure 6a. vehicle control

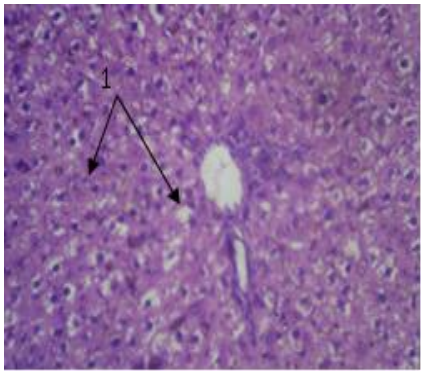

figure 6 b.PCM control

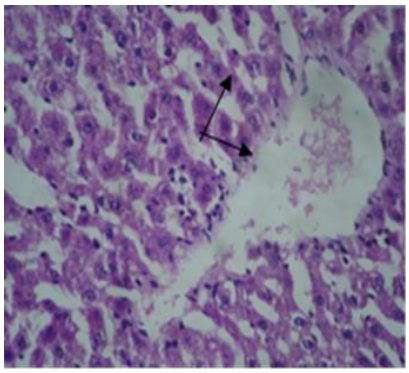

figure 6 c. Standard drug

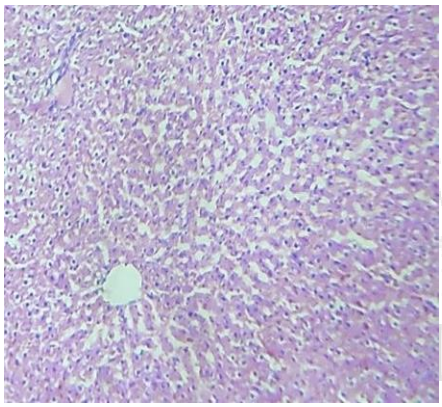

Figure 6 d. BABE-200

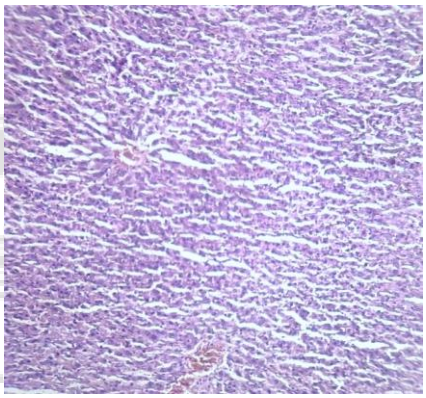

Figure 6 e. BABE-400

EFFECT OF LEAF EXTRACT OF *BAUHINIA ACUMINATA* ON TAM INDUCED HEPATOTOXICITY

a. EFFECT ON SERUM ENZYMES LEVEL

The experimental procedure for this specific model witnessed prophylactic treatment with the group like toxic control (TAM) demonstrated extremely significant (P <0.001) increase in marker enzymes such as AST, ALT, ALP, total bilirubin and direct bilirubin when compared with vehicle control. Further results documented that standard drug (silymarin) demonstrated extremely significant (p<0.001) decrease in biomarkers such as AST, ALT, ALP and bilirubin (total and direct) when compared with toxic control(TAM). The low dose and high

dose of *Bauhinia acuminata* leaf extract demonstrated moderately significant($p<0.01$) decrease in biomarkers such as AST, ALT, ALP and bilirubin (total and direct) when compared to toxic control groups(TAM).

b. HISTOPATHOLOGY

Histopathological observations performed in this study demonstrated that the vehicle control group showed normal lobular architecture and normal hepatic cells. The section of TAM intoxicated liver exhibited massive necrosis of the hepatocytes and presence of haemorrhage. BALE (200 mg/kg) +TAM (45mg/kg) shows moderate hepatocyte damage with less hemorrhage. BALE (400 mg/kg) +TAM(45mg/kg) showed effective inhibition of TAM induced hepatic damage.

c. STATISTICAL ANALYSIS

Table 3: Effect of silymarin and BALE on serum ALT, AST, ALP, bilirubin (total and direct) in TAM induced hepatotoxicity

Treatment	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Total bilirubin (mg/dl)**	**Direct bilirubin (mg/dl)**
Vehicle control	126.427±1.7571	40.015± 2.068	144.04±1.502	0.403± 0.036	0.173± 0.018
TAM control	225.52±1.7896* **	186.64± 3.675***	187.85±1.62***	1.636±0.084***	0.6633± 0.013***
Silymarin(100 mg/kg)	155.48± 2.22$^{###}$	116.66±2.229$^{###}$	152.89± 1.13$^{###}$	0.938±0.032$^{###}$	0.41±0 . 0 1 0 6 $^{###}$
BALE-200	209.095±3.27$^{##}$	161.472±5.629 $^{##}$	169.00±4.26$^{##}$	1.358±0.058$^{##}$	0.548± 0.026$^{###}$
BALE-400	206.62±3.6018$^{#}$ $^{#}$	158.72± 5.643$^{##}$	167.28±4.36$^{##}$	1.301±0.023$^{###}$	0.531±0.0275$^{##}$ $^{#}$

Results are expressed as Mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. P<0.05 was considered significant. The mean values ± SEM will be calculated for each parameter.

All values are mean ±SEM,n=6,**p<0.01, ***p<0.001 when compared to vehicle control.$^{\#\#}$p<0.001, $^{\#\#\#}$P<0.001 compared to TAM control.

BALE-200(*Bauhinia acuminata* leaf extract 200mg/kg), BALE-400(*Bauhinia acuminata* leaf extract 400mg/kg), Silymarin 100mg/kg.

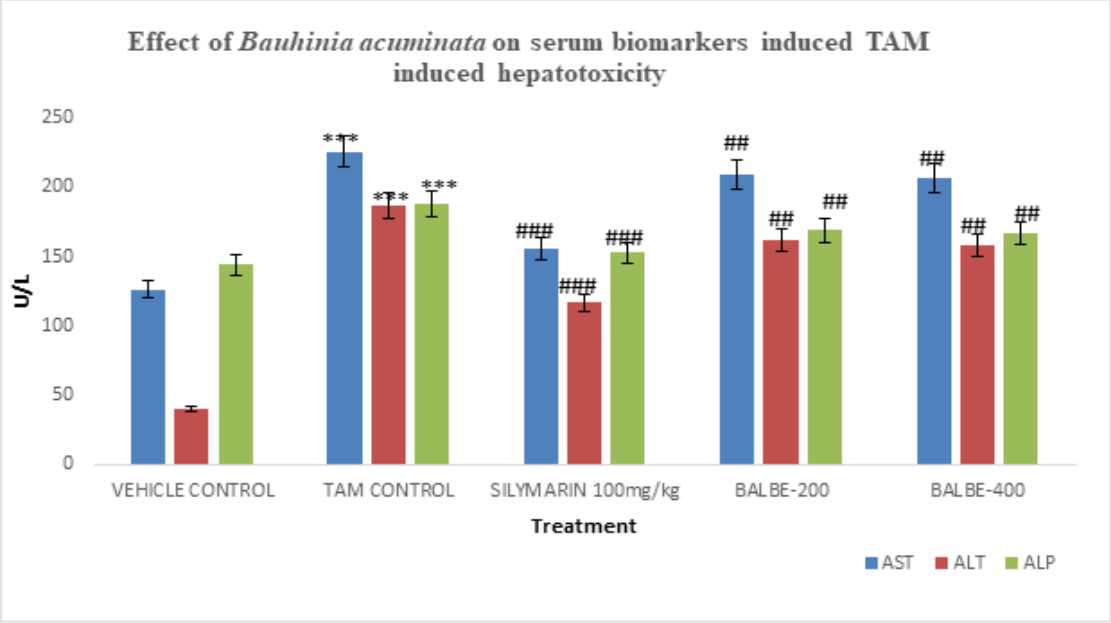

Figure:7; Effect of BALE on serum AST, ALT, ALP on TAM induced hepatotoxicity.

All values are mean ±SEM, n=6, **p<0.01, ***p<0.001 when compared to vehicle control, $^{\#\#}$p<0.001, $^{\#\#\#}$P<0.001 compared to TAM control

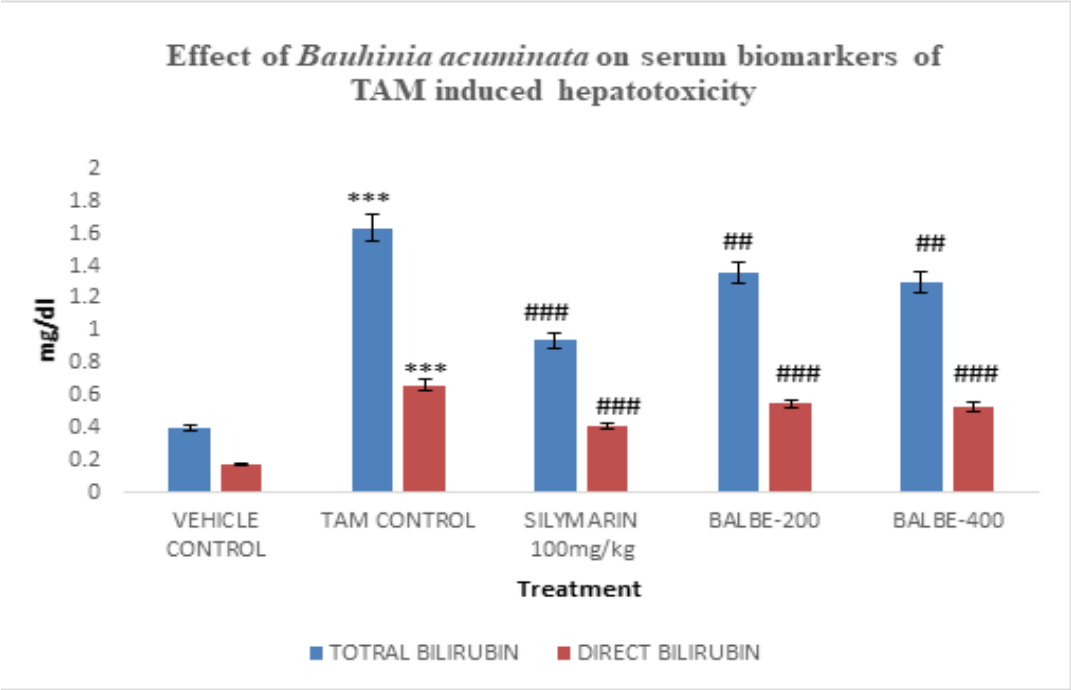

Figure:8; Effect of BALE on Total and Direct Bilirubin of TAM induced hepatotoxicity

All values are mean ±SEM, n=6, $^{**}p<0.01$, $^{***}p<0.001$ when compared to vehicle control, $^{\#\#}p<0.001$, $^{\#\#\#}P<0.001$ compared to TAM control.

b. HISTOPATHOLOGY

Histopathological observations performed in this study demonstrated that the vehicle control group showed normal lobular architecture and normal hepatic cells. The section of TAM intoxicated liver exhibited massive necrosis of the hepatocytes and presence of haemorrhage. BALBE (200 mg/kg) +TAM (45mg/kg) shows moderate hepatocyte damage with less hemorrhage. BALBE (400 mg/kg) +TAM(45mg/kg) showed effective inhibition of TAM induced hepatic damage.

c. STATISTICAL ANALYSIS

Results are expressed as Mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P<0.05$ was considered significant.

The mean values ± SEM will be calculated for each parameter.

Figure 9; Haematoxylin and eosin (H&E) stained section of liver in TAM induced liver toxicity. Photographed at magnification 400X.

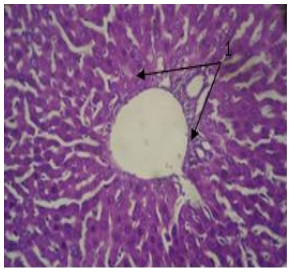

Figure-9a: Normal control.Normal tissue

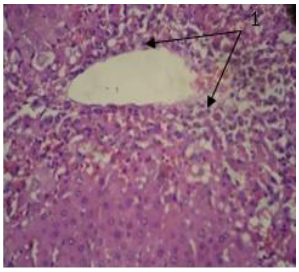

Figure-9b: Toxic control. Moderate to severe tissue degranulation

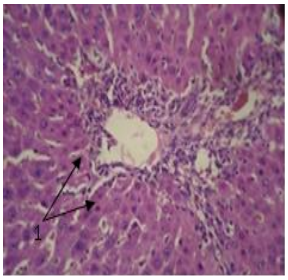

Figure-9c: Standard drug. Mild to exture of liver moderate liver tissue degranulation

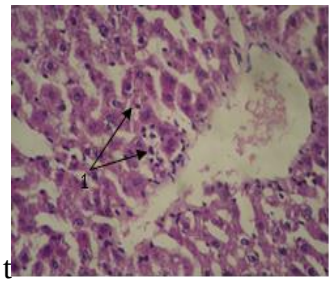

Figure-9d: BALE-200. Moderate Liver Tissue degranulation

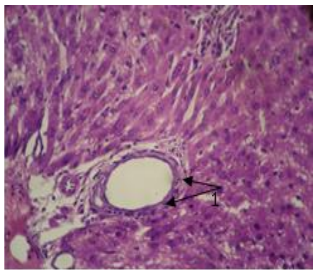

Figure -9e: BALE-400. Mild to moderate liver Tissue degranulation

EFFECT OF BARK EXTRACT OF BAUHINIA ACUMINATA ON PCM INDUCED HEPATOTOXICITY

a. ***Effect on serum enzymes level***

The experimental procedure for this specific model witnessed prophylactic treatment with the group like toxic control (PCM) demonstrated extremely significant (P <0.001) increase in marker enzymes such as AST, ALT, ALP, total bilirubin and direct bilirubin when compared with vehicle control. Further results documented that standard drug (silymarin) demonstrated extremely significant (p<0.001) decrease in biomarkers when compared with toxic control (PCM). The low dose and high dose of *Bauhinia acuminata* bark extract demonstrated moderately significant(p<0.01) decrease in biomarkers such as AST, ALT, ALP and bilirubin (total and direct) when compared to toxic control groups (PCM).

b. HISTOPATHOLOGY

Histopathological observations performed in this study demonstrated that the vehicle control group showed normal lobular architecture and normal hepatic cells. The section of PCM intoxicated liver exhibited massive necrosis of the hepatocytes and presence of haemorrhage. BABE (200 mg/kg) +PCM (2g/kg) shows moderate hepatocyte damage with less hemorrhage. BABE (400 mg/kg) +PCM(2g/kg) showed effective inhibition of PCM induced hepatic damage.

.c. STATISTICAL ANALYSIS

Results are expressed as Mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. P<0.05 was considered significant.

The mean values ± SEM will be calculated for each parameter.

Table 4: Effect of silymarin and BABE on serum ALT, AST, ALP, bilirubin (total and direct) in TAM induced hepatotoxicity

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Vehicle control	126.427±1.7571	40.015± 2.068	144.04±1.502	0.403± 0.036	0.173± 0.018
TAM control	225.52±1.7896* **	186.64± 3.675***	187.85±1.62***	1.636±0.084***	0.6633± 0.013***
Silymarin(100 mg/kg)	155.48± 2.22$^{###}$	116.66±2.229$^{###}$	152.89± 1.13$^{###}$	0.938±0.032$^{###}$	0.41±0 . 0 1 0 6 $^{###}$
BALE-200	206.12±3.27$^{##}$	158.65±5.629 $^{##}$	167.73±4.26$^{##}$	1.254±0.058$^{##}$	0.532± 0.026$^{###}$
BALE-400	203.43±3.6018$^{#}$ $^{#}$	155.87± 5.643$^{##}$	165.41±4.36$^{##}$	1.15±0.023$^{###}$	0.498±0.0275$^{##}$ $^{#}$

All values are mean ±SEM,n=6,**p<0.01, ***p<0.001 when compared to vehicle control.$^{##}$p<0.001, $^{###}$P<0.001 compared to TAM control.

BABE-200(*Bauhinia acuminata* bark extract 200mg/kg), BABE-400(*Bauhinia acuminata* bark extract 400mg/kg), Silymarin 100mg/kg.

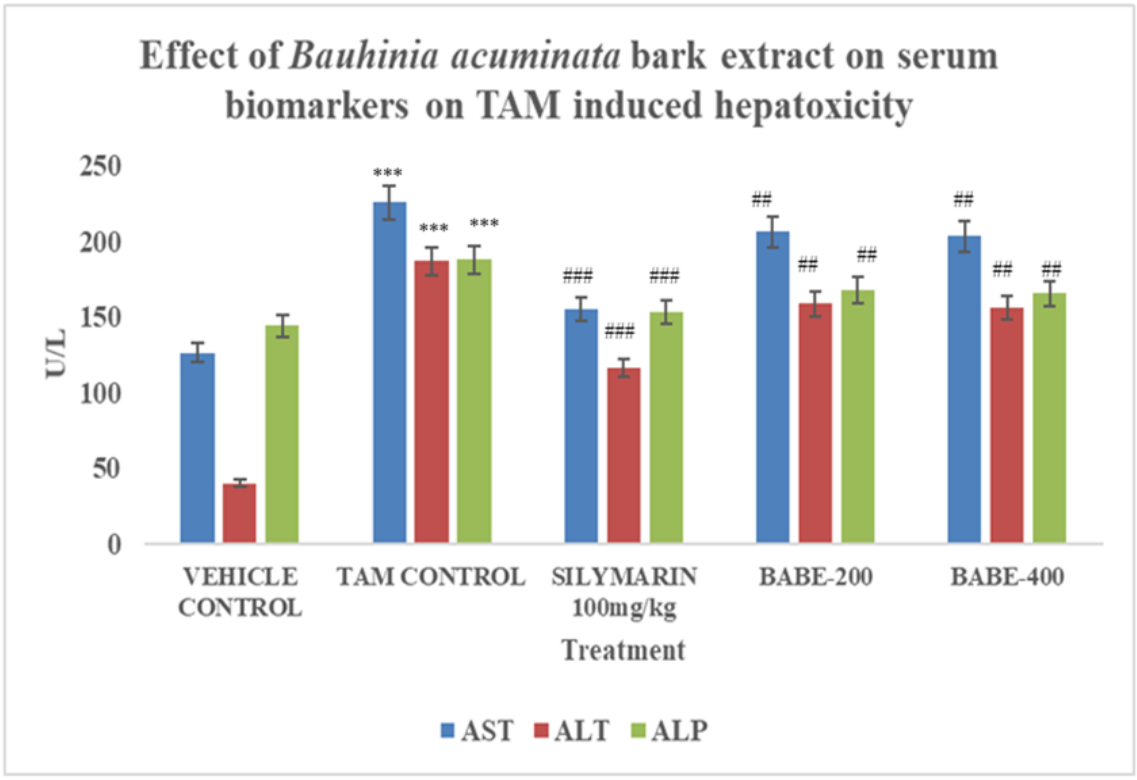

Figure: 10; Effect of BABE on serum AST, ALT, ALP on TAM induced hepatotoxicity.

All values are mean ±SEM, n=6, $^{**}p<0.01$, $^{***}p<0.001$ when compared to vehicle control, $^{\#\#}p<0.001$, $^{\#\#\#}P<0.001$ compared to TAM control

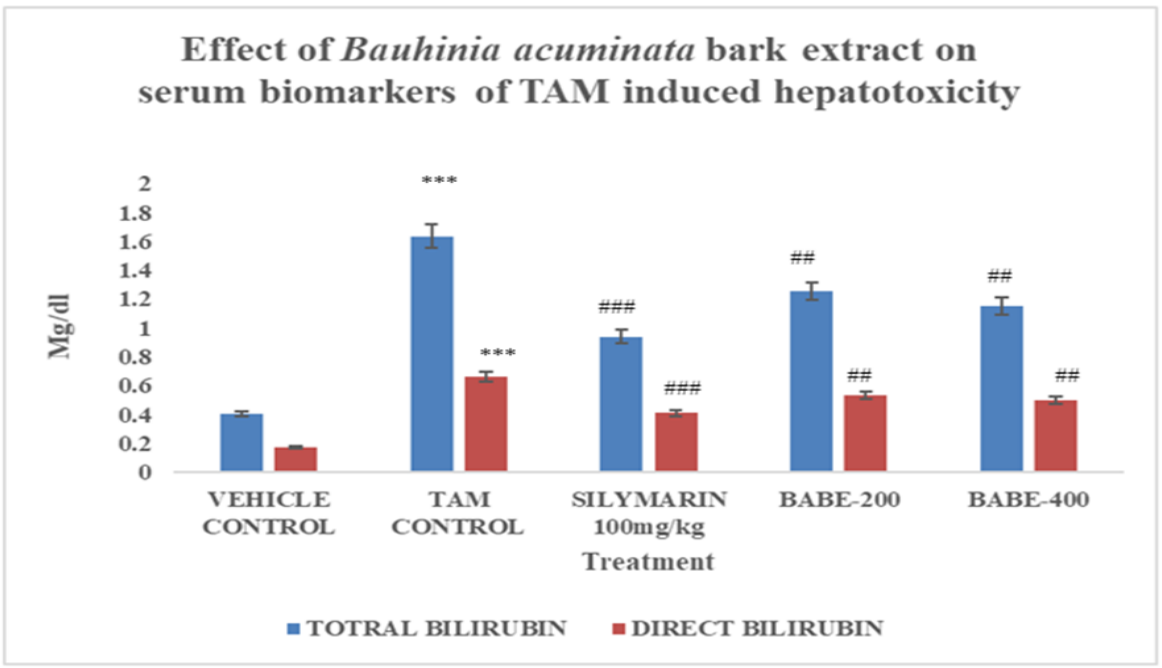

Figure:11; Effect of BABE on Total and Direct Bilirubin of TAM induced hepatotoxicity

Figure 12: Haematoxylin and eosin (H&E) stained section of liver in TAM induced liver toxicity. Photographed at magnification 400X.

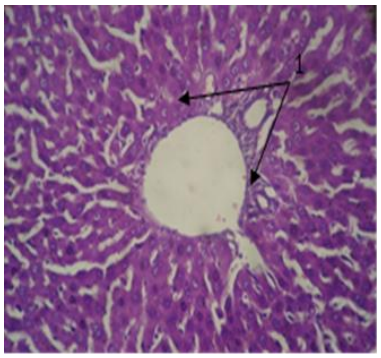

Figure 12 a. vehicle control

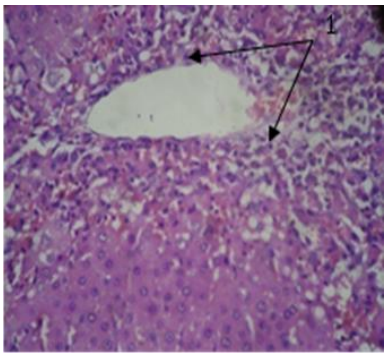

Figure 12 b. TAM control

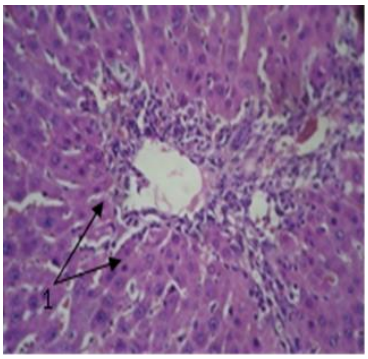

Figure 12 c. standard drug

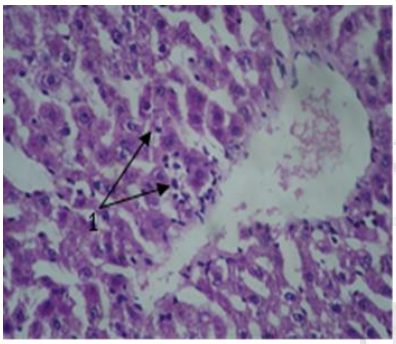

Figure 12 d. BABE-200

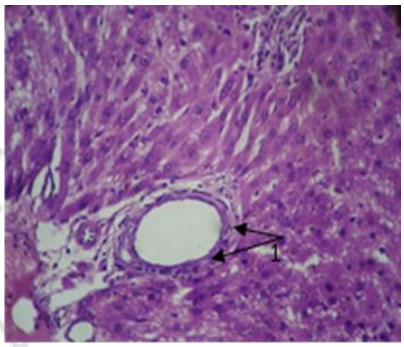

Figure 12 e. BABE-

DISCUSSION

Liver disease are some of the fatal diseases in the world. Toxicities occurs in the liver more often than any other organ. Drugs are the most contributing factor towards liver disease.[20,21]

The present study was designed to evaluate the hepatoprotective activity of *Bauhinia acuminata* leaf and bark extract during hepatic damages induced by various toxic substances like Paracetamol(PCM) and Tamoxifen(TAM) in rat liver.

Liver damage induced by PCM is commonly used model for the screening of hepatotoxic drugs. Paracetamol is an analgesic, which is safe at therapeutic dose but causes liver damage when taken in overdose. Rats are treated with paracetamol in order to induce hepatotoxicity by metabolic activation by cytochrome P_{450} enzymes to a reactive metabolite that depleted

glutathione (GSH) and covalently bound to protein. It was shown that replication of GSH prevented the toxicity. The reactive metabolite was subsequently N-acetyl-p-benzoquinone imine(NAPQI). Liver injury is accompanied by the increase in serum level of biomarkers such as AST, ALT, ALP and bilirubin (total and direct) due to paracetamol toxicity.[96] This model evoked the hepatoprotective activity of *Bauhinia acuminata* in PCM induced hepatotoxicity. The reason for the hepatoprotective activity of *Bauhinia acuminata* may be due to antioxidant property of the chemical constituents present in it. The hepatoprotective effect was supported by histopathological changes produced by the experimental animals.

In the second model, Tamoxifen(TAM) is used for inducing hepatotoxicity in rats. Tamoxifen (TAM), a non-steroidal antiestrogenic drug, is currently the most important endocrine therapeutic agent used in the treatment and prevention of all stages of hormone-dependent breast cancer. In the present study, many histological alterations were recorded in liver of rats treated with TAM. In the current study, the administration of TAM induced a significant elevation in serum level of biomarkers such as AST, ALT, ALP and bilirubin (total and direct). Administration of TAM resulted in an increase in thiobarbituric acid reactive substance that inhibits hexose monophosphate shunt in liver. The inhibition of hexose monophosphate shunt led to increase in the level of MDA and decrease in the level of GSH and in the activities of SOD and CAT or may be attributed to reactive oxygen species (ROS) that can damage the cellular elements like damage in the endoplasmic reticulum and oxidation of membrane component.[97]The recorded results suggested that the leaf and bark extract of *Bauhinia acuminata* demonstrating the hepatoprotective activity by reducing the serum enzymes level against the TAM induced hepatotoxicity which were supported by histopathological studies.

In all experimental models of the present study suggested that both high and low dose of *Bauhinia acuminata* leaf and bark extract (200 and 400 mg/kg) reported significant level of protection and the reason is the antioxidant property due to presence of flavonoids and phenolic as a chief constituent of the plant extract.

The main property of antioxidant is its ability to capture free radicals. These are capable of stabilizing or deactivating free radicals before they attack cells. Antioxidant compounds like flavonoids, phenolic compounds scavenge free radicals and thus inhibit the oxidative mechanisms that leads to degenerative diseases.[60]

In this study, Silymarin, a natural antioxidant made from a mixture of flavonol–lignin extracted from milk thistle is used as standard drug.

Bauhinia acuminata contains flavonoids such as quercetin, Apigenin and kamepferol. Flavonoids interfere with nitric oxide synthase activity which is useful to release nitric oxide for maintaining the dilation of blood vessels. Flavonoids scavenged peroxynitrite radicals produced by reaction of nitric oxide with free radicals. Flavonoids inhibit xanthine oxidase which is a source of free radicals.[61]

The present study reveals that low and high dose of *Bauhinia acuminata* leaf and bark extract decrease the toxic effects of liver induced by Paracetamol and Tamoxifen. The exact mechanism behind this reported activity is not clear but may be due to the presence of some of the fraction of phytoconstituents such as flavonoids and phenolic compounds which is responsible for the induction of metabolic enzymes.

CONCLUSION

The present investigation demonstrated the hepatoprotective activity of *Bauhina acuminata* leaf and bark extract (BALE-200 mg/kg and 400 mg/kg, BABE-200mg/kg and 400mg/kg) against paracetamol(PCM) induced hepatotoxicity and Tamoxifen(TAM) induced hepatotoxicity in experimental rats. The leaf and bark extract of *Bauhinia acuminata* was found to be have hepatoprotective activity due to the presence of flavonoids, tannins and phenolic compounds which exhibits high antioxidant and free radical scavenging activities. The liver protective effect of BALE and BABE may be attributed to the individual and combined action of phytoconstituents present in it. Ethanolic extract of *Bauhinia acuminata* has shown significant reduction in hepatotoxicity. Therefore, further investigations need to be carried out to isolate and identify the antioxidant constituent present in the plant extract.

REFERENCES

1.Hukkeri VI, Akki KS, Sureban RR, Gopalakrishna B, *et al*, Hepatoprotective activity of the leaves of Nyctanthes arbor-tristis linn. Indian Journal of Pharmaceutical Sciences. 2006;68(4).
2. Pandit A, Sachdeva T, Bafna P. Drug-induced hepatotoxicity: a review. J Appl Pharm Sci. 2012 May 28;2(5):233-43.
3. Eidi A, Mortazavi P, Bazargan M, Zaringhalam J. Hepatoprotective activity of cinnamon ethanolic extract against CCI4-induced liver injury in rats. Excli Journal. 2012;11:495.
4. Muluye AB, Ayicheh MW. Medicinal plants utilized for hepatic disorders in Ethiopian traditional medical practices: a review. Clinical Phytoscience. 2020 Dec;6(1):1-1.
5. Shaik AA, Elumalai AA, Eswaraiah MC, Swathi S. An updated review on hepatoprotective medicinal plants. Journal of Drug Delivery and Therapeutics. 2012 Mar 13;2(2).

6. Merlin NJ, Parthasarathy V. Antioxidant and hepatoprotective activity of chloroform and ethanol extracts of Gmelina asiatica aerial parts. Journal of Medicinal Plants Research. 2011 Feb 18;5(4):533-8.
7. Hiraganahalli BD, Chinampudur VC, Dethe S, Mundkinajeddu D, *et a*l, Hepatoprotective and antioxidant activity of standardized herbal extracts. Pharmacognosy magazine. 2012 Apr;8(30):116.
8. Khan MF, Shilpi RI, Rashid RB, Rashid MA. In vitro Antioxidant, Cytotoxic and Membrane Stabilizing Activities of *Bauhinia acuminata* L. Bangladesh Pharmaceutical Journal. 2014;17(1):99-101.
9. Akhter ms. micropropagation of *Bauhinia acuminata* L. International Research Journal of Applied Life Sciences. 2012 Jan 1;1(3).
10. Prabhu R, Razali NH, Dhandapani N, *et al* in vitro anthelmintic study of *bauhinia acuminata* linn. leaf extracts against the housefly worms. Indo American Journal of Pharmaceutical Sciences. 2018 Jun 1;5(6):5082-9.
11. Brynie FH. One Hundred One Questions about Food and Digestion that Have Been Eating at You--Until Now: That Have Been Eating at You... Until Now. Twenty-First Century Books; 2002.
12. Refaey MS, Mustafa MA, Mohamed AM, Ali AA. Hepatoprotective and antioxidant activity of Odontonema cuspidatum (Nees) Kuntze against CCl4-induced hepatic injury in rats. Journal of Pharmacognosy and Phytochemistry. 2015 Jul 1;4(2).
13. Mbhele N. Antioxidant activity and hepatoprotective potential of leaf extracts from Morella serrata (Lam.) Killick (Myricaceae) (Doctoral dissertation, University of the Free State (Qwaqwa Campus)).
14. Eidi A, Mortazavi P, Bazargan M, Zaringhalam J. Hepatoprotective activity of cinnamon ethanolic extract against CCI4-induced liver injury in rats. Excli Journal. 2012; 11:495.
15. Kumar P. Antioxidant and hepatoprotective activity of tubers of Momordica tuberosa Cogn. against CCl 4 induced liver injury in rats.
16. Li S, Tan HY, Wang N, Zhang ZJ *et al*. The role of oxidative stress and antioxidants in liver diseases. International journal of molecular sciences. 2015 Nov;16(11):26087-124.
17. Yahya F, Mamat SS, Kamarolzaman MF *et al*. Hepatoprotective activity of methanolic extract of Bauhinia purpurea leaves against paracetamol-induced hepatic damage in rats. Evidence-Based Complementary and Alternative Medicine. 2013 Jan 1;2013.
18. Timbrell J. Introduction to toxicology. CRC Press; 2001 Nov 15.
19. Kaplowitz, N. and Tsukamoto, H., 1996. Oxidative stress and liver disease. Progress in Liver Diseases, 14, pp.131-159.