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BARK EXTRACT OF *BAUHINIA ACUMINATA* AND BIOLOGICAL ACTIVITY USING DIFFERENT MODELS IN ANIMALS

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ABSTRACT

Biological activity of ethanol extract of bark of *Bauhinia acuminata* against hepatic damage induced by tamoxifen (TAM) in albino rats. In this study we investigate both protective and curative effect of the plant. 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* 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. It was concluded that high and low dose of *Bauhinia acuminata* bark extract demonstrated reduced serum biomarkers activity significantly which was supported by histopathological study.

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

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 acuminataLinn. 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 rats.

OBJECTIVES

The objective of study is to investigate the possible Hepatoprotective effects of *Bauhinia acuminata* bark extract using different experimental models in animals.

Animal models selected for the study include:

- Tamoxifen induced hepatotoxicity.

Biochemical evaluation was carried out in each animal model. Experiment was performed as described in the standard bibliography, literature, text book etc.

Estimations

The parameters evaluated are

1. SGPT, SGOT, ALP and Bilirubin (Total and Direct) activity in serum.
2. Histopathological Studies: Scoring in hepatocytes will be determined from H-E transverse stain.

Specific objectives

- To carry out the extraction of *Bauhinia acuminata* bark using suitable solvents and extraction procedure.
- To explore the curative effect of *Bauhinia acuminata* bark extract against Tamoxifen (TAM) induced liver toxicity in rats.

Experimental 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. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol (SDCP/IAEC/14/2020)

Plant Material and Extraction:

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 water bath to a stick.

Phytochemical Investigation of the Extract

Extract of *Bauhinia acuminata* leaves and bark were subjected to qualitative analysis to investigate the presence of various phytochemical constituents .

TAMOXIFEN INDUCED LIVER TOXICITY

The animals will be divided into five groups of six animals each. The animals are then subjected to either one of the following treatments for 18 days. Group II will be administered with tamoxifen (TAM) at a dose of 45mg/kg/ day (*i.p*) for 7 successive days. Group III, IV, V will be treated with either silymarin or ethanolic drug extract along with tamoxifen for 18 successive days. At the end of the experiment the animal will be fasted, anesthetized and sacrificed.

Rats of either sex will be divided into 5 treatment groups of six animals each.

A. Leaf extract of Bauhinia acuminata

Group-I: Normal saline.

Group-II: Distilled water + TAM (45mg/kg/day).

Group-III: Silymarin (100mg/kg)

Group-IV: Low dose of *Bauhinia acuminata* leaf extract (200mg) + TAM.

Group-V: High dose of *Bauhinia acuminata* leaf extract (400mg) + TAM.

The parameters to be estimated: 1. SGPT, SGOT, ALP and Bilirubin (Total and Direct) activity in serum.

2. Histopathological Studies: Scoring in hepatocytes will be determined from

H-E transverse stain.

B. bark extract of *Bauhinia acuminata*

Group-I: Normal saline.

Group-II: Distilled water + TAM (45mg/kg/day).

Group-III: Silymarin (100mg/kg)

Group-IV: Low dose of Bauhinia acuminata bark extract (200mg) + TAM.

Group-V: High dose of Bauhinia acuminata bark extract (400mg) + TAM

The parameters to be estimated: 1. SGPT, SGOT, ALP and Bilirubin (Total and Direct) activity in serum. 2. Histopathological Studies: Scoring in hepatocytes will be determined from H-E transverse stain.

PREPARATION OF *BAUHINIA ACUMINATA* EXTRACT

The ethanolic extract of Bauhinia acuminata bark was prepared and found to be 35%.

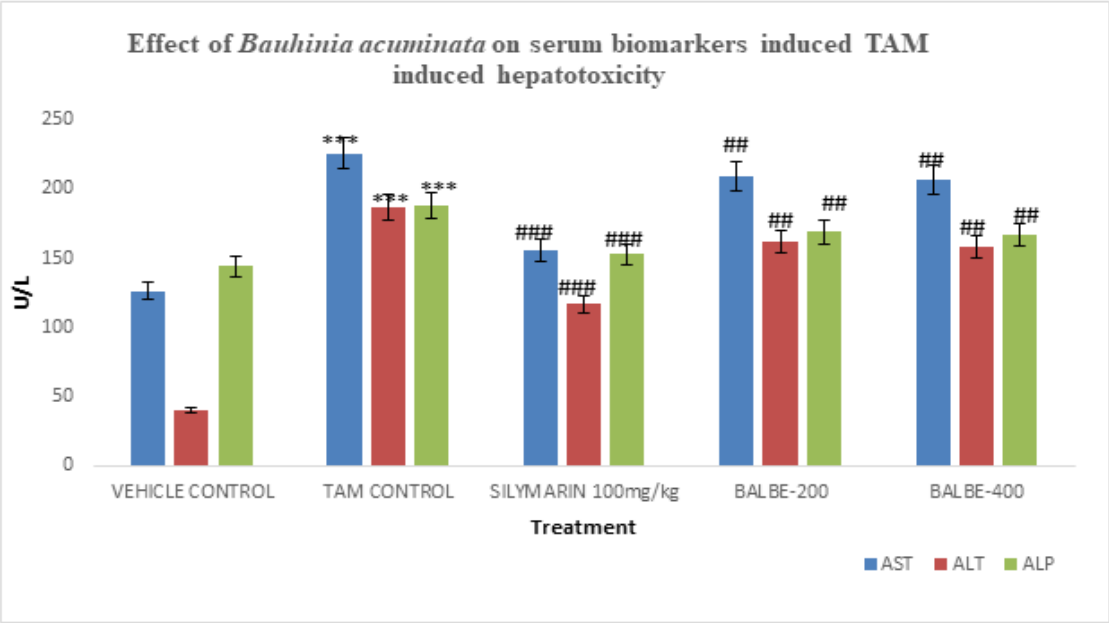
Phytochemical Investigation for Various Compounds:

Table: 1 Phytochemical investigation for various compounds of BABE

SL NO.	TEST	INFERENCE	RESULT
1.Test for alkaloids			

1.a.	Hager's test	Yellow colour	Present
1.b	Cream precipitate	Orange precipitate	Present
1.c	Dragendroff's test	Orange precipitate	Present
1.d	Wagner's test	Red brown precipitate	Present
2.Test for carbohydrates			
2.a.	Molisch test	Violet colour	Present
2.b	Fehling's test	Brick red colour	Present
2.c	Borfoed's test	Red colour	Present
2.d	Benedict's test	Red colour	Present
3.Test for steroids, triterpenoids and glycosides			
3.a	Liebermann-buchard test	Reddish-violet	Present
3.b	Salkowski test	Red colour	Present
3.c	Baljet test	Orange colour	Present
3.d	Keller killani test	Red colour	Present
4.Test for saponins			
4.a	Froth test	1cm foam	Present
4.b	Haemolytic test	No precipitate	Present
5.Test for Tannins			
5.a	Ferric chloride test	Blue colour	Present
5.b	Lead acetate test	Yellow colour	Present
6.Test for Proteins and Amino acids			
6.a	Millon's test	Red colour	Present
6.b	Biuret test	Violet colour	Present
6.c	Ninhydrin test	Violet colour	Present
7.Test for Flavonoids			
7.a	Ferric chloride test	Blackish red colour	Present
7.b	Lead acetate test	Yellow colour	Present
8.Test for specific Flavonoids			
8.a	Test for carotenoids	Deep blue colour	Absent
8.b	Test for phenolic compound	Redish brown colour	Present

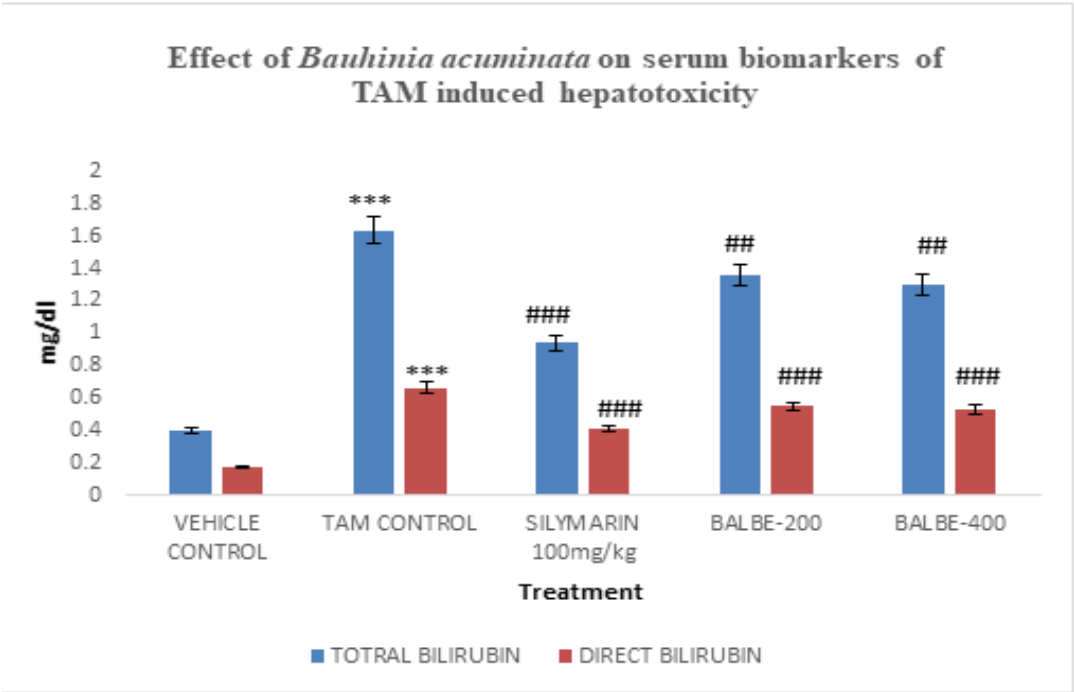
Figure: 1; Effect of BALE on serum AST, ALT, ALP on TAM induced hepatotoxicity.



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: 2; 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.

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.

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

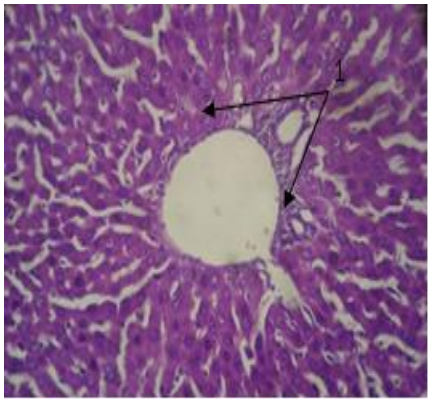


Figure-3a: Normal control.Normal tissue

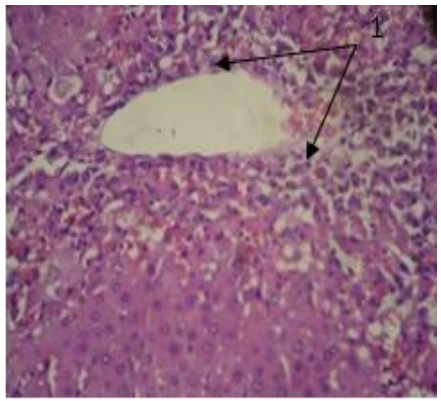


Figure-3b: Toxic control. Moderate to severe tissue degranulation

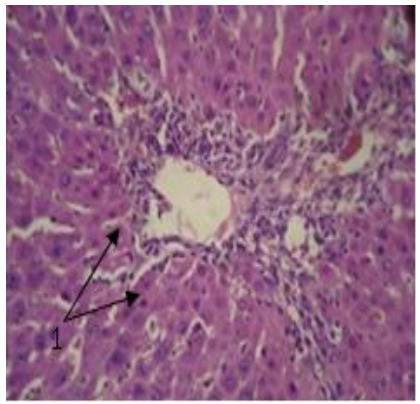


Figure-3c: Standard drug. Mild to texture of liver moderate liver tissue degranulation

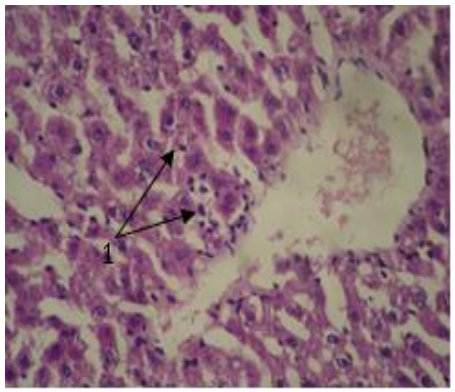


Figure-3d: BALE-200. Moderate Liver Tissue degranulation

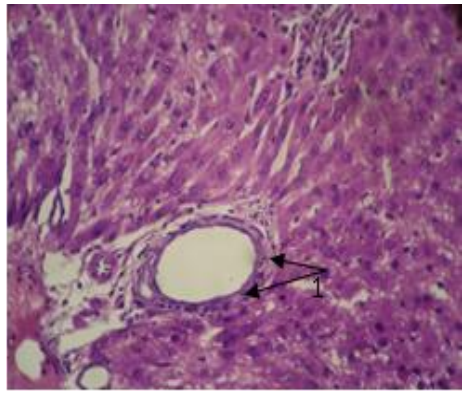


Figure -3e: BALE-400. Mild to moderate liver tissue degranulation

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(100mg/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###

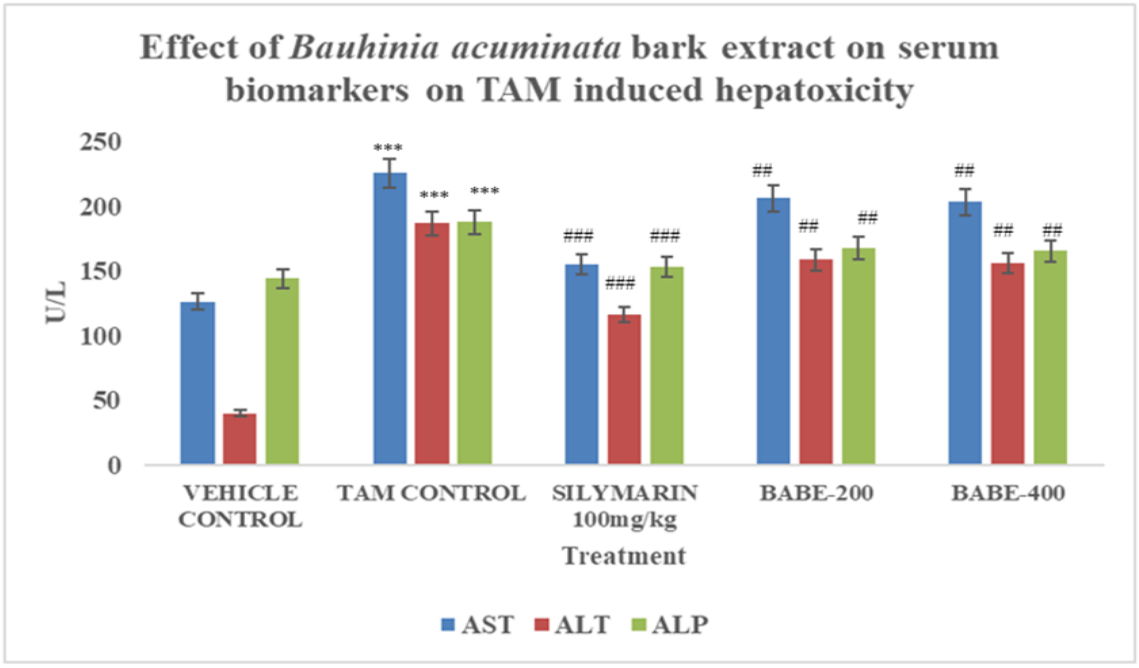
BALE-400	203.43±3.6018$^{\#\#}$	155.87± 5.643$^{\#\#}$	165.41±4.36$^{\#\#}$	1.15±0.023$^{\#\#\#}$	0.498±0.0275$^{\#\#\#}$

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.

Table 2: Effect of silymarin and BABE on serum ALT,AST,ALP,bilirubin(total and direct) in TAM induced hepatotoxicity

Figure:4; 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: 5; Effect of BABE on Total and Direct Bilirubin of TAM induced hepatotoxicity

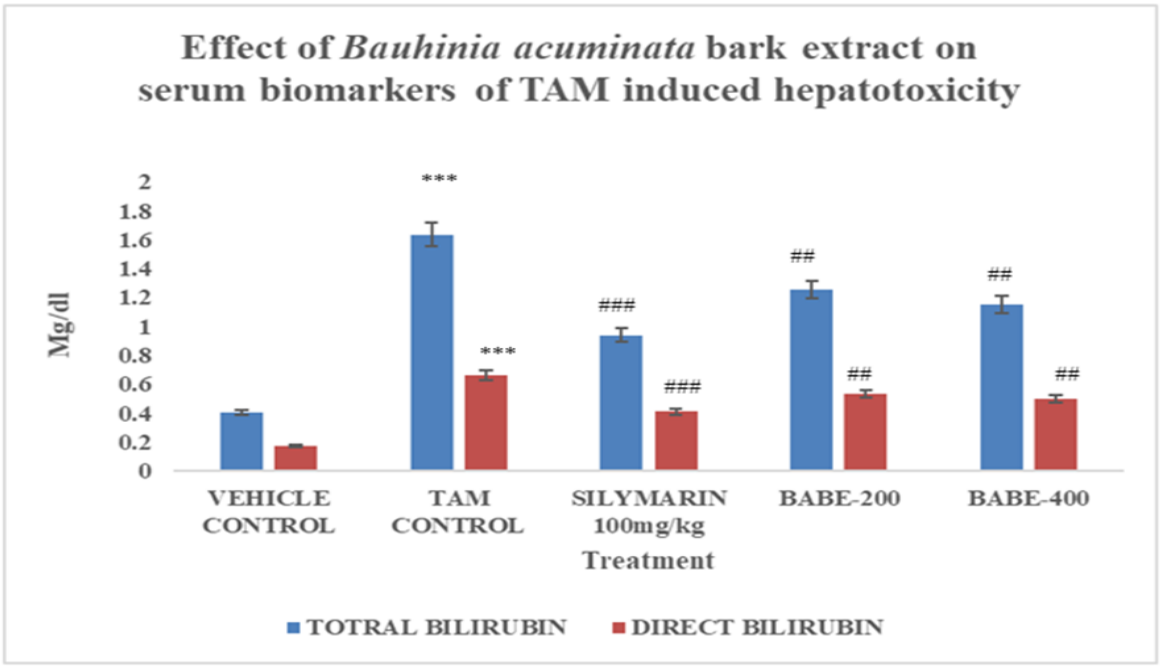


Figure 6: Haematoxylin and eosin (H&E) stained section of liver in TAM induced liver toxicity. Photographed at magnification 400X.

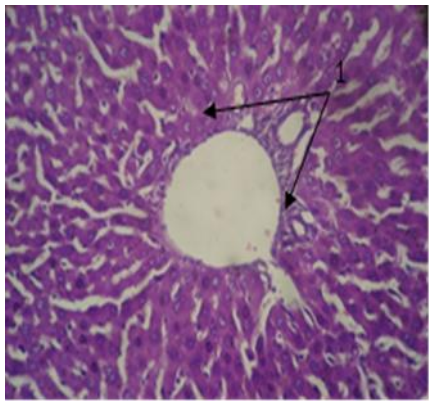


Figure 6a. vehicle control

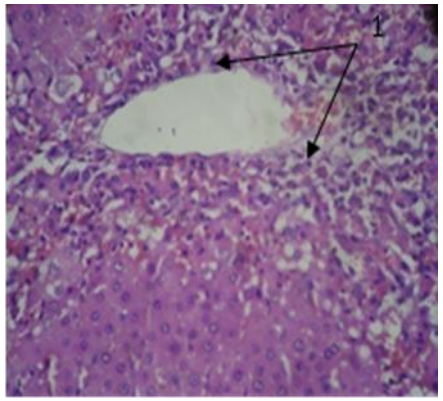


Figure 6 b. TAM control

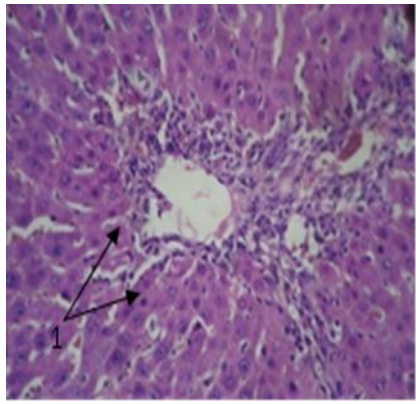


Figure 6c. standard drug

Figure 6 d. BABE-200

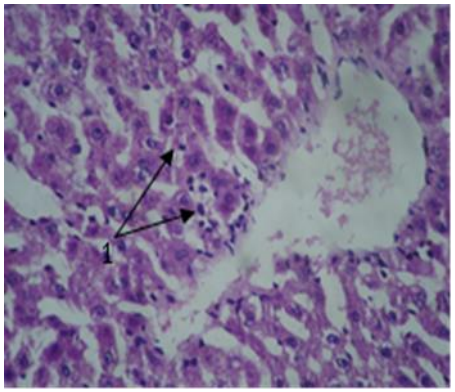
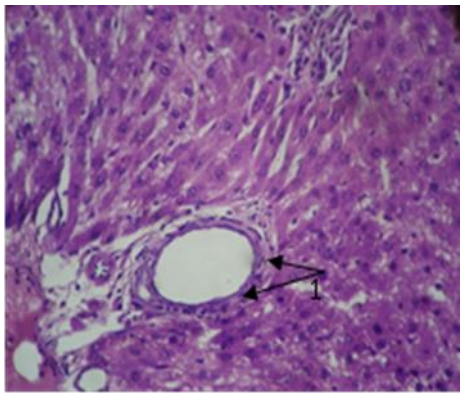


Figure 6e. BABE-400



DISCUSSION

Liver diseases 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.

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

In this 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. The recorded results suggested that the 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 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. 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.

CONCLUSION

The present investigation demonstrated the hepatoprotective activity of *Bauhina acuminata* Tamoxifen (TAM) induced hepatotoxicity in experimental rats. The bark extract of *Bauhinia acuminata* was found to be having 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.

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