

## Effects of Purified Bauhinia Variegata Leaves and Methanolic Extract on Diabetic Type-2 Alloxan-Induced in Male Albino Rats

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### Abstract

The anti-diabetic effects of purified Bauhinia variegata leaves and methanolic extract were Compared with standard drug Insulin. In vivo study reveals that animal treated with methanolic extract of Bauhinia variegata show reduction in blood glucose significantly, body weight, triglycerides, HDL, cholesterol in Alloxan induced diabetic rats. In Bauhinia variegata treated group, there are no significant increase in urea and creatinine level when compared to the control group which reveals the protective nature of the plant source. It concludes that In vivo antidiabetic activity of whole plant extracts of Bauhinia variegata on normal and Alloxan induced diabetic rats was evaluated and concluded that it possesses anti-diabetic activity. From the above results, it is conducted that purified leaves and methanolic extract of Bauhinia variegata whole plant possess significant hypoglycemic and hypolipidemic effects in alloxan induced diabetic rats.

**Keywords:** *Bauhinia variegates, Hypoglycemic, Hypolipidemic, Methanolic extract.*

### Introduction

Certain Plant and their constituent were used for the treatment of diabetes mellitus throughout the world; especially in countries where access to the conventional treatment of diabetes mellitus is inadequate. Diabetes mellitus is a disease that is expressed by a set of clinical syndrome as it influences the metabolism of carbohydrates, fats and blood proteins to produce chronic hyperglycemia. It is classified into two types, namely type I diabetes characterized by the massive destruction of  $\beta$  cells resulting in insulinopenia and the type II corresponding to a decrease in insulin secretion and insulin resistance [1].

Diabetics suffer not only from hyperglycemia, but also the hyperlipidemia and dyslipidemia. Hypercholesterolemia, high low density lipoprotein cholesterol (LDLc) and low high density lipoprotein cholesterol (HDLc) put the patients at an increased risk of coronary artery disease. Dyslipidemia is a major threat to the diabetics for it one of major risk factors of CAD, brain stroke, etc. Dyslipidemia is a change in the lipid fractions and predispose the patients to the CAD. [2, 3]. The mountain ebony, Bauhinia variegates L. belongs to the family

Leguminosae. It is distributed. Widely and planted in the tropics and warm regions of the world. (The Wealth of India Raw Material, 1952) belonging to the more than 200 species in the genus Bauhinia. It grows best in the full moon or partial shade. It easily propagates easily from seed and by air layering [4]. Many scientific studies showed that the B. variegata act as anticancer, antioxidant, hypolipidemic, antimicrobial, anti-inflammatory, nephroprotective, hepatoprotective, antiulcer, immune modulating, molluscicidal and wound healing activities [5].

Previous phytochemical investigations revealed that Bauhinia variegata contained many active gradient sterpenoids, flavonoids, tannins, saponins, reducing sugars, steroids and cardiac glycosides [5, 6, 7]. Show that the Bauhinia active compounds have the ability to reduce the level of sugar in the blood by creator alloxan as well as the treatment of other diseases associated with diabetes [8].

Found that the Streptozotocin (STZ)-high-fat diet (HFD) induced diabetic rabbits exhibited a significant increase in blood glucose, lipid peroxidation, liver enzymes and renal functions as well as decrease in insulin,

antioxidant enzymatic activities and catalase mRNA expression compared with the control group. However, concurrent administration of *Moringa Oleifera* leaves normalized the levels of all these parameters compared with the diabetic group. In this study the prolonged effect (up to 30 days) of the methanolic extracts of whole plant of *Bauhinia variegata* in fasting blood glucose (FBG) and biochemical parameters such as serum total cholesterol (TC), LDL, HDL, creatinine, urea and Total Serum Bilirubin (TSB) were studied in Alloxan induced diabetic rats. Thus the present study is an attempt to test the anti-diabetic activity of whole plant of the *Bauhinia variegata*.

## Materials and Methods

### Plant Material

*Bauhinia* tree leaves collected from garden Baghdad University in October 2016 and identified by the College of Sciences/ university of Baghdad. The leaves was washed the dried in shadow and then crushed by electric machine to a powder and leave it into airtight container.

### Extraction and Purification of Flavonoids

The extraction of flavonoids from the *bauhinia* tree leaves was done by several methods as follows

It was prepared according to the method of [9] with a certain modification: 100 gram of powder of the *Bauhinia* leaves was dropped in a bag of cloth then put it in the soxhlet extractor with 700 ml of the Methanol solvent for extraction and it conducted by using Soxhlet extractor at the temperature 50°C for 6 hours and the solution have been filtered and dried by rotary at 40°C. The dried extract have been weighed and stored at 4°C.

### Column Chromatography (Partial Purification)

A partial purification of the flavonoids is preceding using open glass column (2 x 30) cm was filled with silica gel G60 special for column chromatography. Five ml of methanol extracted of the leaves of *bauhinia* was subjected to column and eluted with methanol solution, and the flow rate regulated to be 60ml / min [10]. All fractions were tested for FeCl<sub>3</sub> 1% solution as

acolorimetric method for polyphenols identification [10, 11].

## Animals

Twenty-five rats average weight (250-300gm) was used in this experiment. Animals were housed in plastic cages in cooled room in the house of animal. They were selected randomly and caged in five groups of 5 rats for each group. The animals were leaving them for physiological adaptation. For 4 weeks:

Group I: Normal control (Control negative C)

Group II: Control positive C+ by given alloxan (150mg/Kg.ip).

Group III: Alloxan (150mg /Kg.ip)+ methanolic extract of *Bauhinia variegata* extract (400 mg /Kg.ip).

Group IV: Alloxan (150mg/Kg.ip) +.Purified leave (200 mg/ Kg.ip).

Group V: Alloxan (150mg/Kg.ip) +Standard drug, Insulin (0.1ml insulin 0.5g/ml orally)

## Biochemical Assays

The blood samples were collected and centrifuged at 3000rpm for 5 minutes using cooling centrifuge. The serum was kept at -20°C until analysed. Levels of Serum creatinine, urea, total cholesterol, triglycerides (TGL), HDL and total serum bilirubin were determined with an analytical instrument (Shimadzu, Japan).

Determination of serum Glucose Concentration (mg/dL) according to [12]. The cholesterol (mg/dL) had determined after enzymatic hydrolysis and oxidation according to [12]. The serum triglycerides concentration (mg/dL), serum HDL-Cholesterol concentration (mg/dL) and LDL-cholesterol was calculated according to [13]. The serum creatinine (mg/dL), serum urea (mg/dL) and Total serum bilirubin (mg/dL) had determined according to [14].

## Histopathological Studies

At the end of the thirty day, rats all five groups were sacrificed to take the liver, spleen, kidney and pancreatic tissues which processing after fixation by formaline 10% for histopathological examination according to the methods of [15] using paraffin sections technique. The organs were fixed in, and then dehydrated using increasing strengths of ethanol (70%, 80%, 90% and 100%) for

each two hours .Clearing of tissues using xylene, then impregnated with paraffin wax, heated for one to two hours in the oven at 60C and embedded L-shaped models. Blocks were cut into 5µm thick section by a microtome. Sections were mounted on glass slides using Haemtoxyaline and eosine stain. After staining, the sections were examined by light microscope under 100x and 400x magnification and photographed taken.

## Statistical Analysis

The Statistical Analysis System-[16] program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant coding mpare between means in this study.

## Lipid Profile

## Results and Discussion

Table (1) shown that the levels of triglyceride, HDL-C, VLDL-C and LDL-Cin the diabetic rats which significantly ( $p < 0.05$ )

increased except Cholesterol as compared with normal rats (Control positive C+). The leaves purified extract of Bauhinia variegata treated rats showed a significant ( $p < 0.05$ ) decrease in the content of lipid profiles when compared with diabetic induced rats. Also HDL-C level decreased in alloxan diabetic rats as compared with normal rats. Its hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis which is usually associated with diabetes [17].

HDL was found decrease in alloxan-induced diabetic rats compared to that of control rats group which is similar to previous study [18]. In light of the results, our study indicates that leaves purified component of Bauhinia variegata have good anti-diabetic activity [19]. Shown that the abnormal serum lipids in high concentrations of serum lipids in diabetic animals are mainly due to an increase of free fatty acids from peripheral fat deposits.

**Table 1: The Effect of difference treatments in lipid profile**

Treatment	Mean ± SE				
	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL	LDL	VLDL
Leaves Methanolic extraction	87.67 ± 0.67	68.33 ± 3.75	52.00 ± 2.00	22.00 ± 1.52	13.67 ± 0.75
Leaves Purified	101.25 ± 6.36	79.75 ± 7.51	39.25 ± 2.92	42.00 ± 5.22	15.90 ± 1.53
Insulin	105.00 ± 0.00	102.00 ± 0.00	33.00 ± 0.00	50.00 ± 0.00	20.40 ± 0.00
Control negative C-	68.00 ± 0.00	103.00 ± 0.00	44.00 ± 0.00	50.00 ± 0.00	20.60 ± 0.00
Alloxan Control positiveC+	97.00 ± 4.00	91.33 ± 11.21	43.00 ± 1.52	31.67 ± 4.40	19.27 ± 1.33
LSD value	12.778 *	20.564 *	6.265 *	11.311 *	3.429 *

\* ( $P < 0.05$ ).

## Effects of Leaves Methanolic Extract (ME) and Leaves Purified (LP) on Blood Glucose Level

Alloxan group of diabetic rats, the blood glucose levels was higher in Compared to the normal control group. Oral administration of leaves methanolic extraction (ME) and leaves purified (LP) at doses 400 and 200 mg/kg BW decreased blood glucose level in diabetic rats significantly ( $P < 0.05$ ) compared to the diabetic control group (Table2). These level of reduction was as near as insulin administered group ( $P < 0.05$ ). These results are agree with other study of diabetes induced by insulin resulted in asinificant elevation in blood glucose level in comparison with the control group after administration of extraction flower of Bauhinia variegata L. To

the diabetic mice for 10 days [7]. Alloxan was destroyed and decreases the population of pancreatic β-cell through the formation of oxygen species [20].

## Effects of Methanolic Extract (ME) and Leaves Purified (LP) of Bauhinia Variegata Extract on Blood Creatinine and Urea Level

In diabetes, the level of blood urea and serum creatinine were increased which may be due to renal damage caused by abnormal glucose regulation or elevated glucose and glycosylated protein tissue levels [21]. In present study, Non-significant increase in serum urea and creatinine levels were observed in diabetic rats compared to normal control rats which in dicate impaired renal function in diabetic rats.

The treatment with methanolic extract of Bauhinia variegata extract (ME) and Leaves purified (LP) of Bauhinia variegata lowered the above parameters significantly compared to diabetic control rats and it showed protective effect of Leaves extraction (ME) of Bauhinia variegata on the kidneys.

## Urea and Creatinine

Blood urea and serum creatinine were elevated in Group2 (control negative) as in

compared to the groups leaves methanolic extraction (ME), Leaves purified (LP), insulin group and positive control with control negative, as shown in (Table2). This shows that in the groups which received Bauhinia variegata extract there was no significant increase in urea and creatinine. These results are agreed with other study of diabetes induced by streptozotocin [22].

**Table 2: Effect of difference treatments in serum Creatinine, Urea, Glucose and TSB level**

Treatment	Mean $\pm$ SE			
	Creatinine	Urea	TSB	Glucose
Leaves Metnaolic extraction (ME)	0.566 $\pm$ 0.14	33.00 $\pm$ 2.00	0.533 $\pm$ 0.12	97.33 $\pm$ 5.33
Leaves purified (LP)	0.550 $\pm$ 0.15	32.50 $\pm$ 3.17	0.675 $\pm$ 0.11	109.50 $\pm$ 9.86
Insulin	0.600 $\pm$ 0.00	30.00 $\pm$ 0.00	0.300 $\pm$ 0.00	119.00 $\pm$ 0.00
Control	0.600 $\pm$ 0.00	33.00 $\pm$ 0.00	0.500 $\pm$ 0.00	81.00 $\pm$ 0.00
Alloxan positive (C+)	0.433 $\pm$ 0.03	32.33 $\pm$ 2.96	0.500 $\pm$ 0.06	103.33 $\pm$ 8.95
LSD value	0.335 NS	7.425 NS	0.266 *	22.533 *

\* (P<0.05), NS: Non-significant.

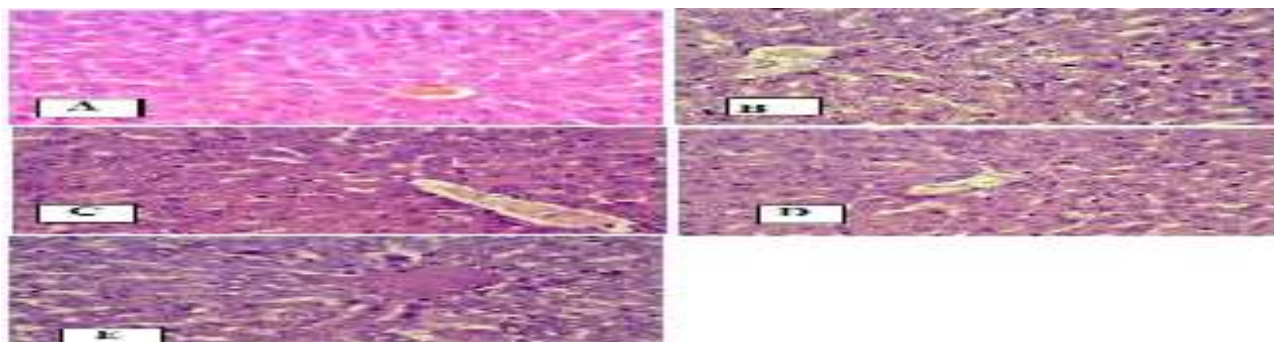
## Histopathological Studies

Histology of the liver sections of rats of different groups is shown in Figure (1). Figure (1A), for the negative control, shows that liver tissue is composed of hepatocytes red cells with nucleus round purple. Groups of hepatocyte cells are separated by sinusoid, with start accumulation of fatty changes.

The negative control did not reveal any characteristics of cell death or necrosis. Figure (1B) shows that sinusoid is widening or dilate, there is hemorrhagic or bleeding, and cell necrosis (positive control). Treatment with Bauhinia variegata supplement in Figure (1C) shows that liver lobule can still be observed, cell hepatocytes appears purplish red and the flecks are purple-black, sinusoid in dicates regularity that leads

toward the central vein, and cell necrosis. Figure (1D) shows that hepatocyte cells are pink and are arranged regularly and radial, but there is asinusoid which is widening or dilate. Cell necroses appear. Acai treatment (Figure1E) shows pink hepatocytes with rounded cell nuclei, hepatocyte plates are clearly visible and organized, sinusoidal dilation does not appear, and there is visible presence of cell necrosis [7].

Shown that the Bauhinia active compounds have the ability to reduce the level of sugar in the blood by creator alloxan as well as the treatment of other diseases associated with diabetes. And they concluded that the plant insulin (Glucokinin) may be responsible for some of the actions as plant extracts for their antidiabetic properties.

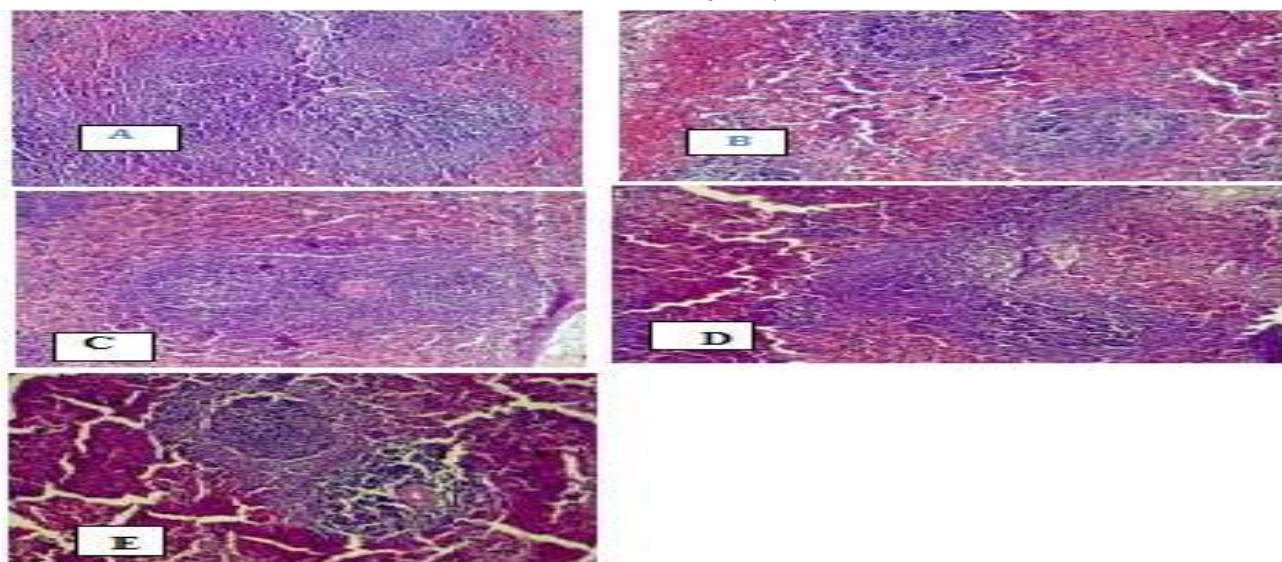


**Figure 1: Photomicrographs of liver sections. A: Livertissue of rat negative control, B: Liver tissue of rats treated with alloxan as positive control, C: Liver tissue of rats treated with crude leaves of Bauhinia variegata, D: Livertissue of treated with purified leaves extract, E: Section showing atrophy of hepatocyte cells with dilatation of hepatocyte cells and few dispersed apoptotic cells treated with insulin.(X400 H&E)**



## The Spleen Histopathology

Histological appearance of spleen sections as represented in Figure (2). Figure (2A) shown that the normal spleen structure appearance of white and red pulp but congestion and haemorrhage as a negative control. Figure (2B) the Section showing prominent widening of white pulp with no more red pulp structure exists but with congestion and haemorrhage as positive control with treated alloxan.

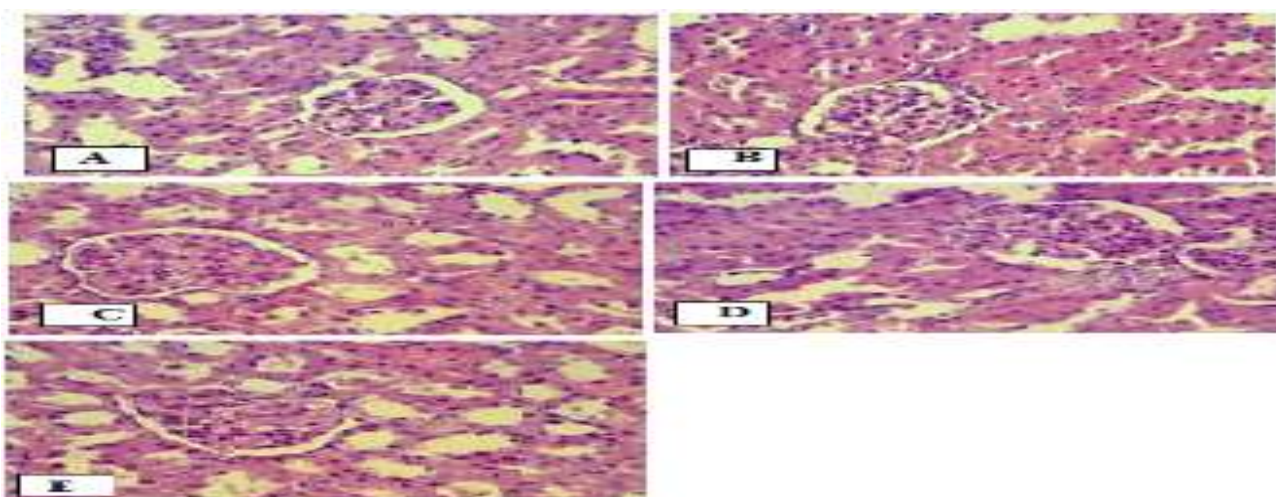


**Figure 2: Spleen Histopathology.** A: Spleen tissue of rats as control negative without treated, B: Spleen tissue of rat Positive control treated with alloxan, C: Spleen tissue of rats treated with methanolic leaves extract of *Bauhinia variegata*, D: Spleen tissue of treated with purified leaves extract, E: Spleen tissue of rats treated with insulin ;. (X100 H&E)

**The Kidney Histopathology:** Kidney histopathology was done qualitative observation used to describe the Kidney cells among the treatment groups (Figure 3). Figure (3A) Kidney: Section showing look like normal structure appearance as a negative control. (3B) Section of the kidney showing normal structure appearance of glomeruli and renal tubules as a positive control. (3C) Section showing slight degenerative changes

Figure (2C) the spleen showing widening of white pulp with no more red pulp and congestion with hemorrhage treated with methanolic leaves extract. Figure (2D) showing widening of white pulp with no more red pulp seen with congestion and haemorrhage treated with pure leaves. Figure (2E) the section showing widening of white pulp with no more red pulp and still congestion and haemorrhage treated with insulin.

of renal tubules especially proximal convoluted tubules after treated with crude leaves. (3D) section showing normal structure like appearance which consist of glomeruli and renal tubules after treated with pure leaves of *Bauhinia variegata*. (3E) Section showing mild degenerative changes of renal epithelial cells especially proximal convoluted tubules after treated with insulin.



**Figure 3: Kidney Histopathology.** A: Kidney tissue of rats as control negative, B: Kidney tissue of rat positive control treated with alloxan, C: Kidney tissue of rats treated with methanolic leaves of *Bauhinia variegata*, D: Kidney tissue of treated with purified leaves extract E: Kidney tissue of rats treated with insulin. (X400 H&E)

The Pancreas Histopathology: Figure 5 shows pancreas histopathology differences among the treatment groups. Figure (4A) section showing normal structure appearance with rare apoptotic cells of endocrine cells control negative. Figure (4 B) showing the dispersed apoptosis of endocrine cells treated with alloxan. And Fig (4C) showing the: Section showing look like normal structure

appearance of endocrine cells treated with leaves methnaolic extraction. Fig (4D) showing few dispersed apoptotic endocrine cells but the majority were look like normal structure treated with pure leaves. Figure (4E) the section showing still there were apoptotic of endocrine cells with fatty treated with insulin.

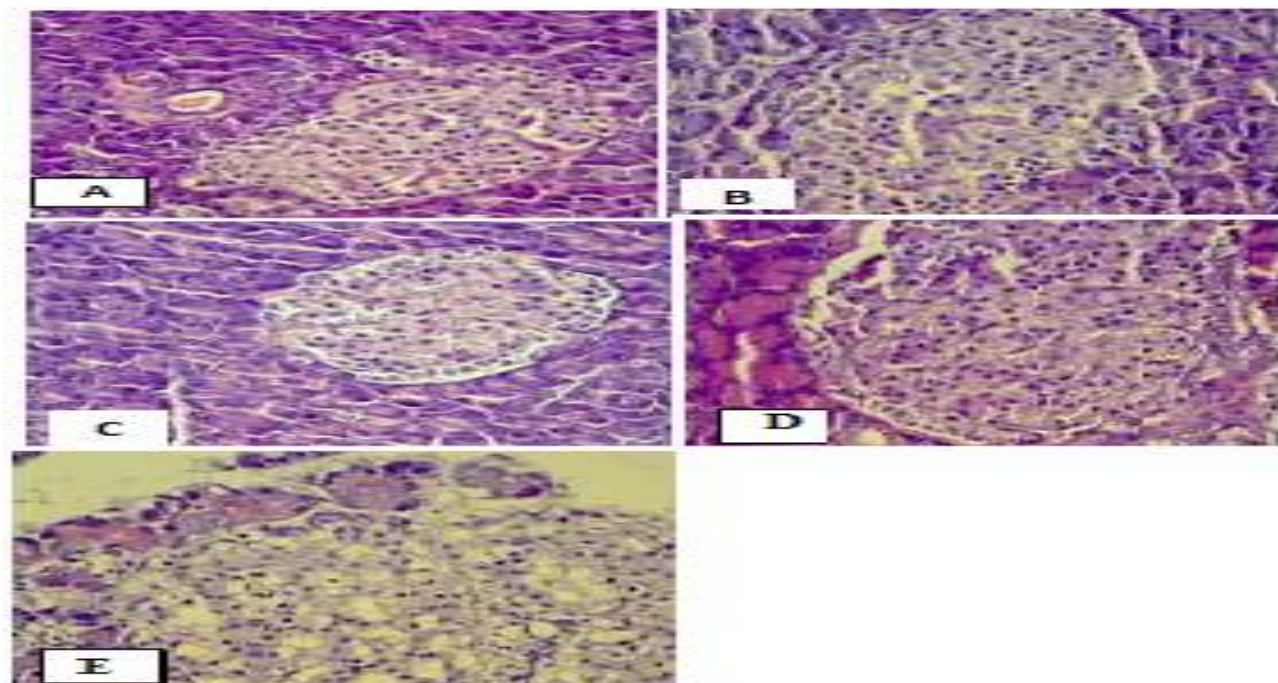


Figure (4): Pancreas Histopathology. A: Pancreastissue of rats as control negative, B: Pancreas tissue of rat positive control treated with alloxan, C: Pancreas tissue of rats treated with methanolic leaves extract of *Bauhinia variegata*, D: Pancreas tissue of treated with purified leaves extract, E: Pancreastissue of rats treated with insulin. (X 400 H&E)

## Conclusion

In-vivo studies of *Bauhinia variegata* extract revealed the glucose lowering effect and increase the activity of carbohydrate metabolizing enzymes. Photomicrographical

data of histopathological studies in our studies extract of *Bauhinia variegata* pure leaves treated groups show that a dose 200 mg/Kg/BW per day has higher effect than the methanolic leaves extract of 400 mg/Kg/Bw per day.

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