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Biochemical, Haematological, and Histopathological Changes caused by Acacia Nilotica on Albino Rats

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Abstract: **This current study aimed to evaluate the hypoglycemic, hypolipidemic, hematological, thermogenic, biomedical, and toxic effects in Albino Rats caused by Acacia nilotica ethanol extract. Acacia nilotcia pods were harvested in Sudan. The animals used in the study were 24 albino Rat males (90-125g). 10 grams of each powdered plant sample were refluxed with 100 ml of 80% of ethanol four 4 hours. The cooled solution was filtered and enough 80% ethanol was passed through the volume of the filtrate to 100 ml. This prepared extract (PE) was used for the various tests. Rats plasmas were tested for Lipid profile, whole blood count, renal functions, and liver functions. Histological sections were taken from the liver and kidneys of the Rats. Result were expressed as mean+_SEM . The data were subjected to one –way analysis of variance (ANOVA). Acacia nilotica administration significantly raised the levels of Proteins, Albumin, ALT, Hemoglobin, RBCs, PCV, and MCV. Acacia also significantly lowered the levels of Blood sugar (RBs), WBCs, Platelets, and caused a marked loss of body weight in albino rats. The changes recorded in Triacylglycerols, Cholesterol, AST, ALP, Urea, or Bilirubin, were statistically insignificant. Administration of Acaia extract to rats can improve their blood profile and protein content, reduce their weights, but also increase their bleeding tendency.**

Keywords: **Acacia nilotica, Ethanol extract, Albino Rats, Blood levels.**

1. INTRODUCTION

Acacia nilotica (family leguminosae, sub family mimosoidease) is naturally widespread in the drier areas of Africa, from Senegal to Egypt and down to South Africa, and in Asia from Arabia eastward to India , Burma and Sri Lanka; see figure (1). Mostly it occurs as an isolated tree and rarely found in patches. It also could be found to a limited extent in India subcontinent as a species of Southern Tropical deciduous forests and Southern tropical thorn forests [1]. Acacia species contains highly effective biologically active substances; (pods) contain secondary metabolites including amines and alkaloids, cyanogenic glycoids, cuclitols, fatty and seeds oils, fluroacetate, gum, non protein, and protein amino acids [2]. The leaf contains apigenin, 6-8 – bis D- glucoside, rutin, and crude protein. The Acaia roots contain ocata consanol, betulin, B-amyrin and B- sitosterol. Gum is of Acacia composed of galactoaraban which gives on hydrolysis L- arabinose, D-galactose,-L-rhamnose, D- glucuronic acid and 4-O- methyl- D-glucuronic acid [3]. *Acacia nilotica* also has numerous medicinal uses. The medicinal traits and pharmacological activities endorsed to various parts of Acacia are detailed as follows:

- Anti- hypertensive and anti – spasmodic activities [4].
- Antibacterial and antifungal activities also were shown at administration of Acacia [5]; *Acacia nilotica* demonstrates higher activity against three bacterias: *Eshirechia coli*. *Staphylococcus aureus* and *Salmonella typhi*) and two fungal strains: *Candida albicans* and *Aspergillus nigar* [6].

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- Anti plasmodial activity: The early acetate extract of Acacia holds the highest activity on *plasmodium falciparum*. The root extract of *Acacia nilotica* reveals significant activity against *plasmodium berghei* in mice [7].

- Antioxidant activities: Water extract / fractions of *Acacia nilotica* possess the peroxyl radical scavenging capacity and results prove the anti-oxidant activity of the plant. Other antioxidants, which can be used as supplement to aid the therapy is the phenolic compounds that can scavenge the free radicals [8, 9].

- Survival of rats: ethanol extracts of Acacia nilotica of 187 mg/kg/bw killed two of five (40%) of experimental rats, while a dose of 500 mg/kg/bw killed 100% [10].

The potential effects of the Acacia are still to be explored. This current study aimed to evaluate the hypoglycemic, hypolipidemic, hematological, thermogenic, biomedical, and toxic effects in Albino Rats caused by *Acacia nilotica* ethanol extract.

Figure (1): Shows *Acacia nilotica* pods.

2. MATERIALS AND METHODS

This was an experimental animal study performed in the Faculty of Science and Technology in Omdurman Islamic University, Sudan, in the period from April to August 2019.

- **Plant collection:**

Acacia nilotcia pods were harvested in Sudan, bought pods from Omdurman market. They were pounded to powder, using a pestle, 100mg/kg/bw were weighted for each rats.

- **Animals:**

The animals used in the study were 24 albino Rat males (90-125g), they were maintained in an experimental animals house at aromatic plants research institute (MAPRI), Khartoum Sudan. They were kept in rat cages and fed on commercial rats food, on the start of the experiment all the animal were weighted.

- **Preparation of the extract:**

10 grams of each powdered plant sample were refluxed with 100 ml of 80% of ethanol four 4 hours. The cooled solution was filtered and enough 80% ethanol was passed through the volume of the filtrate to 100 ml. This prepared extract (PE) was used for the various tests.

- **Collection of blood and serum samples and plasma:**

Periodical blood samples were collected by cervical decapitation from diethyl ether anaesthized rats into heparinized bottles for hematological studies. Blood samples collected in clean Non- heparinized bottles were allowed to clot.

- **Histopathology:**

The liver and kidney of all rats were fixed to preserve tissues for degradation and to maintain the structure of organelles, in buffered formalin in labeled bottles. Tissues were processed routine and water removed from tissues after dehydrated and cleared. Tissues were then filtered with embedding in paraffin wax. Sections of 5µ thicknesses were cut with microtome, and stained with hematoxylin and eosin (H&E stains) and examined under the light microscope and Photomicrographs were taken in Khartoum hospital.

- **Hematology:**

Lewis (1991) manual Methods were followed to determine Hemoglobin concentration (Hb%), Packed cell volume (PCV %) , Red blood cells count (RBCs), White blood cells count (WBCs), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular hemoglobin (MCH), and Red cell distribution (RDW).

- **Sero- biochemical analysis:**

The tests involved were performed through spectrometry: Total protein; Total urea and creatinine; serum Albumin; Total bilirubin; Determination of triglycerides; Determination of cholesterol; Glucose determination; Aspartate Amino-transferase (AST); Alkaline phosphatise; and Alanine amino-transferase (ALT)

- **Statistical analysis:**

Result were expressed as mean+_SEM . The data were subjected to one –way analysis of variance (ANOVA) test and differences between samples were determined by Dunnetts multiple comparison test, using the Graph pad prism (statistical) software. Results were considered to be significant at $P<0.05$.

3. RESULTS

After Administration of aqueous solution of *Acacia nilotica* pods in a dose of 100mg/ kg bodyweight to albino rats for 21days, the following results were documented (Table 1):

Effect of *Acacia nilotica* pods on cholesterol and triacylglycerol: Administration of *Acacia nilotica* to rats for 21days caused no significant change on lipids levels (Triacylglycerols and Cholesterol).

Effect of *Acacia nilotica* on plasma glucose: Aqueous Acacia caused significant reduction of plasma glucose ($P<0.05$)

Effect of acacia pods on body weight: there was a significant reduction on gross body weight of the rats ($p< 0.05$)

Effect of A*caica nilotcia* pods on plasma protein and albumin: Administration of Acacia solution increased protein and albumin levels significantly ($P<0.05$)

Effects of A*cacia nilotica* pods on liver enzymes and histology: A*cacia nilotica* pods caused significant elevation of ALT; an indicator of liver damage.

Effect of A*cacia nilotica* pods on hematology: In this study administration of aqueous Acacia nilotica pods100mg/kg/bw to rats for 21 days showed significant elevation of RBCs , PCV , Hb , MCV. On the other hand, Acacia caused significant reduction in WBCs and platelets ($P<0.05$).

Effect of Acacia on Liver histology: Histology sections in the Rats' livers showed sinusoidal dilatation and dissociation and atrophic changes of hepatocytes; see figure (2).

Effect of Acacia on renal functions and renal histology: There was a significant reduction in creatinine ($P<0.05$) but no significant change in urea level. Histologic kidney sections showed progressive damage. Most kidney sections showed dilatation of bowman space and tubular nuclei not recognized glomerular membrane thickening; see figure (3).

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Table (1): List of the results of Acacia nilotica administration to albino rats for 21 days.

Test	Value before acacia (control)	Value after acacia (test)	P-value of the difference.
Triacylglycerols.	82.8	82.76±081	> 0.05
Cholesterol.	55	50.83±079	>0.05
Blood sugar (RBs)	74	65.81±091	**<0.05**
Body Weight	97.2	85.71±091	**<0.05**
AST (iu)	135	116.6±9.02	>0.05
ALT (iu)	42	70.83±9.08	**<0.05**
ALP (iu)	152.5	82.83±38.6	>0.05
Protein (g/ dl)	7.2	7.467±0.13	**<0.05**
ALB (g/ dl)	4.2	4.433±0.09	**<0.05**
Bilirbin (mg/ dl)	0.1	0.100±0.00	>0.05
RBC ($x10^6mm^3$)	6	6.925±0.11	**<0.05**
MCV (m^3)	55	60.68±0.84	**<0.05**
MCH (pg)	21	20.47±0.40	>0.05
MCHC (%)	33	33.62±0.93	>0.05
WBC (x106mm3)	9.3	8.41±0.93	**<0.05**
RDWSD (%)	30	30.55±1.89	>0.05
HGB (mg/dl)	13	14.28±0.32	**<0.05**
PLT (10^3/ml)	275	25.75±2.04	**<0.05**
PCV (%)	41.8	54.78±0.91	**<0.05**
Urea (mg /dl)	30	27.43±3.49	>0.05
Creatinine (mg/ dl)	1	0.767±0.11	**<0.05**

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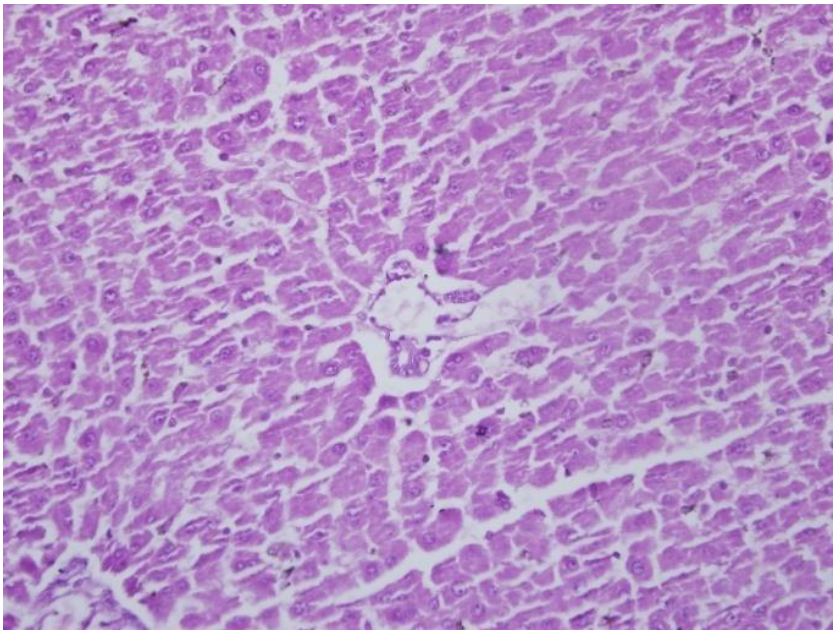

Figure (2) photomicrograph section through the liver of albino rat with A*cacia nilotica* showing sinusoidal dilatation and dissociation of hepatocytes and central dilatation and atrophy in some parts.

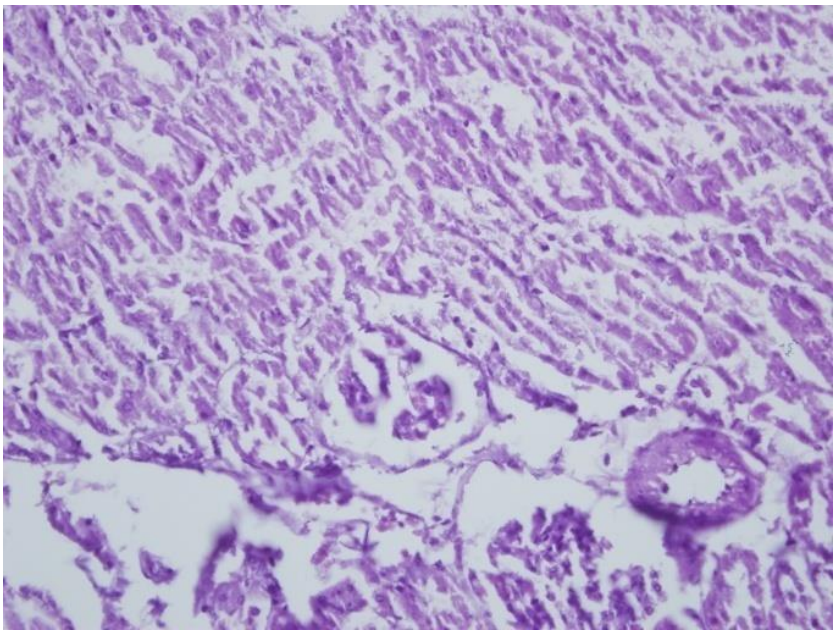

Figure (3) Photomicrograph section through the kidney of albino rat with A*caica nilotica* (0.2%w/v) showing dilatation of bowman space and tubular nuclei not recognized and glomerular membrane thickening.

4. DISCUSSION

Acacia nilotica is a widespread plant in Sudan. The pharmacologic uses of this plant are emerging every day and it still representing a good media for research. The focus of this current research was to detect the potential effect of *Acacia nilotica* in specific chemical, hematological, and tissue parameters.

Acacia nilotica solution at 100mg/kg caused no significant change on lipids levels; TAG and Cholesterol, This report disagrees with other reports Ahmad and Zaman 2008 research where it had been mentioned that *Acacia nilotica* had a lipid lowering effects in the plasma of rabbits with induced Diabetes [11]. Acacia pods contain phytosterol, and this substance is chemically similar to cholesterol and is able to compete with cholesterol for reabsorption. When the

intestine lining cells absorb phytosterol instead of cholesterol, they usually excrete the phytosterol. Therefore Phytosterol may play a role in the above result. Maciejewski et al commented that administration of Acacia to rabbits showed significant reduction in TAG and cholesterol LDL and increase in HDL [12].Tannins which is found in Acacia lead to protein precipitation and this may affect Apo protein in lipoproteins.

Administration of Aqueous Acacia 100mg/kg/bw for 21days caused significant reduction of plasma glucose ($P<0.05$) this report is in agreement with Asad, Munir, and Afzal research of 2011 [13], where fasting rats managed with Acacia and an oral hypoglycemic drug; glyburide, scored a significantly reduced fasting blood glucose. Glucose homeostasis is maintained by a balance between the release and action of insulin on one hand, and the action of its antagonists such of glucagon, catecholamines growth hormones and cortisol. It is possible that Acacia pods may have a multitude of factors that act singly or in combination to exert its hypoglycemic effect. Furthermore *Acacia nilotica* pods have crude fiber and cellulose dietary fiber, this substance may lead to reducing blood sugar.

Acacia nilotica pods extract is rich in tannins and polyphenols. This may decrease the blood glucose level [14].

Flavonoid may affect carbohydrate metabolism by inhibition of glycosidase and amylase, the key enzymes responsible for digestion of dietary carbohydrate to glucose [15]. Some polyphenols are able to regulate the key pathways of Carbohydrate metabolism and hepatic glucose homeostasis including glycolysis that consumes glucose, and glycogenesis that suppresses glucose in blood by elevation of glucokinase.

The WHO (1994) has listed more than 400 herbal plants that effectively decrease blood sugar [16]. In Streptozotocin (STZ) induced diabetes rats phytochemical studies have recently shown that hypoglycemic effect of these plants is due to presence of tannins and polyphenol compounds that have anti-oxidant properties [17].The tannins restore the function of pancreatic beta cells and stimulate release of insulin, while polyphenols reduce the blood glucose level through inhibition of β-glucosidase enzyme from intestine [11].

A significant reduction on rats' body weight was achieved by using the Acacia extract for three weeks. This came in agreement with Xueqing et al (2005) works [18]. Acacia pods may affect intestinal absorption. In the intestine Acacia pods were widely used to treat dysentery that damages the villi .

Acacia contains tannins such as tannic acid that stimulate glucose transport and inhibit adipocytes differentiation [18]; this maybe the reason of body weight reduction. Also Acacia contain flavonoid which acts as a thermogenic substance and may be burn fats [19].

The given Acacia dose increased protein and albumin significantly in Rats plasma. Acacia are very rich in crude protein and amino acids such particularly Cysteine, Methionine, Threonine, Lysine and Tryptophan, all of which participate as building blocks for protein synthesis, this may have lead to elevated albumin and proteins as general [7].

Administration of aqueous A*cacia nilotica* pods100mg/kg/bw to rat for 21 days yielded a significant elevation of ALT. ALT is present moderately in liver and to a lower extent in cardiac and skeletal muscle and other tissues. On the basis of these finding, the results of the present study demonstrate that *Acacia nilotica* may cause sub-acute toxicity [20].

In this study Acacia nilotica induced significant elevation of RBCs , PCV , Hb , MCV and reduction in WBCs and platelets ($P<0.05$). The A*cacia nilotica* pods are very rich in iron and folic acid. These compounds probably have participate in the synthesis of heme and amino acids - like cysteine and tryptophan- which participate in globulin synthesis. For these reasons these compounds may lead to elevated RBCs [21]. WBCs were reduced, but also within the reference ranges. This reduction of WBCs could also be attributed to the tannins in Acacia [22].

In this study administration of Acacia, to rats showed significant reduction in platelets, ($P<0.05$). Usually thrombocytopenia associated with reduction in RBCs and WBCs are primary, but platelets reduction could be secondary process (due to destrution). Although platelets were reduced, there were no thrombocytopenic signs on rats such as ecchymosis. Administration of Acacia for longer time maight lead to symptomatic of thrombocytopenia. Reduction of platelets could appear at its most severe forms as hemolytic anemia and renal failure [23]. Liver enzymes and renal functions test can explain the cause of platelets reduction [24].

Acacia caused significant reduction in creatinine ($P<0.05$) but not in urea. This disagrees with Medani, Samia, and Ahmed research of (2016), as they concluded that oral doses of acacia (1g/kg/day) given to domestic goats lead to significant elevation of both Urea and Creatinine [25]. The high dose of acacia might have played a role in their results.

Histological examination supported these biochemical results and kidney sections showed progressive damage. Most kidney sections showed dilatation of bowman space, tubular nuclei were not well recognized, and there was a visible thickening in the glomerular membrane.

Acacia contains secondary metabolites such as glycosides. These macromolecules may affect kidney, no doubt kidney damage affects kidney function. A progressive glomerulosclerosis and fibrosis may be induced after long time, ending to renal failure [26]. *Acacia nilotica* contains arachidonic acid which play an important role in prostaglandin E synthesis .Administration of Acacia for 21days may lead to over production of prostaglandins E and kallikrein which lead to hyper filtration and vasodilation in the kidney [27].

Tannins are known to lower the rate of protein degradation and deamination, which lead to lower ammonia concentration, hence a lower plasma urea [28].

Tannins increase fecal nitrogen excretion increase by lowering the digestibility of nitrogen and forming of Tannins-protein complex, and this decreased the urinary excretion of nitrogen including Urea nitrogen. According to Edard (2011), saponins of Acaci are able to inhibit urea cycle enzymes and hence decreasing blood urea. On another side, Saponin decrease the level of creatinine [29].

5. CONCLUSION

This was an experimental study in *Acacia nilotica* effects in albino rats. The study involved measurements of biochemical, hematological, and histological changes in rats after exposure to 100mg/kg of acacia solution for three weeks. Acacia nilotica administration significantly raised the levels of Proteins, Albumin, ALT, Hemoglobin, RBCs, PCV, and MCV. Acacia also significantly lowered the levels of Blood sugar (RBs), WBCs, Platelets, and caused a marked loss of body weight in albino rats. *Acacia nilotica* should be avoided in liver and kidney disease, because it affects their functions. *Acacia nilotica also* should be avoided in anemic patients, especially hemophilic patients. Patients, who take Acacia nilotica in a dose more than 100mg/kg/bw need to be careful, because it may lead to kidney damage.

Conflicts of interest: None.

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