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Diuretic and Hepatoprotective Activity of Aqueous-Alcoholic Combined Extract of *Acacia Sinuata* Pods and Bark

Satyanarayana Dammavalam[1]*
1. ASN Pharmacy College, Burripalem road, Tenali, Guntur District, Andhra Pradesh.

ABSTRACT

The current study deals with the investigation of diuretic and hepato protective activity of aqueous alcoholic extract of *Acacia Sinuata* bark and pods. The method and procedure for diuretic activity is followed by Lipschitz *et al*. and the method for hepato protective activity is followed by syed mansoor ahamed *et al.* The present indicates regarding the chemical constituents present in the *Acacia Sinuata* pods and the bark extract. The preliminary phyto-chemical investigation showed the presence of alkaloids, flavonoids, terpenoids, saponins, glycosides, tannins and phenolic compounds in an aqueous alcoholic extract. The result obtained from the diuretic activity indicates the increased urine output and the increased quantities of sodium, potassium and calcium levels in collected urine. The result obtained from the hepato-protective activity indicates that the dose of 500mg/kg possessed significant hepatoprotective activity. It shows that saponins, glycosides, present in this extract may be possibly responsible for the pharmacological action.

Keywords: Aqueous alcoholic extract, saponins, glycosides, diuretic, tannins.

*Corresponding Author Email: satyapharmacology@gmail.com
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INTRODUCTION

From the ancient days the herbal medicines are used for medicinal use, but there is no proper drug to cure the hepatic disorders**.** A perennial, woody, large climbing shrub grows on big trees of acacia sinuate (Lour.) belonging to the family Mimosaceae. Leaves are bipinnate, consisted with sharp prickles on main rachis which is having Pinnate 8 -10 pairs, with small leaflets; small flowers with heads and fruits are like thin pods with 6-10 seeds per pod. The aqueous alcoholic extract of pods of acacia sinuate (lour.) has been used for many medicinal purposes like anti-microbial, anti-oxidant, hair conditioning agent, and as a purgative agent. In this present study the aqueous-alcoholic extract are combined with pods and bark of Acacia sinuate and studied the diuretic[1] and hepato-protective activity. Normal urine contains Na+ and K+[2] as the major cations and excretion varies between 2-4gm /day, the excretion of chloride is about 10g.Renin angiotensin system, glomerular filtration rate(GFR) and atrial natriuretic factor (atriopeptin) regulate body sodium level and thus the blood volume and cardiac output can be maintained in healthy patients. Sodium retention due to failure of this haemostatic mechanism is the important factor that plays a crucial role in causing hypertension,[3-6] and causes chronic renal failure. Increase in K+ levels[7] caused due to renal failure, aldosterone deficiency, diabetic acidosis[8]. The disease conditions among the cardiovascular system, the kidneys, the central nervous system (Na+ plays a crucial role in the regulation of appetite and thirst).

MATERIALS AND METHOD

The collected plant of Acacia sinuate was authenticated by Prof. Dr. K. Sekar, Ph.D. Department of Horticulture, faculty of agriculture, Annamalai University, Chidambaram. The authentication number is AU/HORT/2010/36. The pods were dried in sunlight for two to three days and then dried under shadow. The dried pods were powdered and the powder was passed through the sieve NO. 60. All the chemicals, reagents and solvents as per the laboratory grade.

Extraction Method

A dry round bottom flask was fitted with a surface condenser. Dry porcelain pieces taken in flask followed by 580ml of ethanol with 248ml of water and heating was continued. Then reflux it for 40hours to get the crude extract. The ethanol is directly distilled in to the vessel and used. The powered pods of *Acacia Sianuata* was placed in soxhlet apparatus and temperature is maintained at 70*c .The alcoholic extract was prepared by this process. The alcohol present in the extract was removed by distillation process. The crude extract was collected and then packed in a well closed container .The preliminary phyto-chemical analysis has been done for the identification of various

chemical constituents such as alkaloids, saponins, glycosides, tannins, carbohydrates, sugars, flavonoids, steroids, amino-acids, tri-terpenoids, fixed oils and fats[9].

Pharmacological Studies

Oral Acute Toxicity Studies

Acute oral toxicity was performed by using OECD GUIDELINES -420 and Fixed dose procedure. Fifteen male Albino rats weighed about 100grams were used for the study. The dose has been fixed for about 250 mg, 500 mg, 750mg, 1000mg, 2000mg were given orally to allow the identification of dose producing evident toxicity for the aqueous-alcoholic extract of combined pods and bark of Acacia Sianuata. Up to 7 days the signs for toxicity have been observed. Food was with held for a further of 3 - 4 hours after the administration of drug. The crude extract possess LD - 50 more than 2 gm /kg (LD 50 > 2 gm /kg). Body weight of the rats before and after the administration was noted and any changes in skin, fur, eyes, mucous membrane and also other behaviour pattern were observed. The experiments were carried out in conformity with the institutional animals Ethics Committee (IAEC) after obtaining its permission (Approval number: IAEC/II/02/CLBMCP/2012- dated 19-12-2012).

Procedure for Evaluation of *In Vivo* Diuretic Activity

The experimental animals were divided into four groups and each group consists of six animals. Food and water were withdrawn for 18hrs prior to the experiment. On the day of experiment, the group- I animals receives normal saline (5ml/kg, p.o.). The group-II animals receives furosemide (10mg/kg, i.p.) the group III and IV animals receives aqueous-alcoholic extract of combined pods and bark of *Acacia Sinuata* 250 mg/kg p.o and 500 mg/kg p.o.[10] respectively. After the administration, the animals were placed in metabolic cages (two per cage) which was designed specially to separate urine and faecal matter and placed at room temperature. The urine obtained was collected at the end of 24h. By the time of collecting no water and food was given to the animals. The parameters related to the diuretic activity are total quantity of urine and concentrations of Na^+, K^+ and Cl^- were measured. The measurement of the Na^+ and K^+ concentrations was done by flame photometry and Cl^- concentration was estimated by titration with silver nitrate solution using potassium chromate as indicator[11, 12].

Procedure for *In Vivo* Hepatoprotective Activity

The total number of 30 male albino rats[13] were divided into five groups consist of six animals in each group [14] and first group is a control received normal saline 1 ml/kg. p.o for 7 days. The second group of six animals was treated with aqueous-alcoholic extract of combined pods and bark of Acacia Sinuata 250 mg/kg p.o for 7 days. The third group of animals were received 1% CMC

1ml/kg orally for seven days and on 5th day Acetaminophen 2 gm/kg suspended in 1% CMC has been administered orally as a hepato-toxicity inducing agent. The fourth group were received aqueous-alcoholic extract of combined pods and bark of *Acacia sinuata* 500 mg/kg p.o for 7 days, and on 5th day, Acetaminophen 2 gm/kg suspended in 1% CMC given orally as an hepato-toxicity inducing agent. The fifth group of animals was treated with Silymarin 25 mg/kg body weight for seven days as a standard drug and on fifth day administration of Acetaminophen 2 gm/kg, suspended in 1% CMC as a hepato-toxicity inducing agent[15].

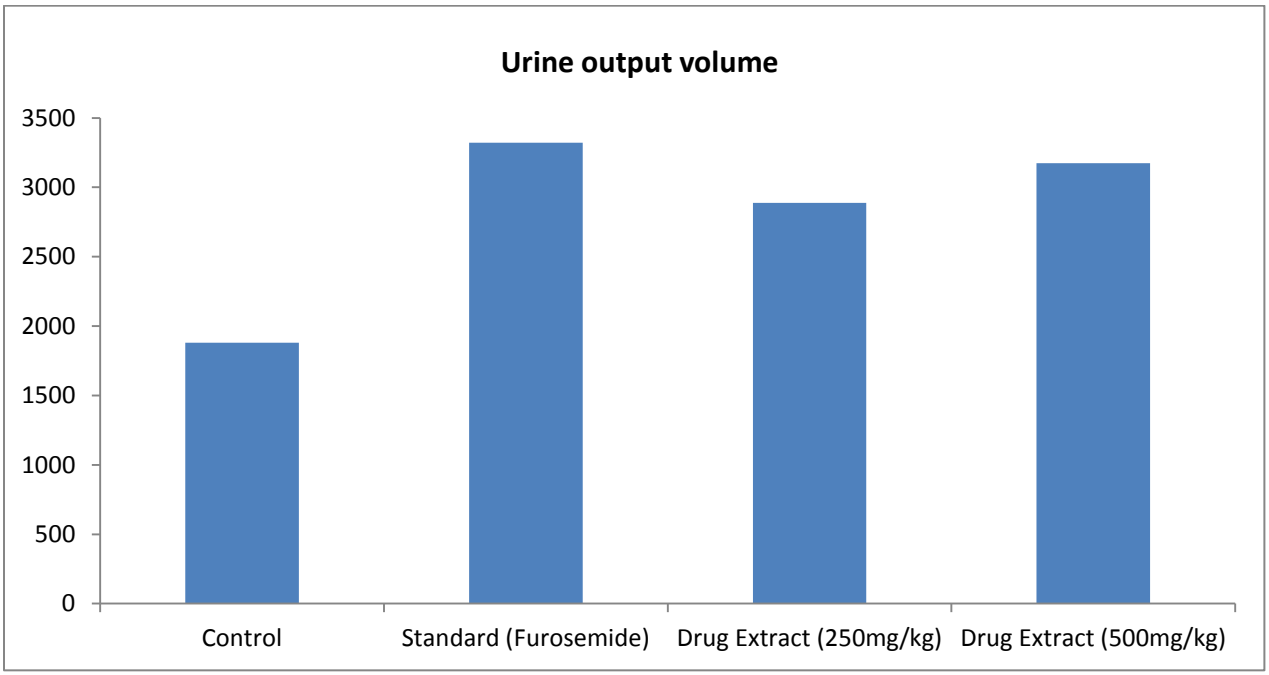
RESULTS AND DISCUSSION

In Vivo Diuretic Activity

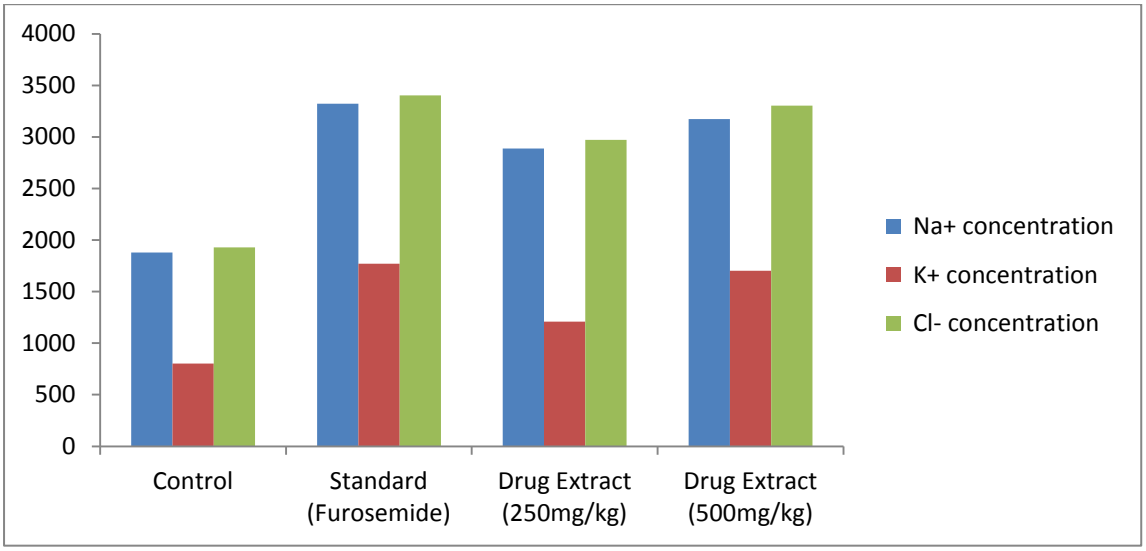
Table-1: Effect of *Acacia Sinuata* Drug Extract on Urine Volume and Electrolyte Concentrations of Collected Urine

S NO	Treatment	Dose(mg/KG)	Urine Volume (ml)	Electrolyte Excretion		
				Na+ µmole/kg	K+µmole/kg	cl-µmole/kg
1.	Control	10 ml/kg	2.4±0.21	1880±35	804±32	1930±21
2.	Furosemide	10mg/kg	4.9±0.75**	3322±66**	1770±301**	3402±59**
3.	Drug extract	250mg/kg	3.2±0.26*	2887±53*	1209±488*	2971±23*
4.	Drug extract	500mg/kg	4.7±1.05**	3174±42**	1701±149**	3302±10*

N=6, data was expressed as mean ± S.E.M; *p<0.05 an d *p<0.001 as compared to normal control (one way A NOVA followed by student "t" test).



Graph 1: Regarding Urine Output Volume of *Acacia Sinuata* Plant Extracts 250 mg/500 mg



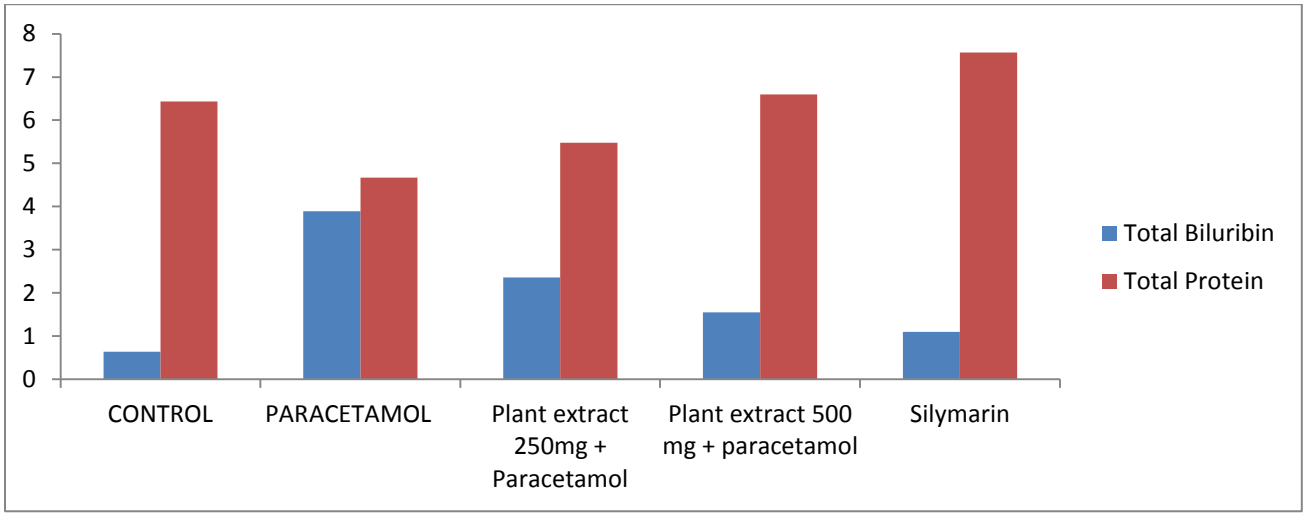
Graph 2: Regarding Na^+, K^+ , Cl^- Concentrations in Urine

In my present study aqueous-alcoholic extract of combined pods and bark of *Acacia sinuata* 250 mg/kg, 500 mg/kg showed the increased levels of Potassium in urine, and this increases the risk of hypokalemia and thus its potassium sparing capacity has to be investigated. The results obtained from my present study indicates that aqueous-alcoholic extract of combined pods and bark of *Acacia sinuata* 250 mg/kg, 500 mg/kg shows the significant diuretic activity and also can be useful in the different disease conditions like hypertension, chronic renal failure and diabetic acidosis. The diuretic therapy sometimes lead to life threatening complications like hypo-natremia, hypo-kalemia, drastic reduction of cl- levels in our body. To avoid these complications the safety profile studies has been carried out, and we can conclude that the aqueous-alcoholic extract of combined pods and bark of *Acacia sinuata* 250 mg/kg, 500 mg/kg can be used as a safe diuretic with a great efficacy. The active principles may be responsible for producing diuretic activity is flavonoids, saponins and terpenoids.

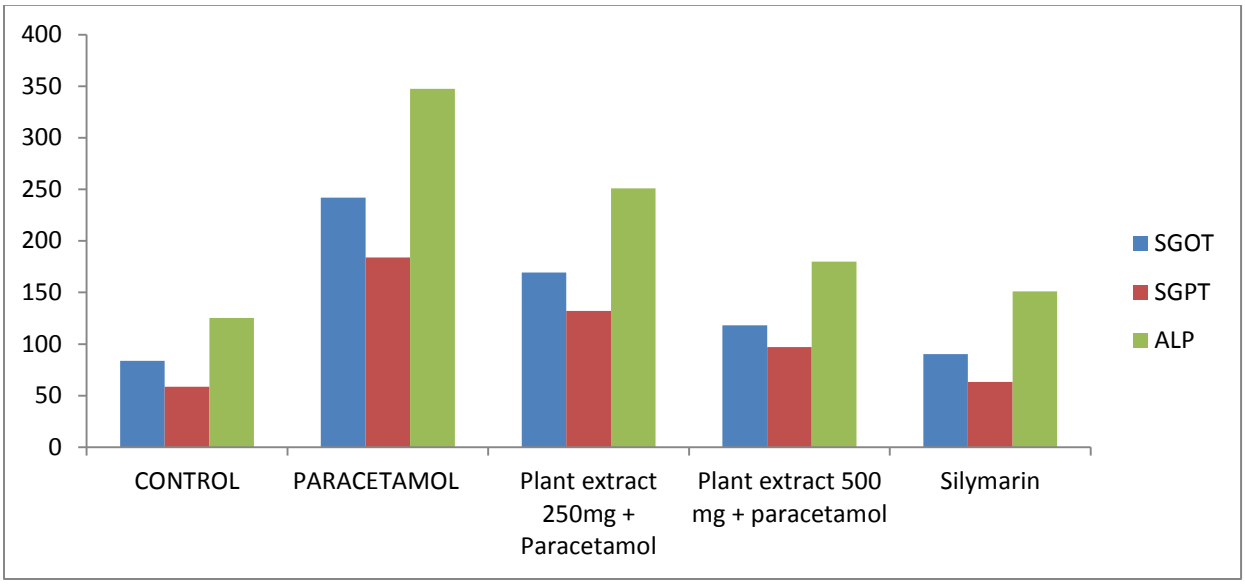
***In Vivo* Hepato-Protective Activity**

Table 2: Evaluation of Parameters Obtained from Haematological Examination of Collected Blood Samples

	Total bilirubin	**Total protein**	**SGOT**	**SGPT**	**ALP**
Control	0.64±0.07	6.43±0.14	83.66±1.31	58.50±1.65	125.33±3.41
Paracetamol	3.89±0.19	4.67±0.14	242±5.74	184±3.51	347.50±4.64
Plant extract 250mg + Paracetamol	2.36±0.06*	5.48±0.06	169.50±1.60*	132±1.94*	251.00±6.54
Plant extract 500 mg + paracetamol	1.55±0.09*	6.60±0.16	118.16±3.35*	97±1.73*	180.00±4.61*
Silymarin	1.1±0.05*	7.57±0.11*	90.16±1.99*	63.16±1.5*	151.16±3.52*



Graph 3: Comparision of Total Biluribin and Total Protein in Collected Blood Samples



Graph 4: Comparision of SGOT, SGPT, ALP in Collected Blood Samples

Histopathological Report

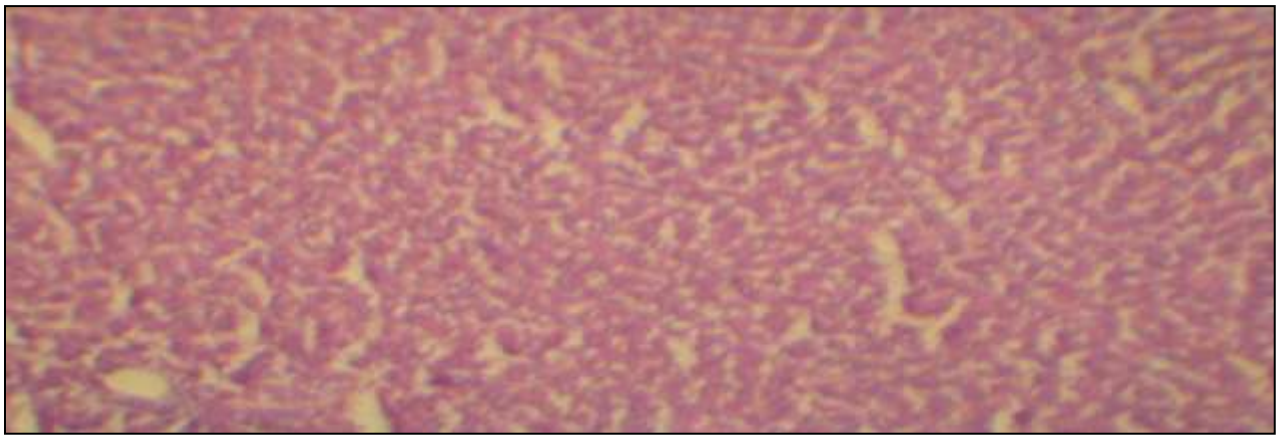


Figure 1: Photomicro Graphs of Control Rat Liver

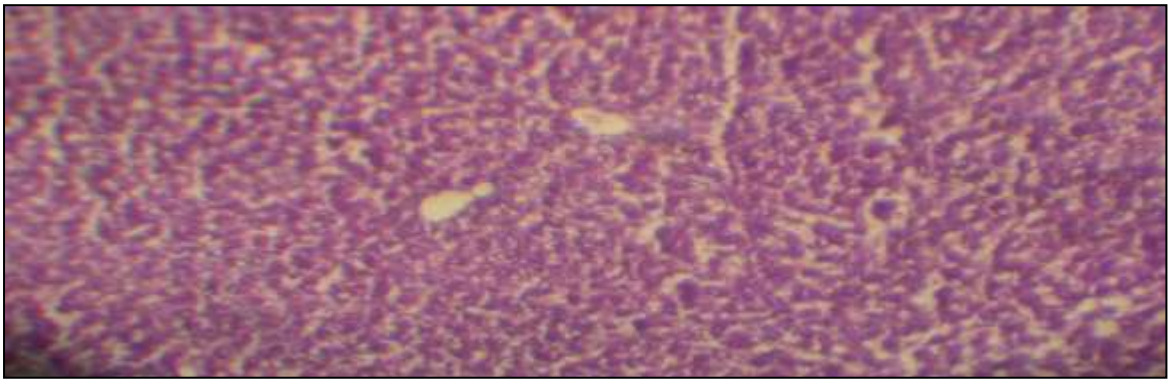


Figure 2: Photomicro Graphs of Drug Extract 250 Mg + Paracetamol Treated Liver

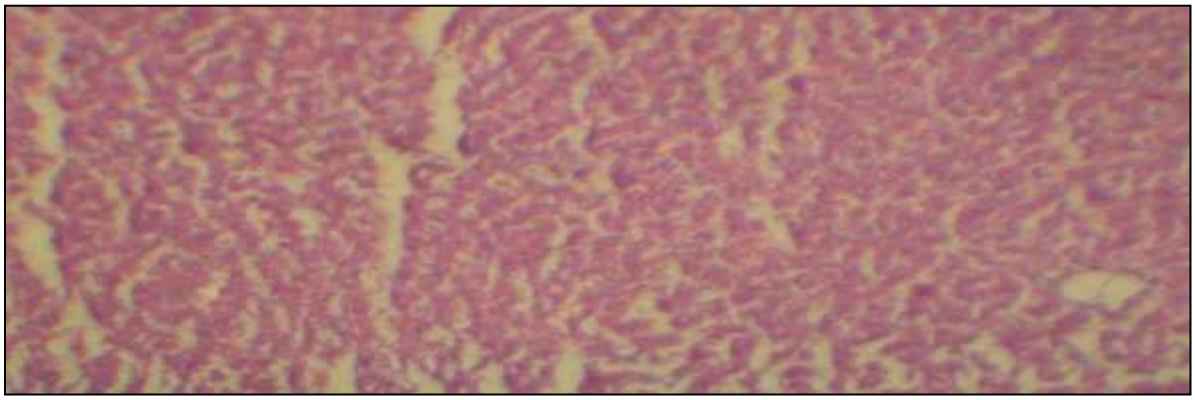


Figure 3: Photomicro Graphs of Drug Extract 500 Mg + Paracetamol Treated Liver

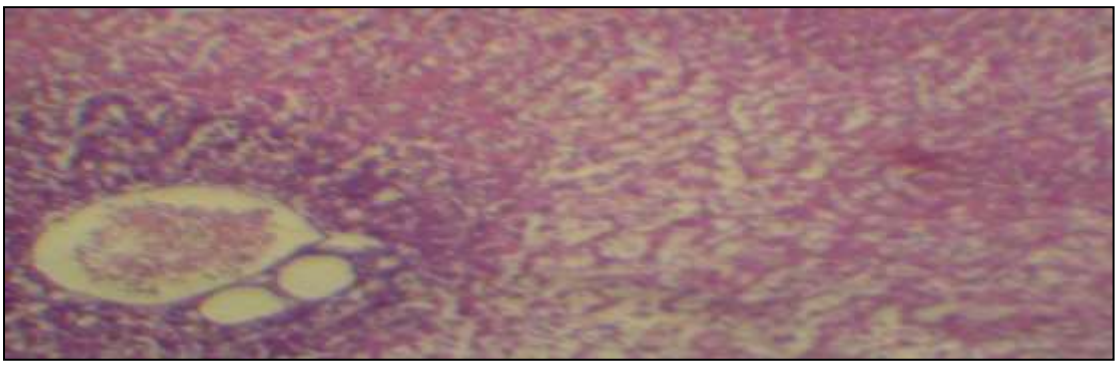


Figure 4: Photomicro Graphs of Paracetamol Treated Liver

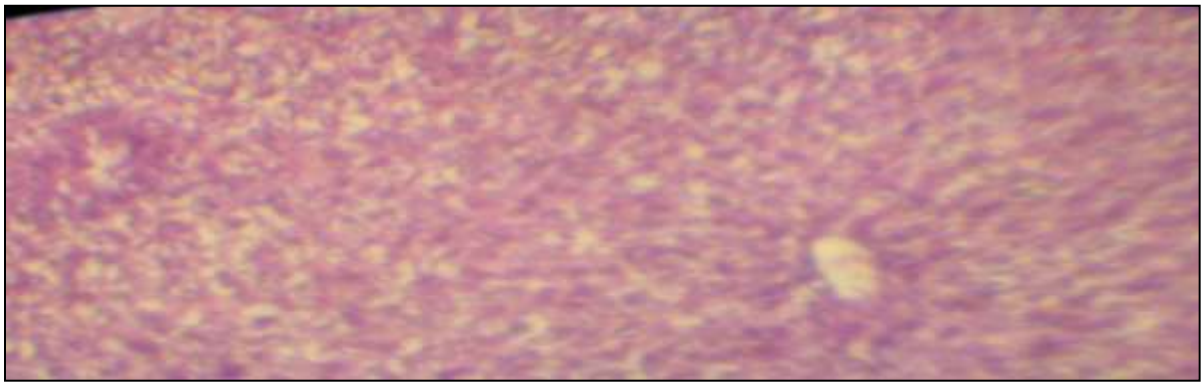


Figure 5: Photomicro Graphs of Silymarin Treated Liver

The light microscopic observation of histopathological examination showed that the liver section of rats treated with toxicant showed intense centriolobular necrosis and vacuolization. Control liver shows normal hepatocytes around central vein and portal triad Paracetamol induced liver shows patchy necrosis, focal clusters of hepatocytes with ballooning degeneration and ccl_4 damage causes inflammatory infiltration. Silymarin shows normal hepatocytes, occasional hepatocytes with degeneration changes. Aqueous-alcoholic extract of combined pods and bark of *Acacia sinuata* 250 mg/kg drug shows hepatic parenchyma with focal clusters of degenerative hepatocytes. Aqueous-alcoholic extract of combined pods and bark of *Acacia sinuata* 500mg/kg drug shows hepatic parenchyma with normal hepatocytes focal inflammatory collections only. The rats treated with aqueous-alcoholic extract of combined pods and bark of *Acacia sinuata* 250 mg/kg and 500mg/kg along with paracetamol showed sign of protection against these toxicants to considerable extents as evident from formation of normal hepatic cords and absence of necrosis and vacuoles. The present study provides significant result regarding hepatoprotective Activity of aqueous-alcoholic extract of combined pods and bark of *Acacia sinuata*.

CONCLUSION

The administration of aqueous-alcoholic extract of 250 mg/kg and 500 mg/kg of pods and bark of *acacia sinuata* exhibited efficient and effective diuretic and Hepato Protective activity because of the presence of flavonoids, saponins and terpenoids.

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