ISSN No:-2456-2165

Hepatoprotective Effect of Parkia Biglobosa Husk Methanol Extract Against Carbon Tetrachloride (CCL_4) Induced Liver Damage in Albino Rats

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Abstract:- ***Parkia biglobosahush extract have*** **traditionally found useful in the treatment of liver and other different ailments. The present study was designed to assess the Hepatoprotective effects of *Parkia biglobosa husk.* The group of rats were orally administered with the *Parkia biglobosahusk extract* with and control group which received only distilled water. The liver function indicators, AST, ALT, Total bilirubin, direct bilirubin, protein and Albumin were measured using spectrophotometric method. Hepatoprotctive activity of methanolic extracts of *Parkia biglobosa husk* was carried out against (CCL_4) induced liver damage. The extract was administered orally at doses of 50,100,150 and 200mg/kg^{-1} body weight once daily. Hepatoprotective activity was measured based on biochemical parameters. Significantly ($p>0.05$) increase level of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase(ALP), total bilirubin(TB) and direct bilirubin(DB) were seeing rats induced with CCL_4.the decrease significantly ($p<0.05$)level of protein and albumin were seeing in rats treated with CCL_4.While normal levels were restored in rats treated with standard drug(silymarin 100mg/kg) and rats treated with the extract. The result is suggested that the extracts have Hepatoprotective effects.**

Keywords:- *Hepatoprotective, Parkia Biglobosa Husk, Carbon Tetrachloride, Transaminase And Alkaline Phosphates, Total Bilirubin, Direct Bilirubin,Albumin And Total Protein.*

I. INTRODUCTION

The liver diseases is a major health problem around the world, receiving special attention from health professionals and scientists. Medicinal plants play a key role in the human health care. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials (IJPSR2011**).**In recent years, many researchers have examined the effects of plants used traditionally byindigenousheders to support treatment of liver diseases. There are no effective drugs that are available in modem medicine that confer protection to the liver against damage or help to regenerate hepatic cells(Chattopadhyay, 2003). Due to the dearth of reliable liver protective drugs in modem medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders (Chatterjee,2000). Sciendfic vdidadons are being made globally to get evidences for traditionally reported herbalplants. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases areinadequate and sometimes have side effects. This is one of the reasons why many researchers turn to complementary and alternative medicine (Guntupallie/*al.*,2006).*Parkia biglobosa* belongs to the family *Fabacea* . The plant is popularly called African locust bean tree, and it is known to occur in a diversity of agro-ecological zones ranging from the tropical rain forest to arid zones (Udobi*et al*., 2010). It is a perennial deciduous plant that typically grows to a height ranging from 7–20 m but can sometimes reach 30 m under exceptional conditions (Sabiti*et al*., 1992). *Parkia* species have traditionally found usefulness as foods and folklore remedies for some ailments (Ajaiyeob*et al*., 2002). The roots and leaves are used in Gambia to prepare lotions to treat sore eyes ((Ajaiyeob*et al*., 2002), for the treatment of dental disorders in Cote d'Ivoire (Kouadio*et al*., 2000) it is used in Nigeria for treatment of liver disease and also a remedy for diarrhea in the northern parts of Nigeria. It has been reported to have anti-hypertensive properties (Millogo*et al*., 2007) and the plant has been used by many tribes as an anti-diabetic, anti-hyperlipidaemic and as anti-snake venom agent (Odetola*et al*., 2006). *Parkia biglobosa* is thus a plant that has shown potential as a source of chemotherapeutic compounds (Udobi*et al*., 2009), while many folkloric and ethanobotanical applications of this plant have been reported. This study therefore investigated the hepatoprotective effect of Pakia biglobosa husk, antioxidants and phytochemical composition, of the plant.The *Parkia biglobosa* husk extract contained a number of phytochemical compound such as flavonoids, tannins, glycosides, alkaloids, cadiac glycosides, saponin , steroids, balsams and volatile oil. (Builders *et al*., 2012).Phytochemical can serve as effective natural agents for preventing and treating free radical mediated diseases. The rate of consumption is high in developing countries especially in Nigeria, because it is relatively chipper and common. The aim of the study was to evaluate the protective effects of Parkia biglobosa husk and establish criteria for safe consumption.

ISSN No:-2456-2165

II. METHODS AND MATERIALS

A. Chemicals

All chemicals and drug used were of analytical grade.

B. Collection and identification of plant materials

The harvested Parkia *biglobosa husk* were collected from Bagega village, Anka local Government Area of Zamfara state, Nigeria. The sample were carried to Herbarium unit of Biological Sciences Department, Usumanu Danfodiyo University Sokoto, Sokoto State Nigeria. The samples were authenticated by Malan Abdul-aziz Salihu Technologist and has the voucher number (UDUS/ANS/0616) was deposited at Herbarium of the same department.

C. Preparation of Plant sample

The*Parkia biglobosa husk* were rinsed in clean water and cut into pieces, and air dry in the laboratory for four (4) weeks. The dried *Parkia biglobosa* husk were pounded to coarse powder using a mortar and pestle.

D. Animals

47 albino rats were obtained from Usmanu Danfodiyo University Sokoto Animal house department of biological science. Albino rat were weight each and range was between (180-220g).The rats were acclimatized for a period of 14 days, at Standard environmental condition and normal temperature. The animals were feed with normal diet (Agro feed Mills Nig. Ltd) and clean water was allowed *ad-libitium* under strict hygienic conditions. Ethical experiments on animals of Institutional Animal Ethics Committee (IAEC) were used. Animals were caged according to their groups.

E. Acute Toxicity

The acute toxicity study was carried out using up and down method according to the Organizational for Economic Cooperation and Development guidelines 425 (OECD, 2001).

The non aqueous methanol extract of Parkia biglobosa husk (5000mg/kg body weight) was administered orally to five groups of rats each three rats (one by one for 48hrs up to 14days) using a feeding needle.

Observation of toxic symptoms such as weakness, food refusal, breathing difficulty and loss of body weight was made and recorded systematically at 2,4,8,12,24,48hrs off to 14days after administration of the Extract.Were determined and no death was recorded for about fourteen days of observation.

F. Sub-Chronic Toxicity Studies

Thirty-five (35) albino rats was randomly divided into seven(7) group of five rats each. Group 1received distill water throughout the period of the experiment, The group 2 received CCL4, group 3 received standard drug(Silymarin 100mg/kg) while remaining groups (4-7) received extract of 50,100,150 and 200mg/kg body weight. The animals was fast for overnight and were sacrificed, the blood samples were collected and was allowed to clot for 30 minutes. Serum was separated using centrifuge at 37^{o}c for determination biochemical parameters.

G. Assessment of Hepatoprotective Activity

The activity of serum aspartate and alanine transaminases, Alkaline phosphatase, albumin and total protein were assayed using method (Randox assay kit) of Reitman and Frankel (1957), Respectively. All these parameters were used to assess the acute hepatic damage caused by CCL_4.

H. Histopathological Examination

Histopathological examination of liver was carried out using the method described by Drury et al., the liver was fixed in 10% buffered formalin solution, then the formalin fixed organ specimens were embedded in paraffin wax and serially sectioned (3-5um) and further stained with haematoxylin and eosin. The stained tissues were observed for pathological changes using light microscopy.

III. STATISTICAL ANALYSIS

Result were presented as Mean and Standard error (Mean +_S. E). The differences between means were carried out using one-way analyses of variance (ANOVA) followed by Duncan multiple comparison test. The statistical package SPSS 20.0 version software. Values were considered statistically significant at $P<0.05$.

Table 2: Effect of administration of different doses of extract on liver function indices.

TREATMENT	AST(U/l)	ALT (U/l)	ALP (U/l)	TB (mg/dl)	DB (mg/dl)	ALB (mg/dl)	TP (mg/dl)
Normal Control(distilled water)	9.83±1.93^{a}	9.63±0.44^{a}	57.00±6.77^{a}	0.9±0.03^{a}	0.6±0.00^{a}	5.93±0.12^{b}	7.03±0.07^{b}
CCl4(1ml/kg bw)	18.67±0.29^{c}	19.33±1.45^{b}	116.33±5.86^{c}	1.8±0.15^{b}	0.9±0.04^{b}	3.20±0.12^{a}	5.06±0.00^{a}
Silymarin(mg/kg)	11.66±1.15^{b}	9.65±0.00^{a}	65.47±5.48^{ab}	0.9±0.03^{a}	0.6±0.04^{a}	5.90±0.06^{b}	6.93±0.12^{b}
Extract(50 mg/kg)	9.50±0.29^{a}	11.33±1.15^{ab}	65.67±11.55^{ab}	0.9±0.02^{a}	0.6±1.01^{a}	5.93±0.03^{b}	7.60±0.10^{b}
Extract(100 mg/kg)	10.16±0.29^{ab}	9.67±0.58^{a}	59.00±2.31^{a}	0.9±0.03^{a}	0.6±0.04^{a}	5.83±0.29^{b}	6.99±0.09^{b}
Extract(150 mg/kg)	11.60±0.29^{b}	11.67±0.00^{ab}	60.33±2.89^{a}	0.9±0.03^{a}	0.6±0.08^{a}	5.87±0.06^{b}	7.01±0.06^{b}
Extract (200 mg/kg)	9.33±0.58^{a}	11.63±0.00^{ab}	71.00±0.58^{b}	0.8.±10^{a}	0.6±0.00^{a}	5.90±0.06^{b}	6.99±0.09^{b}

ISSN No:-2456-2165

Values are expressed as mean ± standard error of mean. Mean values having common superscript letters in a column are not significantly different($p<0.05$) (one-way ANOVA followed by Duncan's multiple range test).

ALP=Alkaline Phosphatase, AST= Aspartate Amino Transferase, ALT= Alanine Amino Transferanse, TB= Total Billirubin, DB= Direct Billirubin, ALB= Albumin, TP= Total Protein.

Table 3: Effect of administration of different doses of extract on kidney function indices.

TREATMENT	Na(mEq/dl)	K(mEq/dl)	Urea(mg/dl)	Creatinine(mg/dl)	Cl(mEq/dl)
Normal Control(distilled water)	145.37±1.15^{b}	4.97±0.35^{c}	37.90±0.00^{b}	0.67±0.00^{a}	33.33±0.33^{a}
CCl4(1ml/kg bw)	144.33±0.59^{b}	4.37±0.11^{a}	35.67±3.29^{a}	0.66±0.80^{a}	33.30±0.58^{a}
Silymarin(100mg/kg)	145.33±0.33^{b}	4.93±0.03^{c}	37.33±3.06^{b}	0.67±0.00^{a}	32.63±0.33^{a}
Extract(50 mg/kg)	144.30±0.58^{b}	4.97±0.06^{c}	37.33±6.06^{b}	0.67±0.00^{a}	33.33±0.88^{a}
Extract(100 mg/kg)	144.30±0.58^{b}	4.93±0.06^{c}	38.37±3.64^{b}	0.73±0.00^{b}	33.33±0.33^{a}
Extract(150 mg/kg)	142.60±0.88^{a}	4.86±0.06^{b}	37.33±3.03^{b}	0.67±0.00^{a}	33.67±0.57^{a}
Extract (200 mg/kg)	142.67±0.00^{a}	4.82±0.15^{b}	37.37±1.18^{b}	0.73±0.00^{b}	33.33±0.88^{a}

Values are expressed as mean ± standard error of mean. Mean values having common superscript letters in a column are not significantly different($p<0.05$) (one-way ANOVA followed by Duncan's multiple range test).

Cr=Creatinine, Na=Sodium ,K=Patassium, Cl=Chloride.

Table 4: Effect of administration of different doses of extract on haematological indices.

Treatment	Normal Control(distilled water)	CCl4 (1ml/kg bw)	Silymarin (100mg)	Extract (50 mg/kg)	Extract (100 mg/kg)	Extract (150 mg/kg)	Extract (200 mg/kg)
WBC(x10^{9}/ L)	5.56±0.98^{b}	1.63±1.19^{a}	5.53±0.86^{b}	5.53±0.35^{b}	5.57±1.76^{b}	5.54±1.62^{b}	5576±4.99^{b}
LYM (%)	55.67±8.72^{b}	69.66±9.00^{c}	55.00±3.49^{b}	50.00±0.58^{a}	54.20±6.84^{b}	55.33±1.44^{b}	55.50±7.22^{b}
MONO (%)	20.73±2.73	20.19±3.00^{b}	19.88±3.00^{b}	20.17±1.15^{b}	19.87±3.52^{b}	14.99±2.60^{a}	14.98±2.52^{a}
GRA (%)	23.93±9.21^{b}	24.53±4.51^{a}	22.00±3.61^{a}	30.33±0.58^{b}	22.03±3.29^{a}	30.00±1.15^{b}	30.67±2.60^{b}
RBC(x10^{12}/ L)	6.80±0.56^{cd}	4.49±1.38^{a}	6.00±0.12^{b}	6.40±0.09^{c}	6.60±0.22^{cd}	6.37±0.04^{c}	6.39±0.18^{c}
HCT (%)	45.67±3.57^{b}	36.00±9.70^{a}	44.50±1.20^{b}	45.67±1.10^{b}	43.99±12.35^{b}	45.53±0.55^{b}	44.99±0.41^{b}
HGB (g/dl)	21.63±2.72^{a}	20.96±3.04^{a}	21.43±0.03^{a}	21.1±0.49^{a}	21.33±0.15^{a}	20.95±2.50^{a}	21.93±0.12^{a}
MCHC (g/dl)	31.23±0.69^{a}	32.16±5.24^{a}	31.33±0.81^{a}	31.1±0.35^{a}	30.77±0.12^{a}	30.53±0.32^{a}	30.05±0.03^{a}
MCH (pg)	21.87±0.06^{a}	21.43±3.28^{a}	21.00±0.55^{a}	20.26±3.67^{a}	20.20±3.67^{a}	21.60±0.35^{a}	20.76±0.81^{a}
MCV (fl)	69.73±1.24^{b}	68.96±8.19^{b}	69.33±0.15^{b}	69.76±0.90^{b}	68.60±1.59^{b}	69.15±0.43^{b}	69.97±2.68^{b}
PLT(x10^{9}/L)	700.33±29.73^{b}	487.33±14.49^{a}	699.00±33.49^{b}	694.33±57.16^{b}	698.67±2.14^{b}	696.50±21.07^{b}	696.33±20.07^{b}

Values are expressed as mean ± standard error of mean. Mean values having common superscript letters in a row are not significantly different($p<0.05$) (one-way ANOVA followed by Duncan's multiple range test).

WBC=White Blood Counts,LYM=Lymphocyte,MONO=Monocyte,GRA=Granulacyte,RBC=Red Blood Cells,HCT=Packed Cell Volume,HGB=Haemoglobin,MCHC=Mean Cell Haemoglobin Concentration,MCH=Mean Cell Haemoglobin,MCV=Mean Corpuscular Volume,PLT=Platelet.

IV. HISTOLOGICAL ANALYSIS

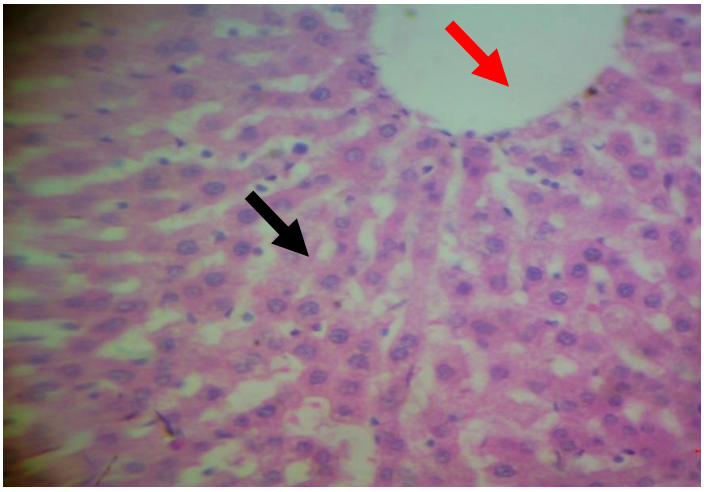

Plate 4.2 x 400.Group 1 Normal Control (distilled water) :Normal architecture of the liver with distinct sinusoidal arrangement with the hepatocytes (black arrow) and hepatic portal vein (red arrow)

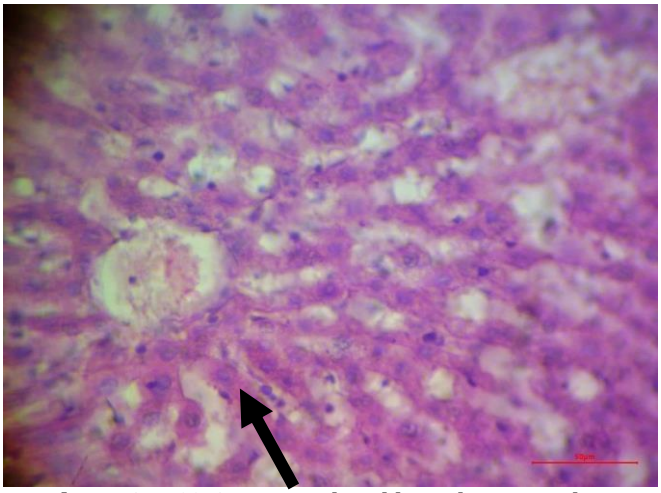

Plate 4.3x 400,Group 2 induced liver damage and no treatment : Normal architecture of the liver with distinct sinusoidal arrangement with the hepatocytes with vacuolation (red arrow) and congestion in the central portal vein (black arrow)

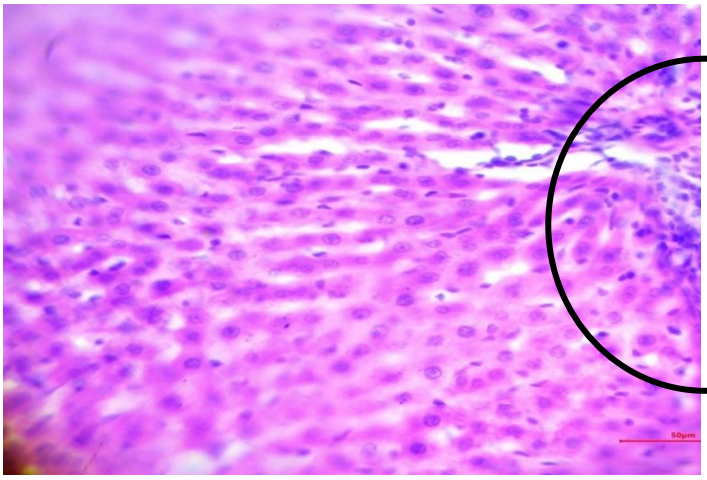

Plate 4.4 x 400,Group 3 induced liver damage and treatment with standard drug (silymarin): Normal architecture of the liver with distinct sinusoidal arrangement with the hepatocytes with slight perivascular infiltration

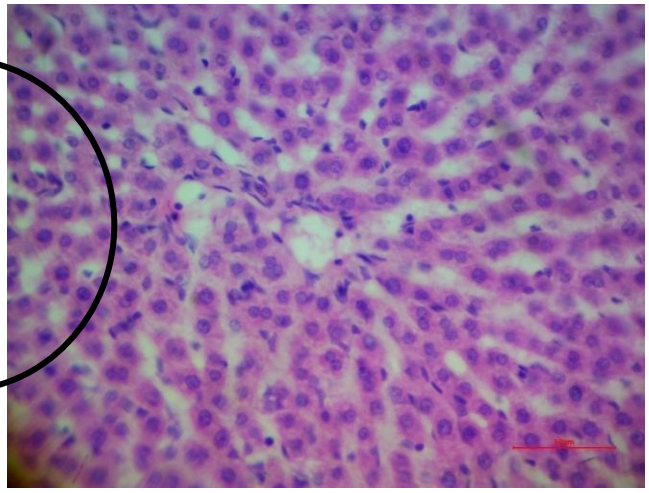

Plate 4.7 x 400,Group 4 induced liver damage and treatment with extract (50mg/kg): Normal architecture of the liver with distinct sinusoidal arrangement (black arrow) with the Hepatocytes (red arrow)

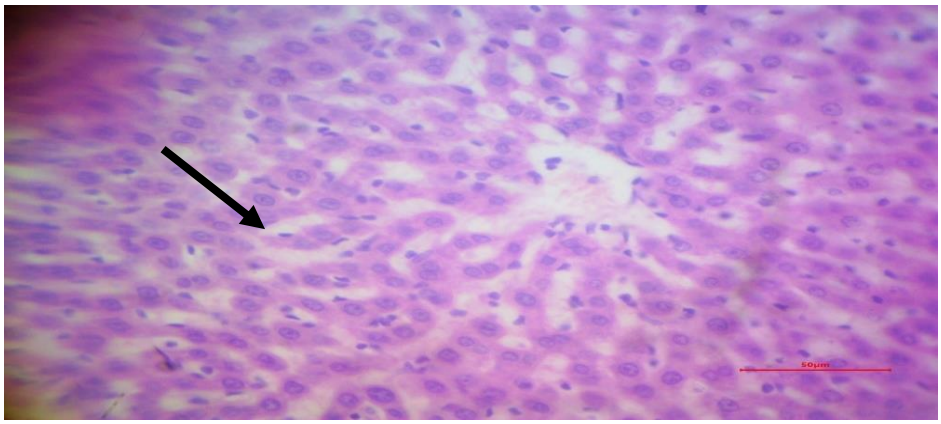

Plate 4.5 x 400, Group 5 induced liver damage and treatment with extract(100mg): liver with distinct sinusoidal arrangement with the hepatocytes (black arrow) and hepatic portal vein (red arrow)

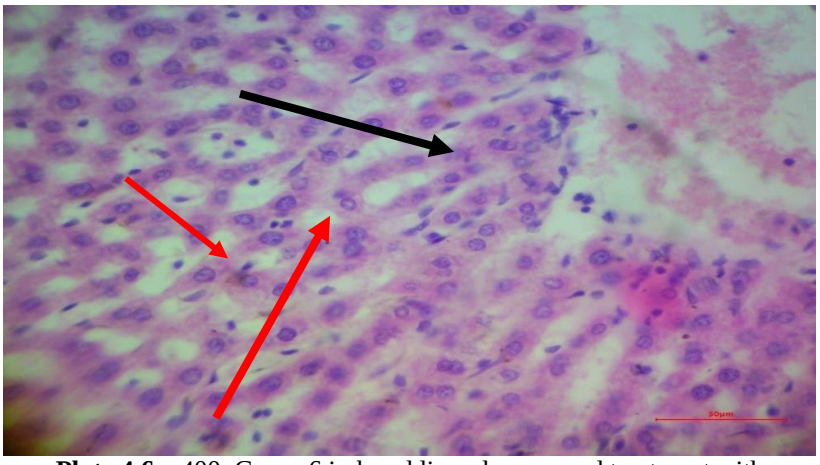

Plate 4.6 x 400, Group 6 induced liver damage and treatment with extract (150mg) : liver with distinct sinusoidal arrangement with the hepatocytes with portal vein (black arrow) with slight perivascular cellular infiltration (red arrow)

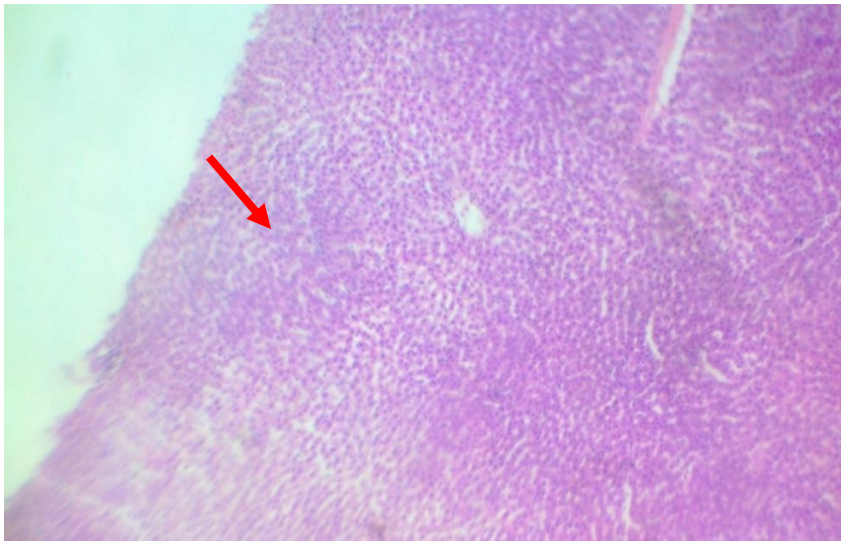

Plate 4.1: x 100,Group 7 induced liver damage and treatment with 200mg/kg extract:sNormalarchitecture of the liver with distinct sinusoidal arrangement with the hepatocytes and portal vein (red arrow).

V. RESULTS

The result of acute toxicity study (LD_{50})using up and down method were no mortality was recorded for the period of fourteen days of observation. Only signs of body weakness, food refusal were recorded. Phytochemical screening is show in (Table 1), Whilesub-chronic toxicity test of liver function test is shows in (Table 2). Rats treated with CCL_4 significantly increase ($p>0.05$) in the activity of liver enzymes ALT, AST and ALP and serum total bilirubin (TB) compared to normal control rats. While kidney function test is in (Table 3).serum urea, creatanine, chloride, sodium and potassium are restored were urea and potassium levels is significantly reduced ($p<0.05$) in rats treated CCL_4. Haematological test result is in (Table 4). WBC,HCT and PLT are significantly ($p<0.05$) reduced in rats treated with CCL_4 compared to rats treated with standard drug and extract. Were LYP is significantly($p>0.05$) increased in rats treated CCL_4. Histological analysis of liver shows that all the livers in normal control, standard drug and treated with extracts are of normals while group that induced liver damage(CCL_4) and not being treated shows hepatocytes with vacuolation and conjunsction in the central portal vein which is an indication of liver injury.

VI. DISCUSSION

Death of the liver leads to increase of the serum marker enzymes which are released from the liver in to blood(Ashok Shenoy *et al*; 2002). AST,ALT and ALP are considered markers for liver function. They are the serum hepatobillary enzymes present normally in the liver while high concentrations is an indication of liver injury(Tolman and Rej, 1999; Hilaly *et al., 2004).* ALT is located primarily in the cytosol of hepatocytes. This enzyme is considered as more sensitive marker of hepatocellular damage than AST. AST is found in the cytoplasm and mitochondria in different tissues, chiefly in the heart, skeletal muscles, liver, kidney,

ISSN No:-2456-2165

pancreas and erythrocytes (Aniagu *et al.*,2004). From our results, increases of serum ALT, AST and ALP is seeing in rats treated with only CCL_4 this is an indication of liver injury (Achiliya *et al.*, 2004; Hassan *et al.*, 2005). membrane permeability of the cells The increase of ALT in the CCL_4 treated group may be due to the release of enzyme from cell of the damage organ. Determination of serum protein and albumin can act as a criterion for assessing synthetic capacity of the liver, since nearly all of them are synthesized in the liver. In our study, shows decrease in serum proteins and albumin which is an indication of liver damage due it malfunction in the synthesis of proteins and albumin. Increases of enzymatic activity caused by CCL_4 to high level of serum bilirubin observed in this research work Therefore, this research revealed that, administration of methanolic extract of *Parkia biglobosa husk extract* has hepatoprotective activity against the toxic effect of CCL_4.

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