Phytochemical, Antioxidant and Hepatoprotecti ve Activity of *Parkia biglobosa* Husk Methanol Extract in Albino Rats.

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Abstract

Medicinal plants are used in almost every part of world to treat illnesses. Oxidative stresses are known to cause damage to living system of humans and animals.This study assessed the determination of phytochemical constituents present in Parkia biglobosa husk, antioxidants and hepatoprotective effect of husk methanol extract on rats .The phytochemical property of methanol extract was determined using standard methods. The phytochemical screening of the husk methanol extract revealed the presence of alkaloid, saponins, tannins, flavonoids, steroids and glycosides. The quantitative phytochemical analysis revealed high content of tannins 42.8%, flavonoids 35.5%, steroids 32.6%, alkaloids 16%, saponins 14.5% and glycosides 3.2%. The oxidative stress markers as SOD, CAT, GSH, Vit C, and Vit E were increased significantly ($P < 0.05$*) at 50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg of Husk extract of P. biglibosa treated animals and decreased significantly the MDA level as compared to control group.Treatment of animals with methanol husk extract at 50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg exhibited significant hepatoprotection in rats induced with* CCl_4 *compared to* CCl_4 *untreated group. These results suggest that, the husk methanol extract of the plant posses therapeutic potentials in the treatment of liver disease.*

Key words: *Parkia biglobosa husk, Cabon-tetrachloride, phytochemicals, and antioxidants.*

Introduction

Traditional solutions speak to broad run of old characteristic wellbeing care system [1]. Plants provide medications, fuel, nourishments and protection for living forms of life. Healing plants involved a major parcel in human wellbein g care framework. About 80% of the mankind populaces depend on the utilization of traditional medicine which is transcendently based from plant materials [1].

Parkia biglobosa has a place to the family Fabacea. The plant is famously called African grasshopper bean tree, it is found in assorted agro-ecological zones extending from the tropical rain woodland to parched zones [2]. It could be a perpetual deciduous plant that regularly develops toa tallness extending from 7-20m but can some of the time reac h 30m beneath extraordinary conditions [3]. Parkia biglobosa species have customarily found value as nourishments and drugs utilized for treatment of diverse illnesses [4]. The seeds are utilized in arrangement of dawadawa, a protein and fat wealthy nourishment. The yellow boring mash that encompasses the seed is a critical nourishment supplement rich in Vitamin C and carbohydrates. The dried powder is frequently blended with water to deliver a drink called dozim [5].

The roots and stem bark are utilized in Gambia to get ready salves to treat sore eyes [4]. Leaves are utilized for

treatment of dental disarranges in Cote d'Ivoire [6], whereas husk is utilized in Nigeria for the treatment of liver infection and the runs within the northern parts of Nigeria.

It has been detailed to have anti-hypertensive properties [7], and the plant has been utilized by numerous tribes as an anti-diabetic, anti-hyperlipidaemic and as anti-snake poison specialist [8].

To the leading of my knowledges, there's no logical report accessible in back of the phytochemical and antioxidant p revention agents considers of *Parkia biglobosa* husk methanol extricate. Subsequently, this study was carried out to assessed phytochemicals and antioxidant prevention agents impact of husk methanol extract of *P.biglobosa* against CCL4 induced liver harm in albino rats.

2.0 MATERIALS AND METHODS

2.1 Materials
All chemicals and reagent utilized were of expository review.

2.2 Plant Collection and Authentication
Fresh husk of *Parkia biglobosa* was collected amid from Bagega town in Anka Nearby Government Zone of Zamfara State, Nigeria. It was verified by Abdul-aziz Salihu Herbarium unit, Division of organic science, Usumanu Danfodiyo College Sokoto. The example of plant was kept at the herbarium unit of the same division with the voucher number (UDUS/ANS/0616).

2.2.1 Preparation of Plant Sample
The *Parkia biglobosa* husk were cut into pieces and air dry within the research facility for four (4) weeks, the dried test of Parkia biglobosa husk was beat to coarse powder employing a mortar and pestle. The methanol extricate was arranged by splashing 250g of parkia biglobosa husk in a 1L of 95% methanol for 72h at room temperature with energetic shaking. The blend was sifted with Whitman channel paper NO. 1. The filtrate was at that point dried at a temperature of $50C^0$ in stove and put away in fridge for advance utilize and rate surrender was calculated.

2.3 Phytochemical Screening

2.3.1 Determination of Alkaloids
The alkaloids substance of the test was decided utilizing strategy as detailed by [9].

2.3.2 Determination of Saponins
Determination of saponins in husk methanol extract of *Parkia biglobosa* was carryout utilizing method depicted by [10].

2.3.3 Determination of Flavonoids
The detection of flavonoids in husk methanol extract was decided using the precipitation method described by [11].

2.3.4 Determination of Tannins
Tannins were determined by the method of Trease and Evans [12].

2.3.5 Determination of Cardiac Glycosides
Cardiac glycoside was determined utilizing the method of glycosides depicted by [10].

2.3.6 Determination of Steroid
The amount of steroid substance in the test sample was determined using strategy of [9].

2.4 Grouping of Experimental Animals
Fourty five (45) albino rats were gotten from animals house of Biological division Usman Danfodiyo University Sokoto. The rats of both sex weighted (185-220g) were acclimatized for a period of 14 days at standard natural condition temperature (25℃). The animals were nourished with typical count calories

(Agro nourish Mills Nig. Ltd) and clean water ad-libitium under
strict sterile conditions. The animals were separated in to 7 groups of five (5) rats each. Group 1(normal control rats) received 1ml/kg bw of fluid paraffin. Group 2 (untreated) were managed carbon tetrachloride 1ml and 1ml of fluid paraffin. Group 3 received CCL_4 and standard drug (silymarin 100g/kg). Group 4-7 (Test groups) were treated with 50,100,150 and 200mg/kg body weight orally of *P biglobosa* husk methanol extract and 1ml of CCL_4.

2.4.1 Assessment of Antioxidants activity

The liver was perfuse with 0.86% cold saline to totally remove all the ruddy blood cells. It was suspended in 10% (w/v) ice cold 0.1M phosphate buffer (PH 7.4) and cut into little pieces. The required sum was weighed and homogenized employing a Teflon homogenizer. The homogenate was utilized for the estimation of non enzymatic cancer prevention agents such as MDA, Vitamin C, VitaminE and enzymatic such as CAT, GSH and Grass [13].

2.4.2 Liver Function Test Parameters

After the treatment phase all of the subject animals were anaesthetized and sacrificed. Blood was withdrawn from the heart and their serum was separated by centrifugation at 3000 rpm at 30^{o}C for 15 min. This was afterward analysed for a variety of biochemical parameters including. Aspartate Amino Transferase Assay (AST), Alanine Amino Transferase Assay (ALT) [14],total protein (15), total albumin (16), alkaline phosphatase (17) and total bilirubin content (18).

2.5 Statistical Analysis

Result was displayed as mean and Standard deviation (Mean±S.E). Information ware analyzed utilizing one-way analysis of variance (ANOVA) taken after by Duncan multiple comparison test. Values were considered significance difference at $P<0.05$.

3.0 Results

3.1. Qualitative and Quantitative Phytochemical Analysis

Phytochemical analysis of methanol extract of husk *P. biglibosa* indicated the existence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins, balsamic, volatile oils and steroids (Table 1). Quantitative analysis showed that tannins, Steriods, Glycosides was found to be 42.8mg/dl, 32.6mg/dl, 3.2mg/dl in concentration and Flavonoids, alkaloids and saponins was found to be 35.5%, 16.3% and 15.2% (Table 2).

Table 1. Qualitative Phytochemicals Constituents of *Parkia biglobosa* Husk Extract

S/NO	Paramters	Result
1	Flavanoids	+
2	Tannins	+
3	Saponins	+
4	Glycosides	+
5	Alkaloids	+
6	Steriods	+
7	Cardiac glycosides	+
8	Saponins	+
9	Balsamic	+
10	Anthraquinone	-
11	Volatile oil	+

Key: + = present, - = Absent

Table 2. Quatitative Phytochemicals Constituents of *Parkia biglobosa* Husk Extract

S/No	PHYTOCHEMICALS	RESULTS
1.	Flavonoids	35.5%
2.	Tannins	42.8mg/dl
3.	Saponins	14.5%
4.	Glycosides	3.2mg/dl
5.	Alkaloids	16.3%
6.	Steriods	32.6mg/dl

3.2 Effect of Administration of *P. biglibosa* Husk Extract on Antioxidants Parameters

Induction of animals with CCL_4 revealed a significant ($P<0.05$) decrease in The CAT, GSH, SOD, VIT C, VIT, E and increase in MDA concentration compared to normal control. Treatment with *Parkia biglobosa* husk methanol extract revealed a significant ($P<0.05$) increase CAT, GSH, SOD, VIT C, VIT, E and decrease in MDA compared CCL_4 untreated group (Table 3).

Table 3: Effect of *Parkia biglobosa* Husk Methanol Extract on Antioxidants Activities of Liver Against CCL_4 Intoxicated Rats.

Treatments	CAT(units/mg)	GSH(mg/dl)	MDA(mmol/m)	SOD(units/mg)	VIT C(mg/dl)	VIT E(mg/dl)
Group1 Normal Control	3.33±0.01^{c}	55.20±0.42^{c}	37.17±0.44^{a}	0.37±0.02^{b}	218.17±0.44^{c}	60.70±0.65^{b}
Group2 CCl_4(1ml/kg bw)	0.22±0.01^{a}	28.03±0.59^{a}	57.83±0.44^{b}	0.23±0.03^{a}	146.83±0.93^{a}	39.83±0.17^{a}
Group3 Silymarin(100mg/kg)	3.30±0.01^{c}	54.22±0.88^{c}	36.00±0.12^{a}	0.36±0.03^{b}	215.47±0.70^{c}	60.20±0.12^{b}
Group4 Extract(50 mg/kg)	2.85±0.00^{b}	44.97±0.55^{b}	35.00±0.35^{a}	0.34±0.01^{b}	198.60±2.14^{b}	56.50±0.12^{b}
Group5 Extract(100 mg/kg)	2.88±0.01^{b}	45.03±0.17^{b}	35.53±0.29^{a}	0.34±0.05^{b}	198.30±1.33^{b}	58.50±0.06^{b}
Group6 Extract(150 mg/kg)	2.93±0.01^{b}	48.00±0.50^{b}	35.66±0.18^{a}	0.36±0.04^{b}	199.67±0.17^{b}	58.30±0.06^{b}
Group7 Extract (200 mg/kg)	3.29±0.01^{c}	53.19±67.74^{c}	35.83±0.15^{a}	0.36±0.01^{b}	201.00±0.23^{b}	59.50±0.15^{b}

Values are expressed as mean ± standard error of mean. Mean values having common superscript letters in a column are not significantly different ($P<0.05$). CAT= Catalase, SODs =Superoxide Dismutase,GSH=Reduced Glutathion,MDA=Molondialdehyde, CCL4= Carbon Tetrachloride.

Effect of methanol extract of *Parkia biglobosa* husk on Liver Function Indices

The effect of methanol extract of *P. biglobosa* husk (MEPBH) on serum biochemical parameters in CCL_4-induced hepatotoxicity in rats are presented in (table 4). There was a significant elevation ($P<0.05$) of serum levels of AST, ALT and ALP , direct bilirubin (DB), total bilirubin (TB) in the CCL_4 untreated induced group compared to normal control group. In contrast to Albumin (ALB) and total protein (TP) levels in induced-control group. Treatment of CCL_4 induced groups with the husk extract and standard drug (silymarin) significantly ($P<0.05$) neutralized the effect of CCL_4 when compared to untreated group.

Table 4: Effect of Administration of Different Doses of MEPBH on Liver Function Indices.

TREATMENT	AST(U/l)	ALT (U/l)	ALP (U/l)	TB (g/dl)	DB (g/dl)	ALB (mg/dl)	TP (mg/dl)
Normal Control(5ml distilled H_2O,kg/bwt)	20.86±1.03^{c}	25.63±0.04^{b}	57.90±6.07^{b}	0.90±0.00^{a}	0.60±0.00^{a}	5.93±0.12^{b}	6.70±0.07^{b}
CCl4(1ml/kg bw)	48.67±0.09^{d}	45.63±1.05^{c}	116.33±5.06^{c}	1.80±0.05^{b}	1.00±0.04^{b}	2.20±0.12^{a}	3.06±0.00^{a}
Silymarin(100mg/kg bwt)	19.99±1.05^{b}	23.60±0.00^{b}	57.88±5.08^{b}	0.90±0.04^{a}	0.60±0.04^{a}	5.90±0.06^{b}	6.69±0.02^{b}
Extract(50 mg/kg bwt)	14.90±0.09^{a}	16.60±1.05^{a}	50.87±11.05^{a}	0.90±0.06^{a}	0.60±1.01^{a}	5.89±0.03^{b}	6.66±0.00^{b}
Extract(100 mg/kg bwt)	15.83±0.09^{b}	16.60±0.08^{a}	50.88±2.01^{a}	0.90±0.03^{a}	0.60±0.04^{a}	5.90±0.29^{b}	6.66±0.09^{b}
Extract(150 mg/kg bwt)	16.03±0.09^{b}	19.61±0.00^{a}	51.88±2.09^{a}	0.90±0.06^{a}	0.60±0.08^{a}	5.90±0.06^{b}	6.68±0.06^{b}
Extract (200 mg/kg bwt)	19.94±0.08^{c}	22.63±0.00^{b}	55.90±0.08^{b}	0.90±0.02^{a}	0.60±0.00^{a}	5.91±0.06^{b}	6.68±0.09^{b}

Values are expressed as mean ± standard error of mean. Mean values having common superscript letters in a column are not significantly different ($p<0.05$).

Discussion

The plant kingdom shows up to be a critical asset of phytochemicals that can serves as antioxidant agents. Preliminary phytochemical screening of *Parkia biglobosa* husk methanol extract indicated the presence of Alkaloids, Cardiac glycosides, Flavonoids, Tannins, Steroids,Volatile oil and Saponins. Thus the antioxidant activity of the plant extract may be due to the presence of those secondary metabolites. As reported that Flavonoids, steroids and alkaloids possess hepatoprotective and antioxidant potential agents [19-22]. The free radical conciliate means has been alarmed in the pathogenesis of different diseases. Tetrachloride (CCl_4) is one of the most universally used hepatotoxins in experimental studies of liver diseases [23]. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloro methyl radical [24].

Continue generation of reactive oxygen/nitrogen (ROS/RNS) variety and reduced potential of antioxidant military protection in the body brings about oxidative stress [25, 26]. production of (ROS/RNS) species is unavoidable for aerobic organisms and in healthy cells, and it happened at a restricted time [27]. During oxidative stress situation, ROS/RNS making is considerably raised, and brings about modification of membrane lipids, proteins, and nucleic acids [28]. Oxidative smash up of these biomolecules is linked with aging and a multiplicity of pathological measures, such as atherosclerosis, carcinogenesis, ischemia reperfusion damage, and neurodegenerative disorders [29]. To sustain steadiness in the redox system and guard the body adjacent to ROS and RNS, humans have discharge complex antioxidant systems that work to avoid harmful property of oxidative stress [30]. The body's antioxidant defense systems are of endogenous and exogenous origin [31]. Exogenous and endogenous source of antioxidants are β-carotene, L-ascorbic acid, α-tocopherol, tocotrienols, catalase (CAT), superoxide dismutase (SOD), glutathione reductase, and glutathione peroxidase (GPx) [32-33]. Presently, there is high demand of interest to replace conventional antioxidants drugs with naturally occurring antioxidants from plants seeing as they are noticeably safer, easily reachable, and inexpensive [34, 35]. Therefore, present study evaluates antioxidant potential of Husk methanol extract *P. biglibosa*, and plant extract significantly neutralized the effect of oxidative stress compared to CCl_4 induced group but not treated with plant husk extract. Moreover, this result is in line with other result [36-37]

Liver enzymes such as AST, ALT, and ALP are important biomarkers of the liver damage normally found in large quantities in either plasma or serum when there is hepatic cellular membrane permeability alteration or cellular injury [38]. Serum marker enzymes, such as AST, ALT, and ALP are recognized as liver symbol enzymes which show signs of increased activities when the liver cells are necrotic, during cholestatic disorder or hepatocellular injured [39]. Wills and Asha [40], revealed in their study that whenever liver injures, AST, ALT and ALP continue moving from the cytoplasm to the circulatory system because of the toxicity mediated transformed permeability of the cellular membrane. Moreover, there are other key indices to evaluate the hepatic function, such as TC, TG, HDL, LDL, VHDL, total protein, bilirubin and albumin levels in serum. Present study established that the CCl_4 induced control group showed a significant raise in the activities of liver indices signifying acute hepatocellular damage. A lot of authors reported that the activities of serum enzymes and other indices were significantly high after induction of animals with CCl_4 [41, 42, 43]. On the other hand, daily administration of *P. biglibosa* Husk extract to CCl_4 induced hepatotoxic rats reduced the increased activity of liver marker enzymes and alleviated the loss of functional integrity of the cell membrane, indicating its hepatoprotective activity. Therefore, this study is in line with previous studies reported by some authors that plant extract significantly decreased the activities of enzyme [44-45]

Conclusion

This study uncovered that *Parkia biglobosa* husk methanol extract is reach with secondary metabolites that displayed as antioxidants and hepatoprotective potentials.

Moral Endorsement

Animals ethical committee endorsement has been taken to carry out this study.

Declaration of Interest

Authors declared that no conflict of interest.

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