Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.15, No.1, 2024

Hepatoprotective Effect of Methanol Stem Bark Extract of Parkia biglobosa in Wister albino Rats

Abdullahi Goni
Department of Education, Yobe State University, Damaturu. P.M.B 1144 Yobe Nigeria
*Corresponding author: Abdullahi Goni
Email: agoni20084dad@gmail.com

Abstract
The increase in prevalence rate of liver diseases in developing countries necessitate the search for alternative therapies to prevent such ailments. This research work evaluated the hepatoprotective effect of methanol stem bark extract of *Parkia biglobosa* in Wister rats. The toxicity of the extract was analyzed using oral LD_{50} determination in two phases. In the first phase nine rats were divided into three groups of three rats each and administered with 10 ,100, and 1000mg/kg of extract while in the second phase, four rats were grouped into four groups of one rat each, and were orally administered with 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg, the rats were observed for sign of toxicity and death within 24 hours. For the hepatoprotective study, 25 rats were grouped into five groups (GI – GV) of five rats each. GI served as normal control, GII served as CCl_4 control, GIII, IV and V were administered with the extract at a dose of 50mg/kg, 100mg/kg and 150mg/kg body weight respectively for two weeks. At the end of the second week, the rats from groups II, III, IV and V were attempted to be induced with liver damage using 120 mg of CCl_4 administered subcutaneously. The animals were euthanized after 24 hours of CCl_4 administration and liver function indices were assayed. The result showed oral LD_{50} of the extract to be greater than 5000 mg/kg body weight. A significant decrease ($p<0.05$) was observed in the mean serum ALT, AST and ALP of extract administered group in dose dependent pattern compared to GII. This shows that the extract may protect hepatocytes against CCl_4 hepatotoxicity.
Keywords: CCl_4; hepatoprotective; liver function indices; Methanol and Parkia biglobosa
DOI: 10.7176/JNSR/15-1-05
Publication date: January 31st 2024

1.0 Introduction

The liver is a major metabolic organ only found in vertebrate animals, which performs many essential biological functions necessary for the survival (Abdel-misih *et al.,* 2010). A human liver normally weighs approximately 1.5 kilograms with a width of about 15 cm (Cotran *et al.,* 2005). There is considerable size variation between individuals, with the standard reference range for men being 970–1,860 grams (Molina *et al.,* 2012) and for women 600–1,770 g (Molina *et al.,* 2015). It is both the heaviest internal organ and the largest gland in the human body. It is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm, to the right of the stomach, and overlying the gallbladder (Tortora *et al.,* 2008).

As a result of the strategic metabolic functions of the liver, it is often the targeted organ for chemically induced organ toxicities. Many factors have been reported to functionally and structurally contribute to the susceptibility of the liver to toxicity. These include the high perfusion of the liver to xenobiotics absorbed from the gastrointestinal tract and the high concentration of xenobiotic metabolizing enzymes (cytochrome P450-dependent monooxygenase system) in the liver. Chemical agents such as those used in laboratories and industries, as well as medicinal plants, are capable of inducing hepatotoxicity. More than 900 drugs have been implicated in liver injury. Drug related hepatotoxicity is an important cause of morbidity and mortality. As such, it is the most common reason for withdrawing new drugs from circulation .

Parkia biglobosa (Jacq) Benth and *Parkia bicolor* A. Chev belong to the plant family Mimosaceae of the order Leguminisae. In Yoruba, *P. bicolor* is referred to as IgbaOdo; Dorowa, in Hausa, and in lbo as Origili Okpi. *P. biglobosa* popularly known as the African locust bean tree is known in Yoruba as Igba, or Irugba, in Hausa as Dorowa and in lbo as Origili. The fermented seeds of *P. biglobosa* are used in all parts of Nigeria and indeed the West Coast of Africa for seasoning traditional soups. Similarly, both trees form a crown so are often grown as shade trees (Dalziel, 1937; Hutchinson, 1959).

Liver diseases is one of the most common affecting more fifty percent of populace in the developing countries. This necessitates the search for alternative compounds and therapies of higher efficacy and less toxicity that will prevent the onset of liver diseases. Consequently, there is need to provide scientific basis for the use of such plants in traditional medicine. *Parkia biglobosa* has been used in traditional medicine in many part of the world including Nigeria and it was claimed to prevent and/or cure many diseases, liver damage inclusive. It is therefore imperative to assess the toxicity profile, efficacy of the plant and some bioactive compounds responsible.

2.0 Materials and Methods

2.1 Collection, Identification and Preparation Plant Extract

Stem bark was collected from a farm in Damaturu Local government area of Yobe state, Nigeria. It was authenticated at Biological Sciences Department of Yobe State University, Nigeria. The barks were carefully wash with distilled water and dried at room temperature, after which it was pulverized to coarse powder using mechanical grinder.

Methanol extract was prepared according to Mittal *et al* (1981) and Fernando *et al* (1989) method. One thousand grams (1000g) of the powder was macerated in 2000cm^3 methanol. the content of the flask was subjected to intermittent shaking in an orbital shaker for 24 hours. It was then filtered using Whatman No1 filter paper and concentrated using vacuum evaporator at 60^{o}C in water bath

2.2 Acute Toxicity Study ($LD_{50\ Oral,\ Rats}$) Determination

The $LD_{50(Oral,rats)}$ was determined using Lorke (1983) method. in two phases. In the first phase nine rats were divided into three groups of three rats each and administered with 10, 100 and 1000mg/kg of extract. The rats were observed for mortality and general behavior for 24 hours.

In the absence of any toxicity, four rats were grouped into four groups of one rat each, and were orally administered with 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg body weight of the extract and observed for signs of toxicity which include: paw licking, salvation, rubbing of nose on floor, change in body weight and death within 24 hours. The number of death in each group within 24 hours was recorded and LD_{50} was calculated from the relation

$LD_{50} = \sqrt{}$ min conc. full death × max conc. no death

2.3 Study animals

Male and female (non-pregnant) albino rats weighing between 100g to 120g were used for the study. The animals were housed in well-ventilated cages and were allowed to access to food and clean water. Principle of laboratory animal care and ethical guidelines for investigation of experimental pain in conscious animals were observed during experimentation (NIH, 1996; Zimmermann, 1983)

2.4 Evaluation of the Effect of Parkia biglobosa stem bark extract against CCl_4 Liver Damage.

The rats were randomly grouped into five groups of five (5) rat's each as per the experimental design below

Group I: Normal rats.
Group II: (Test Control) No extract administered
Group III: Administered with 50mg/kg of the extract for two weeks
Group IV: Administered with 100mg/kg of the extract for two weeks
Group V: Administered with 150mg/kg of the extract for two weeks.

On the 15th day, the rats in groups II to V were attempted to be induced with liver damage using 120mg/kg of according to Alhassan *et al* (2009). Rats were euthanized 24 hours after inducement with CCl_4 and blood samples were collected for determination of AST, ALP, ALT activities, Total and Direct Bilirubin, Total protein, Albumin and Globulin. Aspartate aminotransferase (AST) and Alanine Aminotransferases Assay (ALT) were assayed using Reitman and Frankel (1975) method, Alkaline Phosphatase (ALP) activity was assayed using the method developed by Roy (1970), Bilirubin by method of Malloy and Evolyn (1937), and Total protein was determined by Biuret method (1995).

2.5 Data Analysis

Comparison between groups was performed using analysis of variance (ANOVA) with p value <0.05 considered significant followed by Tukey´s post hoc test. Results were expressed as mean ± standard deviation.

3.0 Results

The result for LD_{50} determination was presented in Table 1a and 1b respectively. In the initial phase, no any sign of toxicity and mortality was observed. In the second phase, rat administered with the highest dose exhibit some sign of toxicity, no mortality was recorded.

Table 1a: Phase I of Oral LD_{50} Determination

Doses (mg/kg)	Methanol extract
10	0/3
100	0/3
1000	0/3

Table 1b: Phase II of Oral LD_{50} Determination

Doses (mg/kg)	Methanol extract
2000	0/1
3000	0/1
4000	0/1
5000	0/1

Table 2 and 3 show serum levels of liver marker enzymes (ALT, AST and ALP), total protein, albumin, total and direct bilirubin and globulin of rats administered with the extract for 14 days followed by inducement of liver damage. A significant increase in serum activities of ALT, AST and ALP ($p<0.05$) in CCl_4 induced control group compared to the normal control was shows an indication of successful induction of liver damage using 120mg of CCl_4. Administration of the extract protect against the induction of liver damage in a dose dependent pattern.

Table 3: Liver Function Parameters of Rats Induced with 120mg/kg CCl_4 after 14 days of Extract Administration

	Weight (g)	ALT (U/L)	AST (U/L)	ALP (U/L)
Group I	48.23 ± 7.55^{a}	54.43 ± 5.44^{f}	44.76 ± 5.53^{o}	45.43 ± 7.45^{x}
Group II	$73.67\pm8.21^{a,b,c}$	$78.33\pm6.05^{f,g,h}$	$72.34\pm5.76^{o,p,q}$	$61.56\pm5.63^{x,y,z}$
Group III	66.44 ± 7.16	71.56 ± 2.55	66.45 ± 5.45	58.64 ± 7.53
Group IV	55.56 ± 3.88^{b}	66.44 ± 4.87^{g}	54.76 ± 4.32^{p}	54.87 ± 4.67^{y}
Group V	47.50 ± 6.78^{c}	56.00 ± 3.67^{h}	48.42 ± 6.43^{q}	47.54 ± 4.43^{z}

Values are presented as Mean ± standard deviation, (n=6). Values with the same superscripts in a column are significantly different compared to each other (P<0.05)

Table 4: Liver Function Parameters of Rats Induced with 120mg/kg CCl_4 after 14 days of Extract Administration

	T-Bilirubin (mg/dl)	D-Bilirubin (mg/dl)	T-Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Group I	0.34 ± 0.021	0.02 ± 0.01	7.10 ± 1.37	4.23 ± 1.21	2.88 ± 0.16^{a}
Group II	0.46 ± 0.017	0.03 ± 0.009	6.46 ± 1.34	3.04 ± 1.41	3.06 ± 0.42^{a}
Group III	0.44 ± 0.035	0.03 ± 0.014	6.74 ± 1.08	3.92 ± 1.14	2.84 ± 0.69
Group IV	0.43 ± 0.014	0.04 ± 0.007	6.64 ± 1.03	4.01 ± 1.06	2.64 ± 0.47
Group V	0.39 ± 0.014	0.03 ± 0.008	6.96 ± 1.00	4.18 ± 1.45	2.78 ± 0.44

Values are presented as Mean ± standard deviation, (n=6). Values with the same superscripts in a column are significantly different compared to each other (P<0.05)

Figure 1 shows the spectra of GCMS analysis of the extract. The peak shows the presence of Tetratriacontane, Octadecane, 3-ethyl-5-(2-ethylbutyl), Heptacosane and Hexadecanoic acid, methyl ester as the probable bioactive compounds.

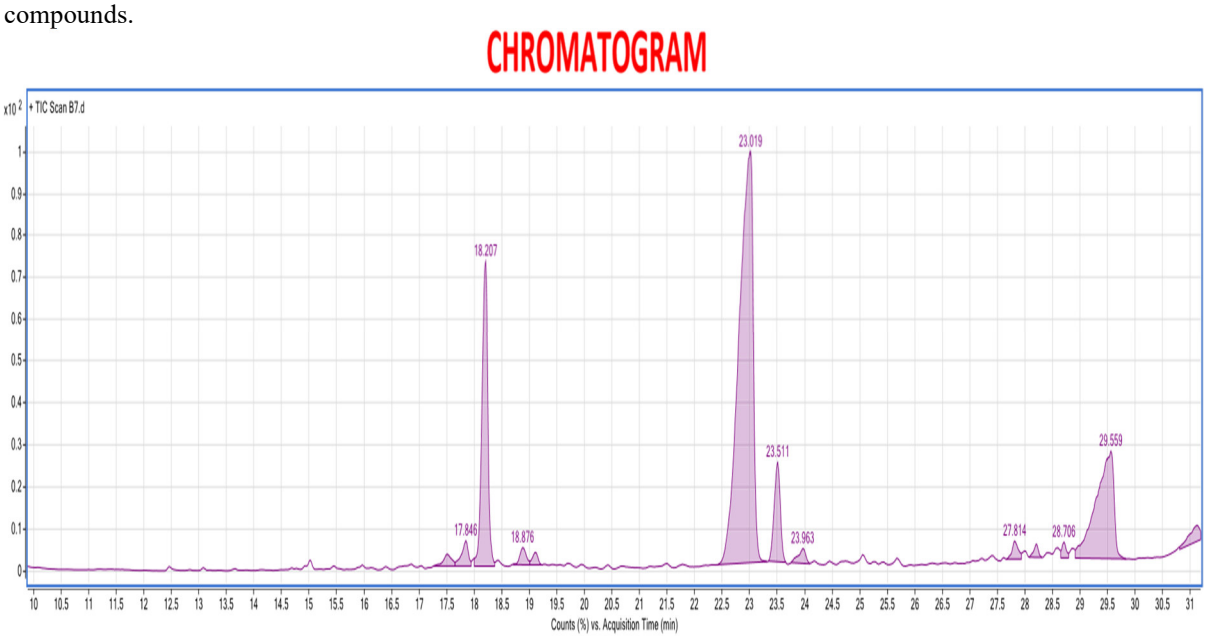

Figure 1: GCMS chromatogram of the extract

4.0 Discussion

Plants have served as the richest source of raw materials for traditional and modern medicine all over the world, particularly in Africa and Asia. Natural products such as plants extract either as pure compounds or as standardized extracts provide unlimited opportunities for new drug discoveries. liver diseases are global problem with increasing prevalence. The problem however remains that some drugs are scarce, costly and unavailable to the common man, in addition to their adverse effects. Hence a preventive approach rather than curative is necessary. *Parkia biglobosa* was chosen in this study to evaluate its hepatoprotective activity against CCl_4 induced liver damage and to characterize its components, scientifically and suggest their use in the development of less costly and effective traditional medicine for management of liver problems.

the oral LD_{50} of the extract was established to be above 5000 mg/kg body weight interpreted as slightly toxic according to Timbrell (2009) scale of classification. The finding is in accordance with the finding of Jules *et al* (2014) who reported the LD_{50} of leave extract to be greater than 5g/kg body weight.

Administration of CCl_4 lead to a significant increase in serum liver enzymes, a result that is consistent with several studies in rats (Muhammad *et al.,* 2015; Alhassan *et al.,* 2009). The CCl_4 induced control group in this research were reported to have an elevated serum enzymes activity when compared to the normal control, an indication of successful induction of liver damage.

Administration of rats prior to induction of liver damage protect the liver tissue against the toxic effect of CCl_4. This finding was in line with a research of Muhammad *et al.,* (2015) who reported aqueous Stem Bark Extract of *Khaya senegalensis* (ASBEKS) to protect the liver against CCl_4 liver damage.

The hepatoprotective mechanism of action of the extract is not established. One possible mechanism may be through its antioxidant properties, which could counteract the toxic effect of CCl_4 by binding to the trichloro methyl-free radical, preventing its covalent binding to microsomal lipid and protein and thereby preventing lipid peroxidation which is thought to be the cause of liver damage by CCl4 (Maiti *et al.,* 2008).

5.0 Conclusion

It may be concluded that, methanol stem bark extract of *Parkia biglobosa* is slightly toxic with oral LD_{50} greater than 5000 mg/kg body weight and can confer hepatoprotective effect against CCl_4 induced hepatotoxicity

6.0 Conflict of Interests

Authors have declared that no competing interests exist

7.0 Acknowledgement

Author's acknowledge the support receive from Tertiary Education Trust Fund (tetfund) for the disbursement of fund for the conduct of the study Institutional Based Research (IBR) Scheme.

References

Abdel-Misih Sherif RZ, Bloomston M, (2010). Liver Anatomy. *Surgical Clinics of North America*. 90 (4): 643–653.

Alhassan AJ, Sule MS, Hassan JA, Baba BA, Aliyu MD. (2009).Ideal hepatotoxicity modal using CCl_4. *BAJOPAS*. 2(2);185

Cotran Ramzi S, Kumar V, Fausto N, Nelso F, Robbins S, Abbas K. (2005). Robbins and Cotran pathologic basis of disease (7th ed.). St. Louis, MO: Elsevier Saunders. p. 878. ISBN 978-0-7216-0187-8.

Dalziel, JM (I 937): The useful plants of West Africa. Crown Agents for Oversea Government and Administration, p. 217.

Fernando MR, Wickramasinghe MD, Thabrew MI, Karnayaka EK (1989). A preliminary Investigation of the possible hypoglycemic activity of *Asteracanthus longifolia*. *J. Ethnopharmacol.* 27:7-14.

Hutchinson J (1959): Families of flowering plants: Dicotyledon. Vol 1. Oxford University Press London, pp. 240 – 247

Jules CN, Assob Dickson S, Nsagha D. (2014). Toxicological Survey of African Medicinal Plants. Elsevier Pg 12

Maiti A, Dewanjee S, Mandal SC (2008). Hypoglycemic effect of *Khaya senegalensis* seeds against type II diabetes. *Int. J. Green Pharm.* 2:224-227.

Mittal GC, Aguwa CN, Eziefe VU, Akabue PL (1981). Preliminary pharmacological studies on antivenom action of *Diodia scandens. Nig. J. Pharm.* 12:32-36

Molina, D. Kimberley D, Vincent JM (2015). Normal Organ Weights in Women. The American Journal of Forensic Medicine and Pathology.

Molina, D. Kimberley; DiMaio, Vincent J.M. (2012). Normal Organ Weights in Men. The American Journal of Forensic Medicine and Pathology.

Molley and Evolyn (1937). Recommendation of a uniform bilirubin standard. *J. Boil. Chem.*:119-121.

Muhammad IU, Alhassan AJ, Wudil AM, Jarumi IK (2015). Toxicological and protective effect of aqueous stem

bark extract of *Khaya senegalensis* (ASBEKS) on Liver of Experimental Rat. *British Journal of Applied Science and Technology*. 2015;9(6):600-605

NIH (1996). Guidelines for the care and use of laboratory animals. National Academic Press, (NIH Publication No. 85:23

Reitman S., Frankel S. (1957). A colorimetric method for the determination of serum glutamate-oxaloacetate and pyruvate transaminase. *Am. J. Clin. Path.* 28: 56.

Roy A.V. (1970) Clinical Chemistry. 16:431.

Tietz N.W. (1995) Clinical guide to laboratory test, (2nd edition), W.B Saunders Company Philadelphia. 554-556

Tortora, Gerard J, Derrickson G, Bryan H (2008). Principles of Anatomy and Physiology (12th ed.). John Wiley & Sons. p. 945. ISBN 978-0-470-08471-7.

Zimmermann M (1983). Ethical guidelines for investigation of experimental pain in conscious animals. *Pain*. 19:109-110