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Hepatoprotective Potential Of Leaf And Bark Extracts Of Gambia ALBIDUM

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***Abstract**: Ethanol and water extract of Gambia albidum were studied for hepatoprotective activity in rats with carbon tetrachloride(CCL_4) induced-liver damage by measuring selected biochemical parameters: aspartate aminotransferase(AST),alanine aminotransferase(ALT),alkaline phosphatase(ALP) and total bilirubin.The rats were grouped into two:A_1/A_2 and A_1/B_2 of ten rats each.Group A_1 consisted of the control rats in which hepatocellular damage wasinduced with oral 1ml/kg body weight of CCL_4 but no treated with any of the extracts.Group A_2 and B_2 rats were also given oral 1ml/kg body weight of CCL_4 but treated with 500mg/kg body weight body weight appropriate extract for 14 days.The rats were sacrificed the next(15^{th}) day,and the serum levels of the above-listed parameters determined.In the control rats,A_1,the mean levels of the parametres were:ALP(0.08+/-0.05mg/kg),AST(52.10+/-1.66u/l),ALT(6.27+/-0.81u/l),totalbilirubin(47.25+/-0.54mg/100ml).In the rats treated with water of Gambia albidum ($_{A2)}$ extract were: ALP(0.08+/-0.05mg/kg), AST(48.10+/-0.94u/l),ALT(53.00+/-0.43u/l),total bilirubin(0.73+/-0.43mg/100ml) and that of the bark were:ALP((0.73+/-0.43mg/kg),AST(43.70+/-u/l),ALT(52.73+/- u/l),and total bilirubin(43.25+/-1.27mg/100ml).The levels in htose terated with ethanol extract of the bark (Group B_2) were:ALP(0.55+/-0.5mg/kg),AST(41.15+/-0.85u/l),ALT(49.80+/-0.05u/l),total bilirubin(38.63mg/100ml) while that of the leaf were:ALP(0.60+/-0.05mg/kg),AST(44.33+/-1.10u/l)ALT(51.60+/-0.57u/l),total bilirubin(40.75+/-0.85mg/100ml).The results of the study indicated a significant ($p<0.05$) reduction in the mean levels of the enzymes measured, indicating hepatoprotection by the extracts of the G,albidum.The effectiveness of the water and ethanol extracts in protecting the hepatic cells did not differ significantly($p>0.05$).In all,G.albidum had a hepatoprotective effect on the CCL_4-treated rats.*

***Keywords:**Ethanol and water extract of Gambia albidum,ALP,AST,ALT,Total bilirubin.*

I. Introduction

The liver is among the most complex and important organs in the human body. It is the central organ for the metabolic and detoxification of drugs and toxins. Consequently, drugs affect the liver more frequently than any other organ and place the liver at great risk for toxic damage (Bussieres and Habra, 1995). After absorption by the intestines, drugs reach the liver via the portal system. In the hepatocytes, these chemicals undergo complex metabolic processes to be converted into hydrophobic substances, readily soluble in the blood stream and easily eliminated thereafter (Lee, 2003). Drug or their metabolites can cause toxic effect on the liver. Many of the intermediate metabolites have a short half-life, some estimated to be less than a minute, which makes detecting them a challenging task (Park et al., 2005). This chemical-driven liver damage is referred to as hepatotoxicity. The use of herbal medicine can be traced back to 2100 BC in ancient China at the time of the xia dynasty and during the vedic period in India. The first written reports are timed to 600 BC with Charaka Samhira in India and to 400 BC with the early notes of the Eatern Zhou dynasty in China (Dhiman and Chawla, 2005). The study of African Medicinal plants has not in the past been taken as seriously, or documented as fully, as Indian and Chinese traditional medicines (Abebayo, 2010). Over 5000 plants are known to be used for medicinal purposes in Africa, but only a few have been described or studied (Adebayo, 2010). *Gambia albidum* belongs to the campodeoidea family and native to the central, Eastern and Western African (Amusa et al., 2003). The plant is specially distributed in Nigeria, Uganda, Niger, Cameroon and Cote d' ivoire (Adewusi, 1997). It is often called the white star apple and distributed throughout the southern part of Nigeria (Idowa et al., 2006). Therefore, medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Adebayo, 2010). Medicinal plants, since time immemorial have been used in virtually all cultures as a source of medicine. Natural products from plants can be another potent source for the discovery of excellent biological activities, that is anticancer and antioxidatant activities (Adebayo, 2010).

Traditionally, Gambia albidum is an important lesser known indigenous tree species that are neither planted nor adequately explored to discover their potentials to be included in agroforestry system, but that,

nevertheless, offer protection to liver injury. However, the genetic base of this species is being reduced as a lack of information on their ethno-botanical and socio economic potentials. This study therefore, seeks to identify and evaluate potential of *Gambia albidum* as practical agricultural tree and the gains from its tree products in the Eastern part of Nigeria.

AIMS AND OBJECTIVES

The general aim of the research was to carry out an evaluation of *Gambia aldidum* (white star apple) and its hepatoprotective potential of liver injury in swiss albino mice. The objectives where

1. To investigate and evaluate *Gambia albidum* as a source of protective measures to liver disease.
2. How the leaf and bark of *Gambia albidum* be treated to enhance their protection.

II. Materials And Methods

PLANT MATERIALS

The fresh stem bark and leaves of Gambia albidum are collected from its natural habitat at Ochigbo John cash crop farm at Offerekpe, Izzi Local government Area of Ebonyi State, Nigeria. It was collected at the month of July 2011. The plant was authenticated at the department of biochemistry, university of Nigeria Nsukka, Enugu, Nigeria where the research was carried out.

PREPARATION OF PLANT EXTRACT

Ethanol extract

The sample (bark and leaf) were miled and measured at 200g each of bark and leaf mixed with 200ml of ethanol into a measuring cylinder and kept for 24hours before sieve. After sieving, the extracts were poured into a beaker and kept in an autoclave to evaporate the ethanol. The dried extracts were transferred in the smaller container and covered with its top and kept at a cool dry place.

Aquous Extracts

50 grams each of back and leaf were mixed with 100ml of distilled water each and allowed to stand for 3 hours. The samples were measured with the help of electronic scale. Reading:

Bark

Weight of crucible 23.25g

Weight of crucible + content 25.88g

Leaf

Weight of crucible. 23.13g

Weight of crucible + content 23.66g

EXPERIMENTAL ANIMALS

Male and female albino rats (15) of wister strain Obtained from the University of Nigeria Nsukka, Enugu, Enugu State, Nigeria weighing between 100-150g were used for the experiment. Animals were maintained at a controlled temperature (25 + 3oC), humidity (60 + 5%) and kept in the animal house of the department of biological sciences, university of Nigeria Nsukka, Enugu State, Nigeria. The animals were allowed to acclimatize for two weeks. Feed and water were given. All animals were treated in accordance with the recommendations of National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH, 1985).

EXPERIMENTAL DESIGN

The rats were divided into three groups (A, B, C). The animals of group A serve as normal control group and were given only vehicle (distilled water, 1ml/kg) for 7 days. The animals of group C (positive control) were administered with vehicle and. CCL_4 (50% solution of CCL_4 in liquid paraffin, 2ml/kg) on the fifth, sixth and seventh day. The animals of group B were administered with 500mg/kg of ethanolic extracts and distilled water for the first four days and with distilled water and CCL_4 on the last three days. Animals were subsequently anesthetized and blood samples were collected for aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALT), and total bilirubin, assays.

BLOOD COLLECTION AND PREPARATION OF SAMPLE

At the end of the treatment period, the rats were anesthetized in diethylether prior to dissection. The blood was then collected by cardiac puncture into lithium heparinized bottles. Plasma was obtained by centrifuges the blood at 10,000 revolutions per minute for 15 minutes into clean bottles and stored at - 20^{o}C until required for biochemical assays (Adebayo,2010). The liver was also collected and fixed with 10% formaldehyde for histopathological examination.

ANALYSIS OF BIOCHEMICAL PARAMETERS

Commercial test kits obtained from Randox laboratories, united kingdom were used for all biochemical parameters measured. Standard methods were used to estimate aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) , acid phosphatase and total bilirubin.

STATISTICAL ANALYSIS

All values were expressed as mean + S. D and Turkey post hoc test was done to, to analyze significant difference between different groups using the statistical analysis software package SPFS (version 1.3).

III. Results

PHYSICAL OBSERVATION

Rats are so friendly when they were feeding well, but if they underfeed can be cruel to each other in condition that they can kill themselves for food. Albino rats are partially blind during the day time that they can't see well, and make sluggish movement during the day light. During administration of the animals with extract of Gambia albidum, they showed a significant decrease in weight, even the effect of ethanolic leaf and bark extract so of G-albidum on CC14 to induce liver injury in rats make the animals to appear physically sick by showing sluggish movement and unable to behave friendly.

Table 4.1: The effect of water extract of leaf and bark of G. albidum in rats with induced liver damage by CCL_4

	ALP (mg/m1)	AST (μ/1)	ALT (μ/1)	Total bilirubin (mg/100m1)
Control	0.89±0.05	52.10±1.66	56.27±0.81	47.25±0.54
Bark	0.73±0.05	43.70±0.70	52.73 ±1.32	43.25±1.27
Leaf	0.80±0.05	48.10±0.94	53.00±0.43	44.00±0.89

From the above table, the bark extract of the plant (*G. albidum*) gives more protection to the hepatic cells than the leaf of the same extract. Total *bilirubin* makes a reasonable decrease than ALP, ALT and AST in concentration in the blood plasma during administration of the extracts while ALP does not actually decrease very fast to show effective protection in the treated rats. In – fact the concentration of ALP, ALT, AST and total bilirubin in the blood plasma reduced hepatoprotective effect on the $CC1_4$ – Treated rats.

Table 4.2 the effects of ethanolic extract of leaf and bark of G. Albidum in rats with induced liver damage by cc_4

	ALP(mg/ml)	**AST (µ/l)**	**ALT(µ/l)**	**Total bilrubin (mg/100ml)**
Control	**0.80 ± 0.05**	**52.10 ± 1.66**	**56.27 ± 0.81**	**47.25 ± 0.54**
Bark	**0.55 ± 0.05**	**41.15 ± 0.85**	**49.80 ± 0.05**	**38.63 ± 0.63**
Leaf	**0.60 ± 0.05**	**44.33 ± 1.10**	**51.60 ± 0.57**	**40.75 ± 0.82**

From the above table, it is observed that the effect of ethanol extract in the treated rats is more effective than that of water extract in the protection of the hepatic damage. The bark extract of G. albidum gives more protection than that of leaf of the same extract. Although, all the targeted enzymes decreases in blood plasma to show protection from the extract but total bilirubin still decreases faster than other enzyme during administration of the extracts.

OBSERVATION DURING ADMINISTRATION

Effect of ethanolic leaf and bark of *Gambia albidum* on carbon tetrachloride (CCL_4) induced liver injury in rats with reference to biochemical changes in plasma. The CCL_4 treated (positive control) group showed a significant increase in activity of aspartate amiotransferase, alanine amino transferase, alkaline phosphatase and plasma total bilirubin, indicating liver injury significant decrease in the activity of AST, and ALT similarly, the activity of ALP and total bilirubin were significant lowered across the groups when compared with the CCL_4 treated group. Histolorgically, rats induced with CCL_4 showed sinusoidal dilatin and focal centrilogbular necrosis while rats treated with the extract of G. albidum showed a significant protection against liver injury from CCL_4 as evidence in mild centrilobular fatty degeneration and reduced sinusoidal dilation across the all treatment groups.

Table 4.3 shows the percentage yield of ethanol and distilled water extract of fresh leaves of *Gambia albidum*

S/N	Solvent	Initial mass of leaves before extraction (g)	Mass of leaves after extraction (g)	Volume of Mass solvent extract (g) added (ml)	Percentage of yield extraction (%)
1.	Ethanol	200	23.66	200	11.83
2.	Distilled water	50	15.05	100	30.1

Table 4.4 Shows the percentage of yield of ethanol and distilled water extract of bark of *Gambia albidum*

S/N	Solvent	Initial mass of leaves before extraction (g)	Mass of leaves after extraction (g)	Volume of Mass solvent extract (g) added (ml)	Percentage yield of extraction (%)
3.	Ethanol	200	23.25	200	11.63
4.	Distilled water	50	15.05	100	30.1

Table 4.5 shows the average weights of the albino rats used for the experiment

Group A (g)	Group B (g)	Group C (g)
0.1039 ± 0.001	**0.1302 ± 0.003**	**0.1281 ± 0.001**

Values are mean ± standard deviation.

IV. Discussion

The potency of any hepatoprotective agent is dependent on its ability to either reduce the harmful effect (s) caused by a hepatotoxin or maintain normal hepatic physiological mechanism. The results of biochemical parameters revealed the elevation of enzyme level in CCL_4 treated group, indicating that CCL_4 induced damage to the liver. This agreed with the known effect of CCL_4 on the liver elevation in the levels of liver enzyme markers aspirate transaminase (AST). Alamine transaminase (ALT) and alkaline PHOSPHATASE (ALP). The elevated elevated elevels of these biochemical parameter are direct relection of alterations in the hepatic structural integrity. Liver injury by toxicants casues cellular leakage and loss of functional integrity (Sallie et al., 1991). ALT is a cytoplasmic enzyme found in very high concentration in the liver and an increase of the specific enzyme indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function (Adebayo, 2010)

The elevated ALT and AST in CCL4 Treated group was significantly reduced ($p<0.05$) upon treatment with the extract of Gambia albidum indicating hepatoprotection from the toxicant. Unlike the enzymes (ALP, ALT and AST), the plasma bilirubin concentration decreased markedly following the administration of the G. albidum extracts. In fact, the mean concentration of ALP, ALT, AST and total bilirubin in the blood plasma reduced markedly after measurement showing that G. albidum had a hepatoprotective effect on the CCL4-treated rats. The bark extract of G. albidum gave more protection that of the leaves of the same extract although all the targeted enzymes decreases in blood plasma to show protection from the extracts but total bilirubin still decreases faster than other enzyme during administration of the extracts. It was observed that the extract faster than other enzyme during administration of the extracts. It was observed that the extract significantly normalized the elevate3d ALT and AST across the treatment groups. $CC1_4$ treated rats showed elevated ALP activity which was significantly lowered by the extract. High level of ALP is an indicator of obstructive jaundice and intra-hepatic cholestasis (Adebayo , 2010). Rats treated with higher doses of the extract exhibited appreciable reduction in plasma total bilirubin suggesting the absence of jaundice and the effectiveness of the extract in activating a normal functional status of the liver. The incidence of liver damage was reduced after with the plant extract. Drugs or their metabolites have a short cause toxic effect on the liver. Many of the intermediate metabolites have a short half-life, some estimated to be less than a minute, which makes detecting them a challenging task (Park et al, 2005). During administration of the animals with the extracts of Gambia albidum, they showed a significant decrease in weight, even the effect of ethanolic leaf and back extract of *G-albidum* on $CC1_4$ to induce liver injury in rats make the animals to appear physically sick by showing sluggish movement and unable to behave friendly. The results of the study indicate a significant ($P>0.05$) Reduction in the mean levels of the enzymes measured, indicating hepatoprotection by the extracts of the G. albidum. The effectiveness of the water and ethanol extracts in protecting the hepatic cells did not differ significantly ($P>0.05$). In all, G. albidum had a hepatoprotective effect on the $CC1_4$-treated rats.

V. Conclusion

The study has shown that the administration of graded doses of water and ethanol extract of Gambia albidum could protect the liver from CC14 induced liver damage in rats. The present finding has provided information on the possible use of the plant for the treatment of hepatic dysfunction.

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