Toxicity studies of extract of African Mistletoe: Agelanthus Dodoneifolius Polh and Wiens in Rats

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ABSTRACT

gelathus dodoneifolius (AD) which is also known as African mistletoe is widely

used to treat Adifferent diseases such as circulatory and respiratory diseases, malaria, diabetes, hypertension and sterility. The sub-acute toxicity studies of water extract of Agelathus dodoneifolius was undertaken to assess its safety and tolerability profile in long term treatment. Sub-acute toxicity (21-days) studies with Agelathus dodoneifolius were done on rats to determine its consequences on food and fluid intake, body weight, heamatological, biochemical, and mortality. Rats treated with the extracts had progressive decrease in food, fluid intake and body weight which was significantly (P< 0.05) and highly significant (P< 0.01) different from control. The water extract increased both haematological and liver function indices significantly compare to the control. The renal function parameters were not significantly different in all the groups. These preliminary results suggest that water extract of Agelanthus dodoneifolius was likely to be non toxic. However, increase in liver enzymes will require further histopathological and chronic toxicity evaluation to confirm its safety.

Keywords: ***Agelathus dodoneifolius, Subchronic toxicity, Haematological, Biochemical.***

INTRODUCTION

n health care, medicinal plant plays an important role **I**in Africa. However, these medicinal plants are not devoid of toxicity as well as unwanted side effects (Awodele et al., 2015). Agelanthus dodoneifolius, (synonyms – Tapinanthus dodoneifolius, DC Danser (Loranthaceae) is a ubiquist plant, especially parasitizing Mimosaceae which largely grow in West Africa (Boussim et al., 2004). The Loranthaceae constitutes the largest group of parasitic plants with about 950 plants distributed in 77 genera (Engone and Salle, 2006). Loranthacean mistletoe, including A. dodoneifolius (DC) and other species are widely distributed in Nigeria and the plants are found on many host trees such as Mangifera indica, Phyllanthus niruri, Parkia biglobosa, Ziziphus spina-christi and Azadirachta indica trees (Deeni and Sadiq, 2002).

African mistletoe (Agelanthus dodoneifolius [DC]) called ‘Kauchi’ in Hausa is a hemi-plant parasite used ethno medicinally by the Hausa and the Fulani tribes of Northern Nigeria as a remedy for several human and animal ailments that include stomach ache, diarrhoea, dysentery, wound and cancer (Deeni and Sadiq, 2002). The leaves and young twigs of the plants have been used in folklore medicine to treat different diseases such as circulatory and respiratory diseases, malaria, diabetes, hypertension and sterility (Efuntoye et al., 2010). Agelanthus dodoneifolius Polh and Wiens, had been shown to possess antiplasmodial activity (Builders et al., 2012a). The cardiovascular, spasmolytic and antiinflammatory activities of water extract of A. dodoneifolius have been reported (Ouédraogo et al., 2005) Cepleanu et al., 1994 also reported the larvicidal and molluscicidal activities of this plant.

The present study was undertaken to determine the sub-acute toxicity profile of the water of the twigs of A. dodoneifolius parasitic on Parkia biglobosa.

MATERIALS AND METHODS

Plant collection and preparation

The twigs of A. dodoneifolius were collected from host plant P. biglobosa in the month of February, 2009 from Chaza village in Niger state of Nigeria. The plant was identified and authenticated and a voucher specimen (NIPRD/H/6543) was deposited at NIPRD Herbarium for future reference.

Extraction of plant materials:

The plant material was cleaned, air dried under shade and pounded into fine powder using a mortar and pestle. A 100 g quantity of the powder was boiled with 1 l of distilled water for 30 min. The decoction was decanted, centrifuged at 4500 rpm (Hamburg-Eppendorf, Germany) for 30 min and freeze-dried. The total yield of dark brown extract was 11.33% w/w of crude starting material. The freeze-dried powder was stored in an airtight container and used for the study.

Chemicals and Reagents

All chemicals were purchased from Sigma – Aldrich, USA. Phytochemical tests

The phytochemical screening of A.dodoneifolius twig extracts were carried out to determine the presence of the following compounds; alkaloid, flavonoids, tannins, anthraquinones cardiac glycosides, saponins, glycosides, sterols, resins, volatile oil, terpenes and phenols using standard procedures described by (Builders et al.,(2011)

Animals

Forty (40) adult wistar rats (180-250 g) of either sex maintained at Animal Facility Centre (AFC) of the Department of Pharmacology and Therapeutics, Bingham University were used for the study. The animals were fed with commercial pellets with free access to purified drinking water ad libitum, standard conditions of 12h:12h light/dark cycle, and temperature (23˚C-25˚C). All of the applied protocols (BU/125/30) were approved by Bingham University Research Ethics Committee.

Sub Acute Toxicity Study

Twenty four (24) rats were selected by randomization and then divided into four groups of six each. The first group served as control while the remaining three groups were given 125, 250 and 500 mg/kg of A.dodoneifolius single oral dose for 21 days according to the oral median lethal dose (LD50) in mice which was estimated to be greater than 5000 mg/kg by Builders et al., 2012a. The first day

of dosing was taken as D0 whereas the day of sacrifice was designated as D21. This was carried out according to the method of Orisakwe et al., (2003)

Metabolic cage study

Water and food intake were monitored daily for 21days.

Haematological methods

The rats were euthanized in an airtight glass chamber saturated with chloroform and after opening up the rats surgically after 21 days. Blood samples were collected by cardiac puncture into ethylene diamine tetraacetic acid (EDTA) bottles for the analysis of haematological parameters [white blood cell (WBC), packed cell volume (PCV), platelets (PLT) , neutrophils and lymphocytes (LMP)] using Sysmex KX-21N automated hematology a n a l y z e r (S y s m e x A m e r i c a I n c , U S A) . T h e microhaematocrit and cyanmethanemoglobin methods of ReyV ´ azquez and Guerrero, 2007 were used for the assay.

Biochemical analysis of serum

Blood collected into non heparinized tubes were then centrifuged at 3000 rpm for 10 min.

The serum separated was analysed to evaluate the liver enzymes [Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP)], using the method of Pieme et al.,(2006). Serum urea and creatinine were evaluated by the method of Aniagu et al., (2005).

Statistical analysis

The data were statistically evaluated by one way ANOVA. Comparison between treatment and control group were made by Student’s t- test then followed with Fisher’s exact. Differences between groups were considered significant at $P<0.05$ and highly significant at $P<0.01$

RESULTS

Table 1: Phytochemical Composition of water extracts of *Agelathus dodoneifolius*

Table 1 indicates the phytochemical analysis revealed the presence of anthraquinones, glycosides, phenols, saponins, steroids, tannins and terpenes while alkaloids and flavonoids were found to be absent.

Phytochemicals Remarks

–

Alkaloids

+

Anthraquinones

Flavonoids –

Glycosides +

Phenols +

Saponins +

Steroids +

Tannins +

Terpenes +

-Absence , + Presence

Effect of the extract on body weight

There were significant changes in the body weight of the treated rats compared to the control groups during the 21 days observation; this was highly significant from 250mg to 500mg extract /kg body weight as indicated in figure 1.

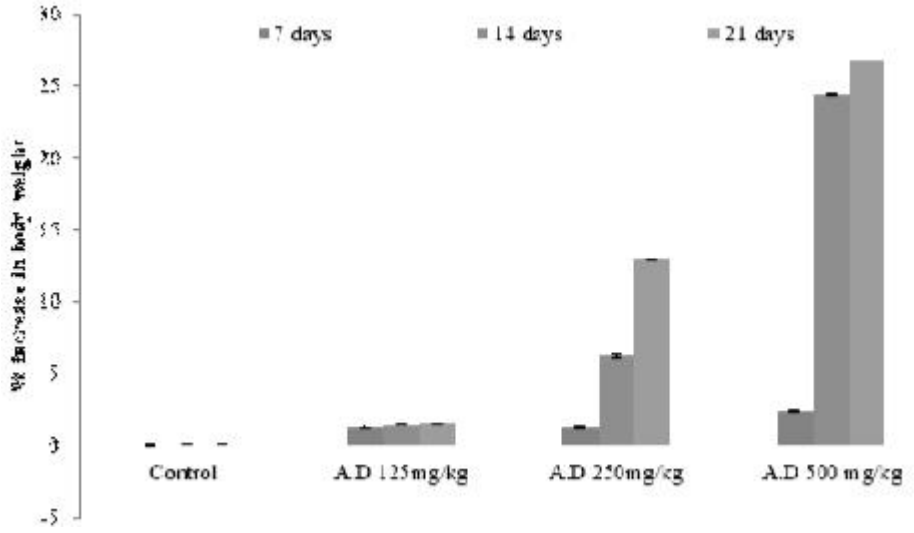

Figure 1 : Effect of Water extract of A.D on body weight

Effect of the extract on water intake

There were significant increases in water intake observed for all the treatment groups when

compared to the control group, this was highly significant after 21 days

(P<0.01) as presented in figure 2.

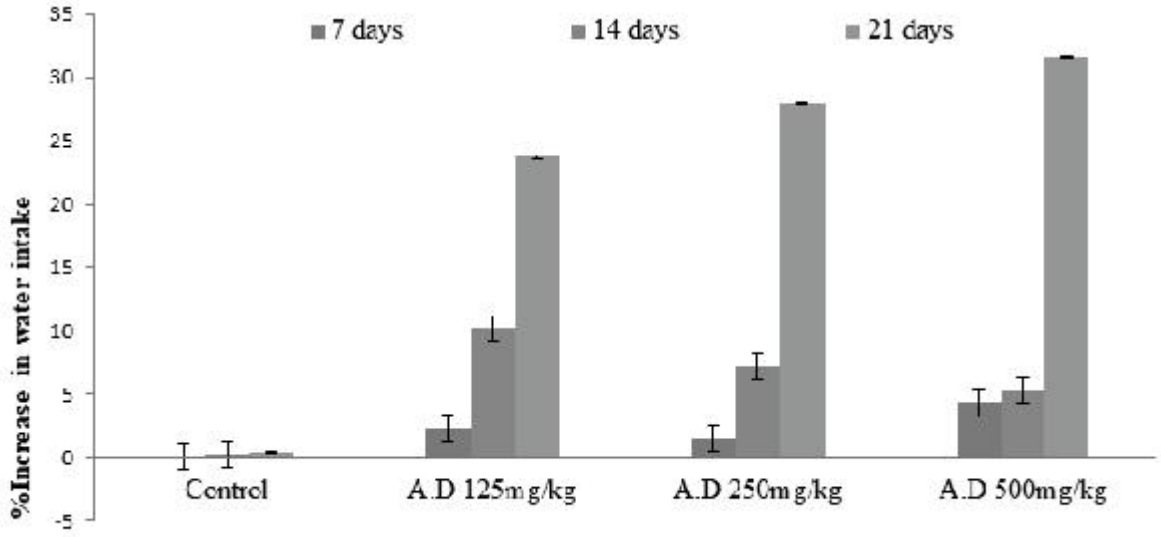

Figure 2 : Effect of Water extract of A.D on water intake

Effect of the extract on food intake

There were significant increases in food intake with the extract treated groups

compared to the control group. This was highly significant after 21 days (P< 0.01) as shown in figure 3.

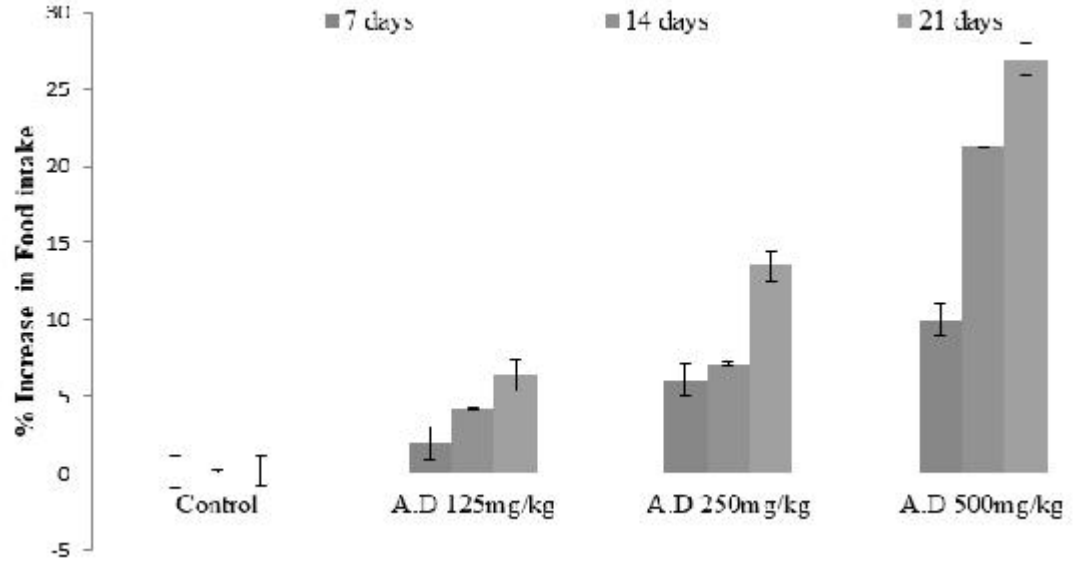

Figure 2 : Effect of Water extract of A.D on food intake

Effect of the extract on haematological parameters in rats

There were increase in white blood cell count, highly significant from 250mg/kg-500mg/kg (P<0.01). A non-

significant increase in packed cell volume was observed in all the treated

groups compared to the control. There

were significant reductions in platelet count, significant increase in neutrophil and lymphocyte level and no

significant changes in monocytes, eosinophil and basophil level as indicated in table 2.

Table 2: Effect of water extract of A.D on haematological parameters

Parameters	Control	125mg/kg	250mg/kg	500mg/kg
WBC	4133.33 ± 0.05	4550.10 ± 1.12.	7000.01 ± 0.89**	7300.21 ± 1.23**
PCV	33.7 ± 0.31	34.8 ± 0.56	36.7 ± 1.3	38.7 ± 1.21
Platelet	591 ± 1.11	268.8 ± 0.67**	484.8 ± 0.72*	114.0 ± 0.90**
Neutrophil	18.7 ± 1.21	15.7 ± 1.10	28.3 ± 0.55**	32.0 ± 0.42**
Lymphocyte	44.2 ± 0.35	75.5 ± 1.12**	68.8 ± 0.98**	120.2 ± 0.20**
Monocyte	5.3 ± 1.00	7.7 ± 0.60	5.5 ± 1.35	5.3 ± 1.09
Eosinophil	2.7 ± 0.44	2.7 ± 1.33	2.7 ± 0.86	2.7 ± 1.42
Basophil	2.5 ± 0.69	1.5 ± 1.37	2.3 ± 1.17	2.0 ± 0.67

n = 6; *significantly different from the control at p<0.05; **significantly different from the control at P < 0.01.

Effect of the extract on biochemical parameters

There were highly dose dependent significant increases in alanine transferase and aspartase enzymes (P<0.01).

No significant changes in the level of urea and creatinine as illustrated in table 3.

Table 3: Effect of water extract of A.D on biochemical parameters

Parameters	Control	125mg/kg	250mg/kg	500mg/kg
ALT	60.1 ± 1.24	130.7 ± 0.41**	104.7 ± 1.00**	314 ± 0.98**
AST	196.7 ± 0.66	218.7 ± 1.20**	302.3 ± 0.45**	774.0 ± 1.11**
Urea	8.92 ± 0.33	8.52 ± 0.86	8.71 ± 1.32	8.50 ± 0.49
Creatinine	41.1 ± 1.12	39.1 ± 1.30	38.0 ± 0.78	36.6 ± 1.17

n = 6; *significantly different from the control at p<0.05; **significantly different from the control at P < 0.01.

DISCUSSION

Ethnopharmacological use of plants can therefore be a basis for phytochemical

and phytopharmacological investigation (Kuria et al., 2001). The phytochemical tests revealed that the chemical composition of water extract of AD included anthraquinones and cardiac glycosides, these phytochemicals have protective /disease preventive properties.

The water extract of the twigs of A. doneifolius is acutely nontoxic according to the research conducted by Builders et al., (2012a) in which the LD50 of the water extract of the twig of A. dodoneifolius is greater than 5000 mg/ kg p.o. The high safety profile obtained may have been responsible for its wide spread use in different ethno-therapeutic interventions.

The increase in body weights of the treated rats is an indication of the improvement of the nutritional state of the animal which may be due to increase in food and water intake, this is similar to research conducted by Orisakwe et al.,(2003) in which progressive increase body weight was also be attributed to growth response.

Increase in haematological parameters of the extract treated groups is an indication of the antianaemic activities of the extract. Study carried out by Onyenyili et al., (1998) showed that anaemia is as a result of breakdown of blood cells and or inhibition of blood cells synthesis.

The dose dependent elevation in white blood cells count implies that the extract has the potential to boost the activity of immune system, this in agreement to the research carried out by Aniagu and co-workers in 2005 (Aniagu et al., (2005).

Specific immune response against pathogens is

lymphocytes while phagocytosis is carried out by neutrophils (Sacher and Mcpherson, 1991) . According to Muhi-eldeen et al., (2008), severe local inflammatory response in muscles is associated with significant increase in neutrophils and lymphocytes count this is in accordance to the findings of our study.

Haemostasis which is a process of reduction of blood loss and vascular injury repair is the responsibility of platelets (Dahlback, 2007). The decrease in platelet number indicates that the extract has the ability to depress the biosynthesis of clotting factors by liver, therefore the extract has antiplatelet activities similar to many bioactive compounds such as garlic, vitamins, carotenoids (Naidu, 2015; Bhowal and Mehta, 2017; Imran et al., 2012) .

Alanine amino transferase (ALT) and aspartate aminotransferase (AST) are markers of liver function; increase in these liver enzyme parameters is an indication of hepatic damage which is similar to study conducted by Builders et al., (2012b)

in which the water extract of the parasitizing plant Parkia biglobosa caused severe histopathological changes in the liver.

The extract of Agelathus dodoneifolius did not interfere with renal function since the

blood urea and creatinine levels were normal; this shows that the renal integrity was preserved. This is similar to research conducted by Builders et al., 2012b in which the water extract of the parasitizing plant Parkia biglobosa did not affect the renal function.

CONCLUSION

These preliminary results suggest that the methanolic extract of Agelanthus dodoneifolius was non- toxic. However histopathological and chronic toxicity evaluations will be required to confirm its safety.

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