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Histopathological assessment of oral administration of aqueous extract of *Cassytha filiformis* in Rats

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ABSTRACT

The effect of aqueous extract of *Cassytha filiformis* leaves were evaluated using histological sections of liver, heart, lungs, kidneys, spleen and testes of albino rats. Experimental albino rats received 250 mg/kg.bw, 500 mg/kg.bw, and 1000 mg/kg.bw of the extract for 28 consecutive days. Administration of extract at 250 mg/kg.bw, 500 mg/kg.bw and 1000 mg/kg.bw to rats was presented with histopathological signs in the liver in the form of focal periportal and pericentral lymphocytic infiltrates when compared with the control. Focal granuloma was observed in the lungs at all the doses when compared with the control. Alterations were observed in the kidney at all the doses of extract in the form of focal lymphocytic infiltrates and focal interstitial hemorrhages in comparison with the control. There were no histological abnormalities in the heart and spleen of the animals at all the doses of the extract when compared with the control. The results of this study suggest that aqueous extract of *C. filiformis* administered at 250 mg/kg.bw, 500 mg/kg.bw and 1000 mg/kg.bw is likely to produce severe toxic effects on the liver, kidneys, lungs and testes and should therefore be used with high degree of caution.

Keywords: *Cassytha filiformis*, aqueous extract, sub-chronic administration, histology, albino rats.

1. INTRODUCTION

Cassytha filiformis (Lauraceae) also known as green or Laurel dodder in English, rumfdar gada in Hausa, aca-agadi in Igbo, soko chenche in Nupe and ominiginigini in Yoruba is a dicotyledonous plant widely distributed in Florida, Central and Southern Peninsula, Texas, Hawaii, Puerto Rico, Austria, Virgin Islands, West Africa [1]. The genus Cassytha is the only parasitic member of the otherwise autotrophic Laurel family. The host range of C. *filiformis* is broad and include *Acacia auriculiformis*, *Anacardium occidentalis*, *Magnifera indica*, *Azadirachta indica*, *Hyptis suaveolens*, *Eugenia aromatica*, *Myristica fragrans*, *Persia americana*, *Tournefortia argentea*, *Scaevola sericea*, etc [2-6]. This twinning parasitic flowering plant absorbs nutrients from the cell membrane of host plant by means of specialized green to orange stem called haustoria [4,5]. Its stem is filiform containing

chlorophyll [6]. The leaves are either absent or reduced to minute scales [1]. The leaves when present are simple, without stipules and usually alternate. The flowers are actinomorphic, usually bisexual and are pollinated by various methods. The flowers are white with three sepals and petals [1,4,5]. The androecium most frequently comprises four whorls of three stamens each, although the inner whorls are often sterile. The filaments of the inner whorls usually have a pair of enlarged glandular appendages near the base.

The anthers dehisce by means of common four, upwardly opening flaps. The single, simple pistil has a superior ovary with single ovule in a solitary locule. The fruit is about 4-6 mm in diameter, soft and green in colour. The fruit is one seeded drupe, often surrounded basally by the short, persistent perianth cup. The seeds require scarification (breaking or scratching of the seed coat) but no host stimulant. Seeds may be dispersed by animals, water, strong winds, farm machinery or crop seeds [6].

Unlike other Magnolidae, the endosperm is completely absorbed by the embryo in Lauraceae [4,5]. The plant has a terrestrial life cycle with various life spans and growth forms as well as various overall height and spread. It requires various water and optimal soil textures and various acceptable pH. The plant tolerates drought, high humidity and sea side conditions but they are shade intolerant. They are insect and disease resistant. They are found in symbiotic associations with butterflies and humming birds [4,5,6].

Ethnomedicinally, it is useful in treating diabetes, ulcer, veneral discharges, haemorrhoids, cough, cancer and African trypanosomiasis [1,3]. It is used as a potential biological control agent for invasive plants [6]. It is used to suppress lactation after still birth [7]. The plant is used to treat jelly fish sting in Fiji, it is used to ease labour pains and quicken labour time and lubricate the birth canal [8]. Non-ethnomedicinal uses of the plant include for dyeing, food plant for humans and animals, vines are used in thatched roof construction [6]. The fruit is ammunition for popguns. The sap from stems is used as shampoo and hair conditioner. The plant is used to line earthen ovens. The plant is used in casual head garlands for picnics. The tips are used for scenting coconut oil [6].

Biologically, the plant has been reported to possess antitrypanosomal property and six aporphine alkaloids that include neolitsine, dicentrine, cassythine, actinodaphine, norneolitsine and cassythidine were isolated and quantified [3]. Preliminary phytochemical screening of the methanol, hot water and n-hexane extracts of *C. filiformis* revealed the presence of glycosides, steroids, terpenoids, alkaloids, carbohydrates, acidic compounds, resins, saponins, tannins, fats and oils and flavonoids [9]. The plant has a number of biologically active chemical compounds with potential human health applications. Ocoteine, a compound isolated from *C. filiformis* was found to be an alpha 1-adrenoceptor blocking agent in rat thoracic aorta which has potential applications for inhibiting certain carcinomas such as prostate cancer. Octoeine and a number of other compounds in C. filiformis have antiplatelet aggregation activity [10]. [11] reported the presence of alkaloids, glycosides, flavonoids, saponins and tannins in cold extracts of methanol and petroleum ether of *C. filiformis*. [12] demonstrated the protective action of aqueous whole extract of *C.filiformis* on plasma biochemical parameters (GOT, electrolytes (Na+, Cl- & K+), total and direct bilirubin, creatinine, glucose level, haematograms (Hb, WBC, RBC, PCV, platelets, MCH, MCHC, MCV and differential values) as well as its hyper cholesterolacmic effects in rats. There is a need for the investigation of the toxicity profile of *C. filiformis* aqueous whole extract was prompted by its rampant use by substantial population in northern Nigeria for various treatment interventions given the proven pharmacological actions of some of the active principles as well as prohibitive cost of synthetic products compared with the prevailing financial capacity of the local population.

2. MATERIALS AND METHODS

2.1 Laboratory animals

Adult Albino male rats (140-250 g) were used for the subacute study. The animals were obtained from Animal Facility Centre (AFC), National Institute for Pharmaceutical Research and Development (NIPRD). They were allowed free access to water and food and were maintained in plastic clean cages under standard humidity (40-60%), temperature (20-22˚C) and 12 hour light and darkness cycle of the prevailing time period in a well ventilated room. Soiled wood shavings in the animal cages were replaced often. The experimental rooms were cleaned and disinfected regularly. The animal cages, feed and water containers were washed regularly. They were acclimated to housing conditions for one week prior to commencement of the study. The animals were handled in accordance with the WHO good laboratory practice (GLP) regulations of 1998 [13].

Principles of laboratory animal care were adopted throughout the study.

2.2 Experimental design

One hundred (100 g) grams of dried whole plant (stems and leaves) and lake salt (red potash) (2 g) were refluxed in 500 ml of sterile distilled water for 6 hours. The mixture was filtered with whatman filter paper No. 1 and filterate was concentrated to dryness at 50˚C with a rotary evaporator. The dried aqueous whole extract was stored in air tight sterile container and refrigerated until it was required for use. The method of [14] was adopted for the subacute study. Twenty four (24) Albino rats were used for the subacute investigation. They were divided into four groups of six rats. The first group was administered water (control) while the three other groups were orally administered 250 mg/kg.bw, 500 mg/kg.bw and 1000 mg/kg.bw of aqueous whole extract consecutively for 28 days. The selection of the dose range was based on previous study of the antidiabetic activity of the extract on alloxan induced diabetic rats (data not shown). The heart, spleen, liver, lungs, kidneys, testes were removed from the rats and fixed in 100% formal saline for at least 48 hours. These were processed routinely and embedded in paraffin wax. Histological sections were cut at 5-6 µm and stained with routine haematoxylin and eosin (HE). The lesions observed were assessed for the following: focal periportal and pericentral lymphocytic infiltrate's, atrophy, focal granuloma. These were graded according to mild (+), moderate (++). Photomicrographs of representative lesions were taken at various magnifications.

3. RESULTS AND DISCUSSION

Table 1 shows the results of histology of organ changes in rats fed aqueous extract of *Cassytha filiformis*. Changes were observed in the liver at 250 mg/kg.bw, 500 mg/kg.bw and 1000 mg/kg.bw in the form of focal periportal and pericentral lymphocytic infiltrates (5/6 rats). Broncho-pneumonia were observed in the lungs in 250 mg/kg.bw group (4/6 rats), 500 mg/kg.bw (5/6 rats). Furthermore, focal granuloma were observed in the lungs at all doses (4/6 rats). Moderate atrophy and focal granuloma were formed in the testes at all the doses (4/6 rats). Changes were observed in the kidney at all doses in the form of focal lymphocytic infiltrates (6/6 rats). Focal interstitial hemorrhages were observed at all the doses of extract (5/6 rats). There were no significant changes seen in the heart and spleen of the animals exposed to aqueous whole extract of *Cassytha filiformis* when compared with the control. Figure.1 shows the photomicrograph of the organs. In the previous study, we demonstrated the haematological and biochemical effects of *C. filiformis* aqueous whole extract [12]. The present study investigates the effect of aqueous whole extract of *C. filiformis* on histological appearance of the liver, kidney, heart, lungs, spleen and testes of rats following 28 days of exposure.

Albumin, total and direct bilirubin, total protein and serum alkaline phosphate (ALP) levels are markers of hepatic cell damage. Although absolute reliance on transaminase enzymes to presume liver disorder is not a good measure of liver function because they are not specific to the liver but are also present in red blood cells, cardiac and skeletal muscles. Alanine transaminase (ALT) and aspartate transaminase (AST) are however raised in acute liver damage, thus serum levels of these enzymes are used to evaluate injury to the liver in individuals with previous history of intact liver function. Elevated levels of transaminases (ALT/AST) have been acknowledged to favor liver cell necrosis [15].

In the study conducted by [12], the serum level of glutamate pyruvate transaminase (SGPT or ALT) was significantly ($p<0.05$) increased dose dependently in animals treated with *C. filiformis*, thus it is safe to presume that the plant may cause dose-dependent hepatotoxicity. This presumption reconciles with the micrograph results obtained from histological analysis of the liver, which showed remarkable lymphocytic infiltration; suggesting that aqueous whole extract of *C. filiformis* adversely affected the structure and physiological functions of the liver. These notable dose-dependent responses observed in the biochemical liver enzyme with commensurate effect on liver integrity as seen in the histology further suggest that aqueous extract of *C. filiformis* may contain compounds acting similarly to medications that are implicated in drug-induced liver injury (DILI). This submission is substantiated by the report of [16], that herbal medicine-related hepatotoxicity represents the second most common cause of drug-induced liver injury (DILI) in Western countries.

Creatinine is a chemical waste molecule that is produced from creatine during muscle metabolisms. Only about 2% of creatine in the body is converted to creatinine daily. The amount of creatinine in rat serum was constant after exposure to aqueous whole

Table 1: Histology of organ changes in rats fed aqueous whole extract of *Cassytha filiformis*

Organ	Group			
	1	**2**	**3**	**4**
Liver	NAD	Focal periportal and pericentral lymphocytic infilterates +	Focal periportal and pericentral lymphocytic infilterates +	Focal periportal and pericentral lymphocytic infilterates ++
Spleen	NAD	NAD	NAD	NAD
Kidney	NAD	Focal lymphocytic infilterates +	Focal lymphocytic infilterates +	Focal lymphocytic infilterates ++ Focal haemorrhage +
Heart	NAD	NAD	NAD	NAD
Testes	NAD	Moderate atrophy + Focal granuloma +	Moderate atrophy + Focal granuloma +	Moderate atrophy + Focal granuloma +
Lung	NAD	Focal granuloma +	Bronchopneumonia, Focal granuloma ++	Bronchopneumonia, Focal granuloma ++, Interstitial fibrotic nodule +

NAD: No adverse effect, Group 1: Control, Group 2: 250 mg/kg.bw, Group 3: 500 mg/kg.bw, Group 4: 1000 mg/kg.bw

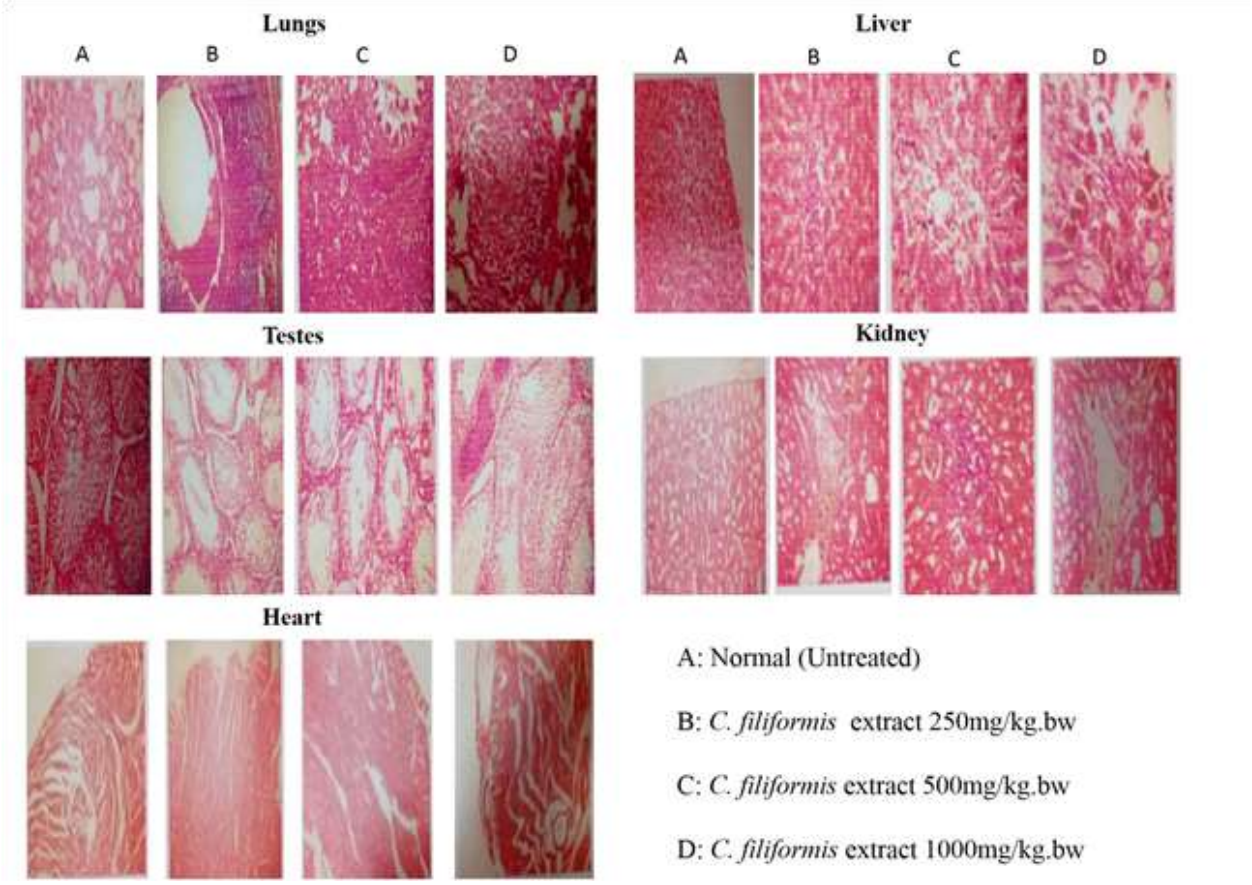

Figure.1. Histological examination of lungs, liver, kidney, testes and Heart of normal (untreated) and *C.filiformis* extract treated group.

extract of *C. filiformis* [12]. This finding supports already established fact that creatinine production remains fairly unchanged because the body muscle mass is relatively constant day to day [17]. In the present investigation, photomicrographs obtained from histological analysis of the kidney showed inflammatory reactions indicated by focal hemorrhage, with severity observed in the treatment with the highest dose. This suggests that exposure to high doses of C. filiformis may produce certain degree of renal injury or gastrointestinal damage. This finding negates the finding of [18], where C. filiformis was found to have haemostatic properties.

Changes in organ weight are acceptable as sensitive indicator of chemically induced changes in organs. In toxicological experiments, comparison of organ weights between treated and untreated groups of animals have conventionally been used to evaluate the toxic effect of test articles [19,20]. The decrease in relative lung weight value obtained in 500 mg/kg.bw and 1000 mg/kg.bw groups by [12] support the remarkable changes observed in the histomorphology of the lungs in the present study. This result is validated by [19,21], who studied the relationship between organ weight to body weight, and posits that the relationship between weights should be proportional, such that an increase in one of the weights should be followed by increase in the other weight proportionally.

Although [12] reported that 500 mg/kg.bw of aqueous whole extract of C. filiformis reduced the relative weight of the heart when compared with the other groups and the control and an increased serum cholesterol level of treated rats. There were no histological abnormalities in the heart of the animals at all the doses of the extract in the present investigation. It is reasonable to deduce that exposure of the animals to aqueous extract was not toxic to the heart. The variance of serum cholesterol level indicates that the increase is not significant to cause damage to the heart. The result of the present study indicates that continuous use of the extract at doses of 250 mg/kg.bw, 500 mg/kg.bw can lead to pathological changes involving the testes of the animals. There were no histological abnormalities observed in the spleen of rats at all the doses of the extract.

4. CONCLUSION

We conclude that the aqueous whole extract of C. filiformis produced no toxic effects in heart and spleen of rats. Although, the extract is reported to have medicinal potentials, it may have long term toxic effects on the kidney, liver, lungs and testes. The present study therefore constitutes safety information that could be useful in the formulation of local phytomedicine products using the crude aqueous whole extract of C. filiformis. The strict respect of safe dosage by local users' needs to be emphasized. Chronic toxicity evaluation and teratogenic studies need to be carried out.

Conflicts of Interest

There are no conflicts of interest.

References

1. Abdullahi, M., Mohammed, G. and Abdulkadir, N. U. (2003). Medicinal and Economical Plants of Nupeland. Jubes-Evans Publications, Bida, Nigeria. Pp. 140.
2. Nickrent, D. L. and Musselman, L. J. (2004). Introduction to Parasitic Flowering Plants: The Plant Health Instructor. www.apsnet.org/education/IntroPlantpath/Pathogen Groups/Parasiticplants.
3. Lerclercq, J. Q., Hoet, S., Block, S., Wautier, M. C. and Stevingny, C. Study on *Cassytha filiformis* from Benin: Isolation, Biological Activities and Qualification of Aporphines (2004). *Proceedings of Bioresources towards Drug Delivery and Development*, 81-106.
4. American Phytopathological Society, APS (2006). Introduction to parasitic flowering plants. http://oak/ppws.vt.edu/ipps/.
5. American Phytopathological Society, APS (2009). Introduction to parasitic flowering plants. http://oak/ppws.vt.edu/ipps/.
6. Nelson, S. C. (2008). *Cassytha filiformis*. Plant Disease, 42.
7. Neuwinger, H. D. African Traditional Medicine (2000). A Dictionary of plants' Use and Applications. *Medicinal Pharmacology*, 99:1-12.
8. Kobayashi, J. (1976). Early Hawaiian uses of medicinal plants in pregnancy and childbirth. *Journal of Tropical Pediatrics*, 22:260-262.
9. Adonu, C. C., Ugwu, O. P. C., Esimone, C. O., Ossai, E. C., Bawa, A., Nwaka, A. C. and Okorie, C. U (2013). Phytochemical analyses of the methanol, hot water and n-hexane extracts of the aerial parts of *Cassytha filiformis* (Linn.) and

leaves of Cleistopholis patens (Benth). Research *Journal of Pharmaceutical, Biological and Chemical Science*, 4(2):1143.
10. Chang, C. W., Ko, F. N., Su, M. J., Wu, Y. C. and Teng, C. M. Pharmacological evaluation of ocoteine isolated from *Cassytha filiformis*, as an alpha (1)-adrenoceptor antagonist in rat thoracic aorta (1997). *Japanese Journal of Pharmacology*, 73:207-214.
11. Ngele, S. O. and Oti, J. O (2016). Preliminary Study of the Phytochemical Constituents of *Cassytha filiformis* (Love Vine). *Global Journal of Pharmacology*, 10(4):101-107.
12. Babayi, H. M., Ijah, U. J. J., Abalaka, J. A., Okogun, J. I., Salawu, O. A., Akumka, D. D., Adamu, A., Zakariya, S. and Inyang, U. S. (2007). Effects of oral administration of aqueous whole extract of *Cassytha filiformis* on Haematograms and Plasma Biochemical parameters in rats. *Journal of Medical Toxicology*, 3(4):146-157.
13. World Health Organization. Basic OECD Principles of Good Laboratory Practice (updated 2004 cited 2006 Nov. 11) http://www.who.Int/tdr/publications.
14. Aniagu, S. O., Nwinyl, F. C., Akumka, G. A., Dzarma, S., Izebe, K. S. and Gamamel, K (2005). Toxicology studies in rats fed nature cure bitters. *African Journal of Biotechnology*, 4(1):70-72.
15. Sareatawong, N., Lertprasertsuke, U. and Scrisawat, S. (2008). Acute and sub-chronic toxicity study of the water extract from *Tilicora trianora* (Colebr.) Diels in rats. Songklanakarin *Journal of Science and Technology*, 30(6):729-737.
16. Evangelos, S. and Konstantinos, T. (2015). Herbal medicine-related hepatotoxicity. World *Journal of Hepatology*, 7(19):2189-2193.
17. Bohacik, J., Kambhampati, C., Davis, D. N. and Cleland, J. G. F. (2013). Prediction of mortality rates in heart failure patients with data in mining methods. *Annales UMCS, Informatic*, 13:1.
18. Dandjesso, C., Klotoe, J., Dougnon, T., Segbo, J., Ategbo, J. and Gbaguidi, F. (2012). Phytochemistry and hemostatic properties of some medicinal plants sold as anti-hemorrhagic in *Cotonou* markets (Benin). *Indian Journal of Science and Technology*, 5(8):3105.
19. Bailey, S. A., Zidell, R. H. and Perry, R. W. (2004). Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint? *Toxicological Pathology*, 32:448-466.
20. Sellers, R., Morton, D., Michael, B., Roome, N., Johnson, J., Yano, B., Perry, R. and Schafer, K. (2007). Society of Toxicology Pathology Position Paper: Organ Weight Recommendations for Toxicologic Study. *Toxicological Pathology*, 35:751-755.
21. Erik, W., Anna, O. and Lena, S. (2013). Use of lung weight as biomarker for assessment of lung toxicity in rat inhalation studies. *Toxicological Pathology*, 41:902-912.