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Biochemical and Histological Studies of Aqueous Extract of *Bidens pilosa* Leaves from Ugandan Rift Valley in Rats

[1,2]J.O.C. Ezeonwumelu, [1]A.K. Julius, [2]C.N. Muhoho, [2]A.M. Ajayi, [2]A.A. Oyewale, [2]J.K. Tanayen, [2]S.O. Balogun, [2]A. Ibrahim, [2]B. Adzu, [2]C.P. Adiukwu, [2]J. Oloro, [2]D.M. Kiplagat, [2]A.D.T. Goji, [1]A.G. Okoruwa, [2]A.N. Onchweri and [3]P.M.K. Reddy

[1]Department of Clinical and Biopharmacy, School of Pharmacy, Kampala International University, Western Campus (KIUWC), P.O. Box 71 Bushenyi, Uganda

[2]Kampala International University Complementary and Alterative Medicine Research (KIUCAMRES) Group, Faculty of Biomedical Sciences, P.O. Box 71, Bushenyi, Uganda

[3]Department of Pharmacology, JIPMER, Puducherry-6, India

Abstract: *Bidens pilosa*, a member of the Asteraceae family, is one of the dominant medicinal plant species worldwide including Uganda. This study aimed at assessing both acute and sub-chronic toxicities of *Bidens pilosa* Leaves Extract (BPLE). Phytochemical tests were done on BPLE to detect the presence of secondary metabolites. In acute toxicity test, a single oral dose of BPLE (500-10,000 mg/kg) was used on each group of Wistar rats. For sub-chronic toxicity study, BPLE (200-800 mg/kg) and distilled water were orally administered daily for 28 days. Signs of toxicity were observed and rats sacrificed, blood and organs collected for biochemical and histological studies. The BPLE was found to contain tannins, flavonoids, phlobatannins, terpenoids and cardiac glycosides. No mortality was recorded during the studies in rats. There was a general reduction in mean percentage body weight gain of test rats compared with control; statistically significant ($p<0.05$) between 400 mg/kg and control and between 400 and 800 mg/kg. Significant increments in mean relative organ weight of the heart occurred between control and 400 mg/kg groups. There were significant ($p<0.05$) increases in the serum levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma Glutamyl Transpeptidase (GGT) enzymes at dose of 800 mg/kg and of cardiac Creatine Kinase (CK-MB) at all three doses when compared with the control. Light micrograph of the liver, kidney and heart tissues at 400 magnifications appeared normal with no changes. It can be concluded that BPLE from the Rift Valley Region of Western Uganda contains secondary metabolites and has high index of safety.

Key words: Acute toxicity, *Bidens pilosa*, phytochemicals, sub-chronic toxicity, tissues, Uganda

INTRODUCTION

Bidens pilosa is a member of the Asteraceae family, which is one of the dominant families of plants contributing to medicinal species worldwide. It is a plant that belongs to the same botanical family as feverfew. It is indigenous to the Amazon rainforest and other tropical areas of South America, Africa, the Caribbean, and the Philippines. It is often considered a weed in many places in Africa including Uganda (Duke, 1997). *Bidens pilosa* (hairy beggar ticks) (Grubben and Denton, 2004); common names include '*Mucege'* (*Kikuyu*, Kenya), *'Enyabarashana'* (*Ankole*, Uganda), *'Ogwunma'* (*Ibo*, Nigeria), '*Guizhencao'* (China), bur marigold (Europe). It is a popular folk medicine in Taiwan for all sorts of illnesses, from influenza to hepatitis. In one study with laboratory animals, Taiwanese scientists showed that this herb has significant anti-oedemic and anti-inflammatory activity (Duke, 1997).

Medicinal plants are re-emerging health aid, which has been observed in developing countries probably fuelled by the rising costs and resistance of orthodox drugs in the maintenance of personal health and well-being (Okeke *et al*., 2005). Generally, the whole plant is prepared in decoctions or infusions for internal use or crushed into a paste for external use. In Southern and Central America (such as Peru, Mexico and Brazil), *Bidens pilosa* is used for foot-and-mouth disease, angina, diabetes, menstrual disorders, hepatitis, laryngitis, pharyngitis, hemorrhoids, as a gargle for mouth blisters,

Corresponding Author: J.O.C. Ezeonwumelu, Department of Clinical and Biopharmacy, School of Pharmacy, Kampala International University, Western Campus (KIUWC), P.O. Box 71 Bushenyi, Uganda

hepatitis, nervous problems, intestinal worms, for internal and external inflammations, tooth-ache, headaches, sores, lacerations, upset stomach in food poisoning, sore throat and water retention (Taylor, 2005; Duke, 1997). In Uganda, the sap from crushed leaves is used to speed up clotting of blood in fresh wounds; a leaf decoction is used for treating headache; sap from the plant is put in the ear to treat ear infection; a decoction of leaf powder is used to treat kidney problems; and a herbal tea made from the plant decreases flatulence (Tadesse, 1994). It is also used in Uganda by traditional healers in the treatment of opportunistic infections of HIV/AIDS patients mostly oral lesions (Theta, 2005). Its roots, leaves, and seeds are reported to have antibacterial, anti-dysenteric, anti-inflammatory, and antimicrobial, antimalarial, diuretic, hepatoprotective, and hypotensive properties (Mvere, 2004; Rojas *et al*., 2006; Wat *et al*., 1980; Dimo *et al*., 2002; Brandao *et al*., 1997; Chiang *et al*., 2004).

Although there is some variation in the level of activity of the different species of *Biden*s, probably due to different levels of active constituents, the general properties appear similar (Andrade-Neto *et al*., 2004). *Bidens pilosa* is used in popular medicine in Uganda for the treatment of several ailments. This suggests that *Bidens pilosa* leaves' aqueous extract contains different active phytochemicals responsible for its uses and perhaps has some level of safety. Despite the popular use of this plant in Uganda, there is dearth of information concerning the toxicity of this local variety. Therefore, this study evaluated the phytochemicals, acute and sub-chronic toxicities and selected histological and biochemical effects of aqueous extract of Ugandan Rift Valley *Bidens pilosa*.

MATERIALS AND METHODS

Preparation of plant extract: The *Bidens pilosa* plant was identified by a botanist from KIUWC. Fresh leaves of *Bidens pilosa* were collected on 30th of March 2010 early in the morning during the rainy season from Ishaka in Bushenyi District, Western Uganda. A voucher specimen of the plant was deposited in the Herbarium of the Pharmacognosy Unit of School of Pharmacy of Kampala International University-Western Campus (KIUWC) Ishaka, Uganda. The leaves were washed with clean water, dried under shade and ground into fine powder. Decoction method of extraction was used; 400 g of the powder was weighed into an empty clean beaker and 1 L of distilled water added and boiled for 4 h. It was cooled at room temperature and filtered using a clean cotton cloth and subsequently through cotton wool and Whatmann No. 1 filter paper plugged in a funnel. The filtrate was poured into clean dry beakers, which were held in the water bath by retort stands and evaporated to dryness at 80^{o}C.

Preliminary phytochemical screening: Qualitative phytochemical tests were conducted on the aqueous BPL extract using standard methods (Trease and Evans, 2002).

Laboratory animal acquisition and maintenance: Wistar rats were bred at the Animal Facility in the School of Pharmacy, KIUWC. Twenty healthy young adult male *wistar* rats weighing between 90 and 200 g were obtained and housed separately for 1 week to allow for acclimatization. The animals were kept in a cage lined with wood shavings, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 h of light and 12 h of darkness. They were fed with standard diet (Nuvita® Animal Feeds Ltd., Jinja Uganda). They had access to clean drinking water *ad libitum.* The animal experiments were conducted according to the National Institute of Health Guide for the care and use of laboratory animals (NIH, 1996) and ethical guidelines for investigation of experimental pain in animals (Zimmerman, 1993).

Acute toxicity study: This test was conducted in two phases according to the Lorke's (1983) method for the evaluation of the safety of herbal medicines. In phase I, nine male young adult *Wistar* rats were randomly divided into three groups of three animals. The animals were deprived of food for 16-18 h prior to administration of the extract. The rats were weighed and grouped in 3 groups as follows: treatment groups (group A for low dose of 500 mg/kg), group B for medium dose of 1,000mg/kg and group C for high dose of 1,500 mg/kg and administered extracts and observed for signs of toxicity immediately and up to 72 h after administration. In Phase II, three male young adult *Wistar* rats were divided into three groups of one animal. The animals were deprived of food for 16-18 h prior to administration of the extract. They were weighed and grouped into 3 groups as follows: treatment groups (group A for low dose of 2,000 mg/kg), group B for medium dose of 5,000 mg/kg and group C for high dose of 10,000 mg/kg.

Sub-chronic toxicity test: Organization for Economic Cooperation and Development (OECD) Test Guidelines (TG) which describe short-term repeat-dose toxicity testing: Repeated Dose 28-day Oral Toxicity Study in Rodents (TG407) was used for the study (OECD, 2006).

Twenty young healthy *Wistar* rats were weighed and grouped randomly into four groups (n = 6) as follows: the treatment group included fifteen rats of three groups (group A for low dose (200 mg/kg), group B for medium dose (400 mg/kg) and group C for high dose (800 mg/kg); the control group comprised five rats to which distilled water was administered. They were given food thirty minutes after administration of the extract by gavage. Body weights of the animals were taken daily for 28 days before administration of the extracts. The rats were observed daily to detect differences in appearance,

Table 1: Mean body weight and S.E.M values of rats (grams)

Group/Days	Day 0	Day 7	Day 14	Day 21	Day28
Control	115.86±6.90	154.12±8.20	166.06±7.04	169.42±6.78	195.04±6.87
200 mg/kg	116.62±6.15	115.12±5.65	166.94±6.12	179.48±6.15	197.32±5.56
400 mg/kg	118.56±5.73	131.00±9.17	142.08±9.34	157.16±10.44	164.44±8.91
800 mg/kg	117.74±6.23	147.66±4.53	157.72±4.75	163.40±5.54	187.68±3.46

N:5; values presented as mean ± standard error of mean (S.E.M.)

discoloured fur, diarrhoea, bloody stool and constipation, loss of appetite and thirst and lack of interest in the environment. After 28 days, all surviving animals were allowed to fast overnight and sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture from the animal into non-heparinized vacutainers for biochemical parameters analysis. Organs were removed, weighed and absolute and relative organ weights determined and organs preserved separately for each animal in 10% neutral buffered formalin to prevent tissue autolysis and kept for histological analysis.

Biochemical analysis: The blood collected into non-heparinized tubes was allowed to clot for 30 min and then centrifuged at 3000 rpm for 10 min. The serum was separated and analyzed for some enzymes. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK-MB) and gamma glutamyl transpeptidase (GGT) were assayed using the Humalyzer 2000, German model.

Histological study: Preserved tissues were histologically analysed at Histology Laboratory of Mbarara University of Science and Technology, Uganda. Tissues (liver, kidney and heart) were processed with microtome (Ernst Leitz Wetzlar GMBH 530497 No. 537, Germany) and automated tissue processor (USA), embedded, placed on a slide and stained with Haematoxylin and Eosin stain (H and E) (Ganter and Jolles, 1970; Lucia *et al*., 2008). The tissues were observed under light microscope for changes in structure and the pictures taken with digital camera (Kodak, USA) attached to the eyepiece of the light microscope. Relative organ weight was determined using the following formula,

Relative organ weight = [Absolute organ weight (g) × /Body weight of rat on sacrificed day g)]×100

Statistical analysis: Data are expressed as mean values ±standard error of mean (SEM) and group data comparisons were evaluated by an independent sample t-test with significance at $p<0.05$ using SPSS version 17.0.

RESULTS

Phytochemical analysis: The phytochemical analysis of the aqueous BPL extract revealed the presence of tannins, flavonoids, phlobatannins, terpenoids and cardiac glycosides.

Acute toxicity evaluation: No animal death occurred in both phases I and II indicating no toxicity. There were no differences in appearance, no discoloured fur, diarrhoea, bloody stool, constipation, anorexia, and no lack of thirst and interest in the environment in the rats.

Sub-chronic toxicity study: There were no differences in appearance, no discoloured fur, no diarrhoea, no bloody stool, no constipation and no lack of appetite, thirst and interest in the environment among the rats.

Table 1 shows results of mean weight values of rats over 28 days of treatment with BPL extract. Here, no mortality was recorded throughout the duration of sub-chronic toxicity studies thus indicating high safety profile for BPL in experimental rats. Increase in body weights of rats were observed along each group of rats from days zero to 28. However, significant reduction in body weights of the rats were recorded at $p<0.05$ on the 28^{th} day down the group between control (195.04 g) and 400 mg/kg (164.44 g) groups and between 200 mg/kg (197.32 g) and 400 mg/kg (164.44 g) groups of rats. But significant increment in body weights of experimental rats occurred between 400 mg/kg (164.44 g) and 800 mg/kg (187.68 g) groups of experimental rats at $p<0.05$ on the same 28^{th} day of the study .

Table 2 displays percentage gain in body weights of experimental rats. Body weight gain was generally observed across the groups. But down the groups, there was reduction in body weight gain among the experimental rats with the highest reduction in the 400 mg/kg group. Significant reduction ($p<0.05$) in percentage body weight gain was observed on day 7 between the control group (33.27 g), 400 mg/kg group (10.57 g) and 800 mg/kg group (25.99 g), and between 200mg/kg (33.53 g) and 400 mg/kg groups (10.57 g). But there was a significant body weight gain at $p<0.05$ (two tailed) on the same day 7 between 400 mg/kg (10.57 g) and 800 mg/kg groups (25.99 g) of rats; on day 14, percentage body weight gain decreased significantly at $p<0.05$ (two tailed) between 200 mg/kg (43.82 g) and 400 mg/kg groups (19.71 g) of animals and increased significantly at $p<0.05$ (two tailed) between 400mg/kg (19.71g) and 800 mg/kg groups (34.76 g); on day 21, body weight gain decreased significantly at $p<0.05$ (two tailed) between 200 mg/kg (55.02 g) and 400 mg/kg groups (32.15 g) and day 28 followed the same pattern as day 14. When data was tested with Pearson's correlation it was found out that percentage gain in body weights of experimental rats correlated significantly at $p<0.01$ (2 tailed) on days 7 and

Table 2: Mean percentage gain in body weight and S.E.M values of rats (%)

Group/Days	Day 7	Day 14[1]	Day 21[1,2,]	Day 28[1,2,3]
Control	33.27±1.14	44.06±3.35	47.09±4.00	69.50±5.02
200 mg/kg	33.53±2.46	43.82±3.86	55.02±6.62	70.75±8.32
400 mg/kg	10.57±5.78[4,5]	19.71±4.36[4,5]	32.15±3.05[4,5]	38.64±2.53[4,5]
800 mg/kg	25.99±2.85[4,6]	34.76±4.53[6]	39.52±4.48	60.68±6.33[6]

N:5; values presented as mean±standard error of mean (S.E.M.) [1]: Pearson correlations are significant at the 0.01 level (2 tailed) and equal variances assumed; [2]: Pearson correlations are significant at the 0.01 level (2 tailed) and equal variances assumed; [3]: Pearson correlations are significant at the 0.01 level (2 tailed) and equal variances assumed; [4]: indicates values significant at p<0.05 (2 tailed) between control and treated groups;[5]: indicates values significant at p<0.05 (2 tailed) between 200 mg/kg and the 400 mg/kg and 800 mg/kg groups; [6]: indicates values significant at p<0.05 (2 tailed) between 400 mg/kg and 800 mg/kg groups

Table 3: Mean relative organ weight and S.E.M values of rats

Group/Organ	Liver	Kidney[1,2]	Heart[2,3]
Control	1.89±0.08	0.20±0.02	0.18±0.03
200 mg/kg	1.75±0.14	0.19±0.03	0.23±0.03
400 mg/kg	62±0.36[5]	0.34±0.06[5]	0.30±0.04[4]
800 mg/kg	2.00±0.09	0.27±0.03	0.21±0.03

N:5; values presented as mean±standard error of mean (S.E.M.); [1]: Pearson correlations are significant at the 0.01 level (2 tailed) and equal variances assumed; [2]: Pearson correlations are significant at the 0.01 level (2 tailed) and equal variances assumed; [3]: Pearson correlations are significant at the 0.01 level (2 tailed) and equal variances assumed; [4]: indicates values significant at p<0.05 (2 tailed) between control and treated groups; [5]: indicates values significant at p<0.05 (2 tailed) between 200 mg/kg and the 400mg/kg groups

Table 4: Mean levels of serum enzymes of test and control rats (μ/L)

Group/ Enzyme	ALT	AST	GGT	CK-MB
Control.	80±1.48	3.20±1.61	32.50±4.46	63.25±9.07
200mg/kg	14.20±7.46	51.18±7.83	40.33±4.20	148.50±14.94[4]
400mg/kg	23.38±6.87	23.00±6.39	26.93±8.73	157.75±21.06[4]
800mg/kg	94.43±23.82[4]	95.42±24.64[4]	50.20±0.64[4]	201.25±33.50

[4] N:5; values presented as mean±standard error of mean (S.E.M.) [4]: indicates values significant at p<0.05 (2 tailed) between control and treated groups

14 for all the groups of animals; days 7 and 21, days 7 and 28, days 14 and 21, days 14 and 28 and days 21 and 28 equally had significant correlations for all the groups of experimental rats at p<0.01 (2 tailed).

Table 3 shows results of mean relative organ weights of rats. Significant increments were noted in mean relative organ weights of the liver and kidney at p<0.05 (2 tailed) between 200 mg/kg (1.75 liver, 0.19 kidney)) and 400mg/kg (2.62 liver, 0.34 kidney) groups of rats. The same pattern of significant increment took place in the heart between control (0.18) and 400 mg/kg groups (0.30). At p<0.01 (2 tailed), Pearson's correlation showed high correlation between liver and kidney, between liver and heart and between kidney and heart for all the groups.

Table 4 has results of selected serum enzyme levels of rats after 28 days of treatment with aqueous extract of BPL. There were significant increases in the serum levels of ALT (94.43 μ/L), AST (95.42 μ/L), GGT (50.20 μ/L) enzymes at dose of 800mg/kg when compared with control at levels of 2.80 μ/L of ALT, 3.20 μ/L of AST and 32.50 μ/L of GGT, respectively when tested at p<0.05. For CK-MB serum levels of (148.50 157.75 and 201.25 μ/L) at all three respective doses of 200, 400 and 800 mg/kg, significant increments were observed at p<0.05 within a 28-day treatment period when compared with the control group of rats having CK-MB level of 63.25 M/L.

Histological examination: Light micrograph of the tissues at 400 magnification appeared normal with no changes when compared with the control.

DISCUSSION

The phytochemical screening of BPL extract tested positive for tannins, flavonoids, phlobatannins, terpenoids and cardiac glycosides. Most of these components ascertained from this work belong to the two major classes of secondary metabolites contained in extract of *Bidens pilosa* namely polyacetylenes and flavonoids as confirmed by the studies carried out by (Sundararajan *et al.,* 2006; Brandao *et al*., 1997; Jager *et al*., 1996; Hou *et al*., 1989). These two main active constituents of *Bidens pilosa*: polyacetylenes inhibit various pathogenic organisms while flavonoids reduce inflammation. The polyacetylenes can also manifest an anti-inflammatory action, probably mediated by a different mechanism than the flavonoids. *Bidens* also has fried lane triterpenes and essential oils which may contribute to the observed therapeutic action of the herb. Analyses of various species of *Bidens* have been conducted in several countries. Although there is some variation in the level of activity of the different species of *Biden*s, probably due to different levels of active constituents, the general properties appear similar (Andrade-Neto *et al*., 2004). These phytochemical components have been found to be responsible for the various medicinal activities of *Bidens pilosa* and these activities include antimalarial activity due to the presence of acetylene and flavonoid (Brandao *et al*., 1997), chemoprotective activity of ethyl acetate and butanolic fractions (Chiang *et al*., 2004); protects liver from cholestatic disease (Suzigan *et al*., 2009), anticancer properties by photo-activated polyacetylenes (Sundararajan *et al.,* 2006; Hou *et al*., 1989); anti–inflammatory properties (Geissberger and Sequin, 1991; Chin *et al*., 1995, 1996); infection-inhibiting and anti-inflammatory properties (Pereira *et al*., 1999); treatment of headache in Zulu land by inhibiting cyclo-oxygenase, an activity associated with the flavonoid components (Jager *et al*., 1996) and activity against chemical- and bacteria-induced gastric lesions and ulcers and to reduce gastric acid secretion (Taylor, 2005).

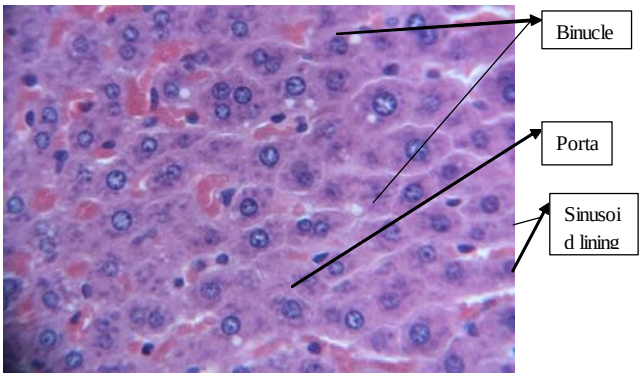

Fig. 1: Light micrograph of liver x 400 (Treated group)

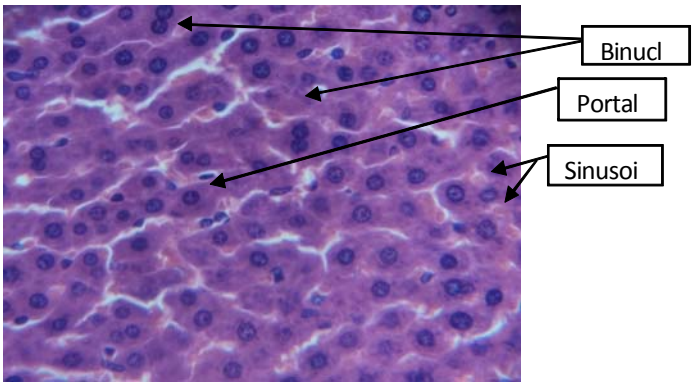

Fig. 2: Ligt micrograph of liver x400 (Control group) liver appears normal with no changes versus the control

Acute toxicity tests showed that the extract can be considered to be practically safe since it did not cause death in rats and there were no signs of toxicity such as alterations of the skin, mucous production and the effects on the eye, circulatory system gastrointestinal, respiratory, central nervous and peripheral and any general signs of toxicity were observed after 72 h. Acute toxicity studies are designed to determine the dose that will produce either mortality or serious toxicological effects or high level safety when given once or over a few administrations. They also serve to provide information regarding doses that should be used in sub-chronic or chronic studies. They can also give an early indication of the possible target organs' toxicity. Therefore the doses used for sub-chronic studies were derived from the knowledge of the results of acute toxicity tests.

In sub-chronic toxicity tests, there was no mortality throughout the duration of the studies thus showing high safety index of BPL in experimental rats. Increase in body weights of rats were observed along each group of rats from days zero to 28. The observed increase in body weight could be attributed to the nutritive components in the plant as revealed by (Duke, 1997). However, there was a general reduction in mean percentage body weight gain of rats a dministered with *Bidens pilosa* extract as compared with control. The observed reduction in body weight gain among the rats down the groups was relatively dose dependent; statistically significant ($p \leq 0.05$) difference was also observed between 400 mg/kg and control and also between 400 and 800 mg/kg. Therefore this pattern of reduction in weight gain caused by *Bidens pilosa* confirms the findings of (Duke, 1997) that the plant can be used as a weight loss aid.

The mean relative organ weights of rats followed the same pattern as the percentage weight gain of rats. The mean relative organ weights of the liver, kidney and heart were highest at 400 mg/kg dose group followed by 800 mg/kg dose group. Significant increments in mean relative organ weight of the heart occurred between control and 400 mg/kg groups. These increments in mean relative organ weights of rats can directly be corroborated to reduction in body weight gain of rats displayed in Table 2 thereby indicating a relative dose dependent activity of BPLE

Selected serum enzyme levels of rats after 28 days of treatment with aqueous extract of BPLE revealed significant ($p<0.05$) increases in the serum levels of ALT, AST, and GGT enzymes at dose of 800 mg/kg when

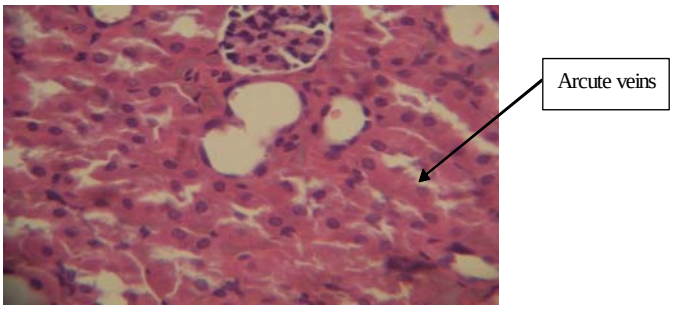

Fig. 3: Light micrograph of Kidney x 400 (Treated group)

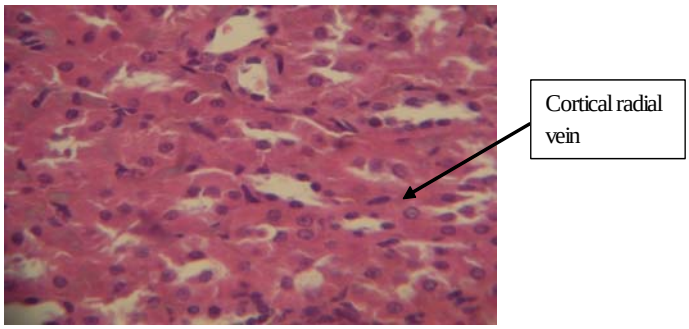

Fig. 4: Light micrograph of kidney x 400 (Control group) kidney appears normal with no changes versus the control

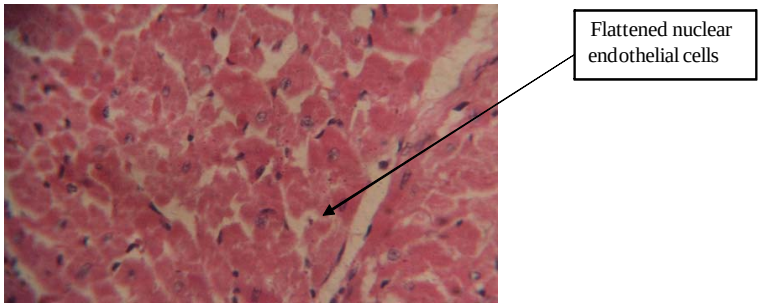

Fig. 5: Light micrograph of heart x 400 (Treated group)

compared with the control. Significant ($p<0.05$) increments in CK-MB serum levels were observed between control and the treated groups. The two most important transaminases are aspartate amino transferase (AST) and alanine amino transferase (ALT). They are present in high concentrations in liver, myocardium, and muscles. AST is more concentrated in the myocardium than ALT, and ALT is more concentrated in the liver than AST and ALT is a cardinal indicator of liver disease while gamma glutamyl transpeptidase (GGT) enzyme catalyses the transfer of the gamma-glutamyl residue of glutathione to other substrates. Its plasma concentration rises in most of the liver disease and it is an early indicator in alcoholic hepatitis (Calbreath, 1992). The major value of CK-MB measurements is in the diagnosis and monitoring of the myocardial infarction (MI). When cardiac muscle is damaged, the cells release enzymes and other materials into the circulation; because CK-MB fraction is present in high amounts in cardiac tissue, the level of this isoenzyme fraction increases after an infarction (Calbreath, 1992). Significant increase in the levels of the enzymes was dose dependent indicating that graded doses of BPL extract could have caused damage in the liver and heart tissues thereby releasing enzymes from these organs to increase the serum level of these enzymes. The increased levels of ALT and GGT and those of AST and CK-MB in the serum show that short term use of BPL extract can respectively cause liver and heart diseases. However, it has been found that BPL extract is hepatoprotective (Suzigan *et al*., 2009; Li-Ping *et al*.,

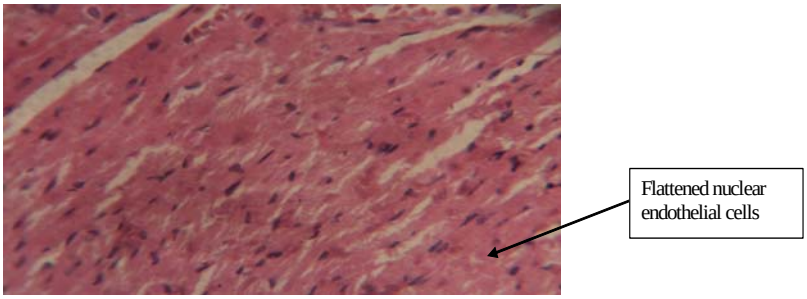

Fig. 6: Light micrograph of heart x 400 (Control group) heart appears normal with no changes versus control

2008; Chin *et al*., 1996) suggesting that short term administration of BPL extract may constitute a challenge to the body and may resolve post-treatment. A study done on evaluation of the acute and sub-chronic toxicities of ethanolic leaf extract of *Spathodea campanulata* P showed elevation of the serum liver enzymes especially the aminotransferases during the 90 days of the studies and later resolved 28 days post treatment indicating that the injury, if any is reversible (Ilodigwe *et al*., 2010). From the Fig. 1-6, it can be seen that the extract did not cause any noticeable effect on the liver, kidney and heart studied when compared with the control. Therefore BPLE can be said to possess high index of safety and its continued use among the rural and urban population can still be greatly encouraged.

CONCLUSION

It can be concluded that *Bidens pilosa* leaves aqueous extract from the Rift Valley Region of Western Uganda contains secondary metabolites such as tannins, flavonoids, phlobatannins, terpenoids and cardiac glycosides. It can also be concluded from this study that the aqueous extract of the leaves of Rift Valley *Bidens pilosa* has a high margin of safety. We recommend therefore that chronic studies should be carried out on this plant to determine its long term effect in animals.

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REFERENCES

Andrade-Neto, V., M. Brandao, F. Oliveira, V. Casali, BNjaine, M. Zalis, L. Oliveira and A. Krettli, 2004. Antimalarial activity of *Bidens pilosa* L. (Asteraceae) ethanol extracts from wild plants collected in various localities or plants cultivated in humus soil. Phytother Res., 18(8): 634-639.

Brandao, M., A. Krettli, L. Soares, C. Nery and H.Marinuzzi, 1997. Antimalarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene and flavonoid compound. J. Ethno. Pharmacol., 57(2): 131-138.

Calbreath, D.F.M, 1992. Clinical Chemistry. Philadelphia W.B Saunders Company, pp: 468.

Chiang, Y., C. Da-Yung, W. Sheng-Yang, K. Yueh-Hsiung, T. Pi-Wen and S. Lie-Fen, 2004. Metabolite profiling and chemopreventive bioactivity of plants extracts from *Bidens pilosa*. J. Ethno. Pharmacol., 95(2-3): 409-419.

Chin, H., C. Lin and K. Tang, 1995. Anti-inflammatory activity of Taiwan folk medicine ham-hong-chho in rats. American J. Chinese Med., 23(3-4): 273-278.

Chin, H., C. Lin and K. Tang, 1996. The hepatoprotective effects of Taiwan folk medicine ham-hong-chho in rats, American J. Chinese Med., 24(3-4): 231-240.

Dimo, T., S. Rakotonirina, P. Tan, J. Azay, E. Dongo and G. Cros, 2002. Leaf methanol extract of *Bidens pilosa* prevents and attenuates the hypertension induced by high-fructose diet in Wistar rats. J. Ethno. Pharmacol., 83(3): 183-191.

Duke, J.A., 1997. The Green Pharmacy: New Discoveries in Herbal Remedies for Common Diseases and Conditions from the World's Foremost Authorities on Healing Herbs. Rodale, Retrieved from: www.greenpharmacy.com.

Ganter, P. and G. Jolles, 1970. In tissus conjonctifs in histichimie normales et pathologique, Gauthier-Villars Paris, 2: 1045-1126.

Geissberger, P. and U. Sequin, 1991. Constituents of *Bidens pilosa* L. Do the components found so far explain the use of this plant in traditional medicine?, Act. Trop., 48(4): 251-261.

Grubben, G. and O. Denton, 2004. Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen; Backhuys, Leiden; CTA, Wageningen.

Hou, D., Q. Guo, Y. Li, L. Li, Q. Wang, Q. Yi and M. Tang, 1989. Effects of anticancer No.8 drugs on the experimental tumours in mice. J. China Pharmaceutical. Univ., 20(6): 348-350.

Ilodigwe, E., P. Akah and C. Nworu, 2010. Evaluation of the acute and subchronic toxicities of ethanol leaf extract of Spathodea campanulata P. Beauv. Inter. J. Appl. Res. Natur. Prod., 3(2): 17-21.

Jager, A., A. Hutchings and J. Van Staden, 1996. Screening of Zulu medicinal plants for prostaglandin synthesis inhibitors. J. Ethnopharm., 52 (2): 95-100.

Li-Ping, G., S. Xian-Yu, T. Guan-Rong, C. Kyu-Yun and Q. Zhe-Shan, 2008. The synthesis and anticonvulsant activity of 1-substituted-7-Methoxy-1, 2, 4-Triazolo [4,3-a] Quinoline. Turkish J. Chem., 32(2): 181-189.

Lorke, D., 1983. A new approach for acute toxicity testing. Arch.Toxicol. 54: 275-287.

Lucia, B., B. Nibia and D. Guillermo, 2008. Annual male reproductive cycle of a Hantavirus reservoir, the long-tailed mouse *Oligoryzomys flavescens* (Rodentia, Crcentigae, Sigmodontinae) from Uruguay. Mastozol. Neotrop. 15(1) Mendoza.

Mvere, B., 2004. *Bidens pilosa* L. In: Grubben, G.J.H. and O.A. Denton, (Eds.), *PROTA* 2: Vegetables/Légumes. [CD-Rom]. PROTA, Wageningen, Netherlands.

NIH, 1996. NIH Guide for the Care and use of Laboratory Animals. NIH Publication, No. 23-83.

OECD, 2006. Repeated Dose 28-day oral Toxicity Study in Rodents; Updated with Parameters for endocrine effects. http://www.oecd.org/dataoecd.

Okeke, I., R. Laxmaninarayan, Z. Bhutta, A. Duse, P.Jenkins, T. O'Brien, A. Pablos-Mendez and K.Klugman, 2005. Antimicrobial resistance in developing countries. Part 1: Recent trends and current status. Lancet Infect. Dis. 5: 481-493.

Pereira, R., T. Ibrahim, L. Lucchetti, A. da Silva and V.Gonçalves de Moraes, 1999. Immunosuppressive and anti-inflammatory effects of methanolic extract and the polyacetylene isolated from *Bidens pilosa* L. Immunopharmacology, 43: 31.

Rojas, J., V. Ochoa, S. Ocampo and J. Munoz, 2006. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Complement. Alter. Med., 6(1): 2.

Sundararajan, P., A. Dey, A. Smith, A. Gana Doss, M.Rajappan and S. Natarajan, 2006. Studies of anticancer and antipyretic activity of *Bidens pilosa* whole plant. Afr. Health Sci., 6(1): 27-30.

Suzigan, M., A. Battochio, K. Coelho and C. Coelho, 2009. An aqueous extract of *Bidens pilosa* L. protects liver from cholestatic disease experimental study in young rats. Acta Cirúrgica Brasileira, 24(5): 347-352.

Tadesse, M., 1994. An account of *Bidens* (Compositae: Heliantheae) for Africa. Kew Bulletin, 48(3): 437-516.

Taylor, L., 2005. The Healing Power of Rainforest Herbs, Retrieved from: http://www.rain-tree.com.

Theta, 2005. Herbs Commonly Used for the Treatment of HIV/AIDS and Related Infections, Retrieved from: www.thetaug.org.

Trease, G.E. and W.C. Evans, 2002. Textbook of Pharmacognosy, 13th Edn., Bailliere Tindal, London.

Wat, C., T. Johns and G. Towers, 1980. Phototoxic and antibiotic activities of plants of the Asteraceae used in folk medicine. J. Ethnopharmacol., 2(3): 279-290.

Zimmerman, M., 1993. Ethical guidelines for investigation of experimental pain in conscious animals. Pain, 16: 109-110.