IOSR Journal Of Environmental Science, Toxicology And Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399. Volume 3, Issue 5 (Mar. - Apr. 2013), PP 37-41
www.Iosrjournals.Org

Biochemical Assessment of the Effect of Aqueous Leaf Extract Of *Euphorbia Heterophylla Linn* on Hepatocytes of Rats

Apiamu Augustine[1], Evuen Uduenevwo Francis [2], Ajaja Uche Ivy[3]
[1, 2 & 3]*(Department of Biochemistry, College of Natural and Applied Sciences, Western Delta University, Nigeria)*

Abstract: *In recent years, the search for biologically active compounds from Euphorbia heterophylla in the treatment of different diseases has always been of great interest to researchers. In this present study, we investigated the effect of the aqueous leaf extract of the plant on hepatocytes using animal models. A total of twenty (20) wistar albino rats (150-240g) were used for the study. The rats were randomly divided into four experimental groups (A, B, C & D) comprising five rats per group. The control group was administered deionised water while the treatment groups were orally administered doses of the aqueous leaf extract of the plant(100mg/kg, 200mg/kg and 300mg/kg body weights) by means of a gavage for two weeks. Total protein, albumin, urea nitrogen, alanine aminotransferase(ALT), aspartate aminotransferase(AST) and alkaline phosphatase(ALP) were the biochemical parameters assessed in this study. The results showed no significant difference($p>0.05$ in the levels of the aforementioned parameters. The aqueous leaf extract of the plant indicated the presence of carbohydrates, saponins, tannins, flavonoids, alkaloids, terpenoids and steroids, but anthracene derivatives were absent. The results obtained in this study, therefore, justify the traditional use of the plant for food and medicinal purposes respectively*.

Keywords: *Alanine aminotransferase, Albumin, Alkaline phosphatase, Euphorbia heterophylla, Hepatocytes*

I. Introduction

Medicinal plants are plants which contain substances that could be used for therapeutic purposes and precursors for the synthesis of useful drugs [1, 2]. Medicinal plants are of great importance to the health of individuals and communities in general. Traditional medicine is an important part of African cultures and local medicinal systems vary between different cultural groups and regions [3]. In developing countries like Nigeria, a vast number of people live in extreme poverty and some are suffering and dying for want of safe water and medicine they have no alternative for primary health care [4].Therefore, the need to use medicinal plants as alternatives to orthodox medicines in the provision of primary health care cannot be over-emphasized. Moreover, herbal medicines have received much attention as sources of lead compounds since they are considered as time tested and relatively safe for both human use and environment friendly [5]. They are also cheap, easily available and affordable [6]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. There is therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the ethno medical use and characterization of compounds which will be added to the potential lists of drugs [7].

Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities in the world [8, 9,10]. Each medicinal plant species has its own nutrient composition besides housing pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of the human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human wants and needs for energy and other life processes [11,12,13]. Many medicinal plants are used by marginal communities to cure various diseases [9,14] as various medicinal plant species are used either in the form of extract or decoction by the local people in different regions, therefore, evaluating their marginal significance can help to understand the worth of these plants species in different ecological conditions. Some of these medicinal plants serve as both food and medicine. *Euphorbia heterophylla* is one of such plants. [7].

Euphorbia plants are widespread in nature ranging from herbs and shrubs to trees in tropical and temperate regions all over the world [15]. It is a local medicinal plant commonly known as'spurge weed' [16] .The family, Euphorbiaceae comprises 280 genera and 730 species with the largest genus, Euphorbia having about 1600 species. Generally, they have characteristic milky latex. *E. heterophylla* leaf is used in traditional medical practices as laxative, anti-gonorrhoeal, migraine and heart cures. The plant latex has been used as fish poison and insecticides [17,18]. The leaves of *Euphorbia heterophylla* are commonly used as a lactogenic agent by taking a decoction of it or massaging the breast with the poultice to induce milk flow [19].

The leaves of *E. heterophylla* have been reported to contain quercertin [20].

Diterpenoids have also been reported in the root of *E. Heterophylla* [21]. The skin irritant, tumour-promoting and anti-tumour /cancer and recently anti-HIV activities of Euphorbia species have also been reported in *E. heterophylla* leaf linn [22].

Objectively, this study is done to ascertain the effect of isolation and characterisation of the bioactive constituents of *E. heterophylla* on the healthy status of the liver tissues via assessment of some biochemical parameters

II. Materials And Methods

2.1 Experimental Animals

Twenty Wistar rats of both sexes weighing between 144g and 166g were used for the study. They were purchased from the animal unit of Anatomy Department, University of Benin, Nigeria. They were then housed in standard laboratory cages at the Department of Biochemistry, Western Delta University, Oghara, Nigeria to acclimatize for two weeks and were fed with pelleted mash (purchased from Anatomy Department, University of Benin) and tap water.

2.2 Preparation of Plant Extract and its Administration

The leaves of the plants were always harvested freshly for preparation of the extract for the fourteen days that the animals were given the extract. The leaves were weighed and macerated using mortar and pestle. A specific quantity of water was added to ensure proper maceration. Thereafter, the solution was filtered using filter paper.

2.3 Experimental protocol

The rats were divided into four experimental groups (A, B, C & D) of five rats each. The "Control Group" (group A) was given 100mg/kg body weight of water only. The other three groups, B, C and D were treated with 100, 200 and 300mg/kg body weight of the aqueous extract of *Euphorbia heterophylla* respectively. The administration of the aqueous extract was done once daily for 14 consecutive days. The freshly prepared extract was administered by the use of gavage to the rats in the experimental groups.

2.4 Collection and Preparation of Sample Homogenate

At the end of the 14 days treatment period, the rats were anaesthetized with chloroform, dissected and the sample tissues (liver) from each group were rapidly excised into a universal container containing little quantity of phosphate buffer at pH 7.4 and kept in a chilled condition to maintain the physiological state of enzymes located in the tissue under consideration. The samples were homogenized in phosphate buffer using pre-chilled mortar and pestle. The homogenates were centrifuged at 4000g for 30minutes and the supernatants were subsequently used for assaying the activities of alanine aminotransferase, aspartate aminotransferase alkaline phosphatase, and estimation of total protein and urea nitrogen respectively.

2.5 Biochemical assays

The activities of tissue aminotransferases were measured by the procedure of Reitman and Frankel [23] as described in the Randox kit leaflet product of Quimica Clinica Aplicada (QCA), Spain. Alkaline phosphatase activity was monitored by the method described by Lee *et al.*[24]. Total protein in liver tissues was estimated by the method of Lowry *et al* [25]. Urea nitrogen levels were estimated by measuring the hydrolysis of urea to ammonia in the presence of urease [26]. The levels of albumin in serum were also determined by the method described by Gustafsson [27]. All biochemical assays done in this study were kit-based.

2.6 Phytochemical and Proximate Analyses:

The crude aqueous extract of *E. heterophylla* was subjected qualitatively to phytochemical screening by method described by Evans [28], and the leaves of the plant were quantitatively subjected to proximate analysis using the procedure described by FAQ [29].

2.6 Statistical Analysis

Data obtained from experimental groups were expressed as mean±standard deviation (S.D). Qualitative variables were assessed by one way ANOVA. The degree of significance between the various groups were tested using the Least Significant Difference (LSD) and Duncan at $P<0.05$.Data were analyzed by means of SPSS package (version 20) such that results with $P<0.05$ were considered significant.

III. Results

The aqueous extract of *Euphorbia heterophylla* at doses of 100, 200 and 300mg/kg orally relative to the control group, revealed no significant effect on the following biochemical parameters

(TABLE 1). The preliminary phytochemical evaluation revealed the presence of carbohydrates, saponins, favonoids, tannins, alkaloids, terpenoids and steroids at varying concentrations in the fresh plant leaves(TABLE 2).On nutritional basis, the fresh plant leaves showed the percentage composition of carbohydrate, protein, moisture, fibre, ash and fat respectively(TABLE 3)

Table 1: Effects of the Aqueous extract of *Euphorbia heterophylla* on some liver Biochemical Parameters of Rats

Parameters	Group A	Group B	Group C	Group D
Total Protein (g/dl)	28.3 ± 19^a	38.4 ± 20^a	37.1 ± 18^a	34.4 ± 24^a
Albumin (g/dl)	2.4 ± 1.0^b	2.2 ± 0.6^b	3.1 ± 1.2^b	2.6 ± 0.6^b
ALT (U/L)	51.2 ± 31^c	56.8 ± 27^c	47.0 ± 29^c	52.5 ± 22^c
AST (U/L)	32.8 ± 6^d	30.0 ± 5^d	27.3 ± 5^d	40.0 ± 11^d
ALP (U/L)	31.8 ± 2^e	29.7 ± 9^e	30.7 ± 6^e	32.6 ± 6^e
Urea nitrogen(U/L)	31.8 ± 2^e	73 ± 10^e	73 ± 22^e	77 ± 6^e

**Expermental data were expressed as mean ± S.D of five rats in each group. p<0.05 was considered significant. Biochemical parameters marked with the same letters across the groups designate no significant difference (P>0.05)*

Table 2: Results of Phytochemical tests.

Constituents →	Reducing Sugar	Carbohydrate	Saponins	Anthracene derivatives	Steroids	Terpenoids	Tannins	Flavonoids	Alkaloids
Intensity	+++	+++	++	-	+	++	++	++	+
Inference	Present	Present	Present	Absent	Present	Present	Present	Present	present

Key: *+ = little, ++ = moderate, +++ = profuse, and - = absent.*

Table 3: Results of proximate Analysis of *Euphorbia heterophylla*

Parameters	Moisture	Carbohydrate	Protein	Fat	ash
Values (%)	11.79	74.84	6.06	2.92	2.57

IV. Discussion

At the onset of the study, *Euphorbia heterophylla* showed laxative property, as proven and reported by researchers. Phytochemical analysis is an indicator of the chemical nature of the constituents of the plant extract, composition among chemical constituents and the one that predominates over the other. It could also be used as a vital tool to search for bioactive compounds as precursors needed for the synthesis of useful drugs [30].

In this present study, the phytochemical investigation revealed that the fresh leaves of *Euphorbia heterophylla* contained important bioactive compounds like carbohydrates, reducing sugars, saponins, steroids, terpenoids, tannins, flavonoids and alkaloids respectively. The results obtained in this study, therefore, correlates with the report of Omale and Emmanuel [7]. The ethnomedical use of *Euphorbia heterophylla* may be attributed to its high content of some bioactive compounds such as tannnins, flavonoids, soponins, to mention but a few, to manage a number of ailments [31]. The significant level of flavonoids present in the plant extract confirms the use of *Euphorbia heterophylla* in the treatment of inflammatory disease conditions such as asthma [32]. Although the revealed levels of steroids in the plant extract is scanty in nature, but it should be noted that steroidal compounds are of importance and interest in Pharmacy due to their relationship with such compounds as sex hormones [33]. More so, the bioactive compounds contained in the analysis conform to the report of Rahilon *et al* [34].

Euphorbia heterophylla, contains 11.79% moisture, 6.06% protein, 1.82% fat, 2.92%, fibre, 2.57% and 74.84% carbohydrate respectively. The nutrient composition of the plant, therefore, reveals its usefulness for food and medicinal purposes. The high carbohydrate content of the fresh plant extract indicates its predominance among the other nutrients. This conforms to the report of Omale and Emmanuel [7] and Falodun *et al* [18] respectively. Contrary to the order of nutrient composition of *Euphorbia heterophylla* reported by Omale and Emmanuel , the increasing order of nutrient composition of the plant on the basis of percentage magnitude is given as: Fat < Ash < Fibre < Protein <Moisture < Carbohydrate. Therefore, the plant is a high source of energy and water, but moderate in protein and fibre contents. This may be advantageous to grazing animals, as source of energy and as an index of increased rate of digestibility. It is generally known that protein

functions in the repair of worn out tissues, stress response, storage, transport regulatory defence, and carbohydrate processes, this could be a reason for the medicinal function of *Euphorbia heterophylla.*

The aqueous crude extract of *Euphorbia heterophylla* showed no significant effect on the levels of total protein, blood urea nitrogen and serum albumin in wistar rats, which is contrary to the report of Kheyrodin and Ghazvinian[35]. The non-significant effect of the aqueous extract may be attributed to adequate drinking of water by the rats because dehydration was not observed among the animal models. More so, the liver is a core site for the biosynthesis of blood urea nitrogen via the urea cycle, and plasma proteins such as albumin with diverse physiological functions. Consequently, an elevation or a depression in the levels of these biochemical parameters may compromise the integrity of liver tissues. It may be justifiable to say, therefore, that the non-significant effect of the aqueous extract of *E. heterophylla* on total plasma protein, serum albumin and blood urea nitrogen respectively as an index of healthy biochemical status of the liver is physiologically and metabolically uncompromised. Again, the aqueous crude extract of the plant showed no significant effect on the activities of alanine amino transferase (ALT), and aspartate amino Transferase (AST), as against the report of Kheyrodin and Ghzvinian [35].

AST is similar to ALT in that both enzymes are associated with liver parenchyma cells. The difference is that ALT is found predominantly in the liver with clinically negligible quantities found in the cardiac muscles, skeletal muscles, kidneys, brains, and red blood cells [36, 37]. Since *Euphorbia heterophylla* extract did not induce a significant effect on AST and ALT activities, it therefore means that the aqueous crude extract of the plant may not be hepatotoxic and other tissue injuries relative to AST. This may confirm the incessant use of the plant as a laxative in traditional medicine.

The aqueous crude extract of *Euphorbia heterophylla* shows no significant effect on the levels of Alkaline Phosphatase (ALP) activity. ALP is reported to be present in large number of cells but only in a few cells is the activity sufficient to be of clinical significance. [38]. It is found in liver cells and is associated with osteoblastic activity in the bone [11, 39,40]. This means that the aqueous crude extract of the plant may not cause any injury on several tissues of the body.

V. Conclusion

In conclusion, it should be emphasized that the phytochemical and proximate evaluations indicated the presence of several bioactive compounds, which are confirmations of the therapeutic effects of *Euphorbia heterophylla*. Although the aqueous crude extract of the plant showed no hepatotoxic potentials and other tissue injuries, however, caution should be taken in its use for medicinal and food purposes for humans and grazing animals. In addition, there is room for further research to consolidate its toxic potentials if there would be any.

Acknowledgement

We are very thankful to Mr. Bright Igere of the Department of Microbiology and Biotechnology, Western Delta University, Oghara, Nigeria and Mr. Cyril Ugbeni, of the Department of Biochemistry, University of Benin, Nigeria, for their enormous contributions and intense supportive roles in this study.

References

[1] O.A. Abolaji, A.H. Adebayo, and O.S. Odesanmi. Nutritional qualities of three medicinal plants (*Xylopia aethiopica,Blighia sapida and Harinari polyandra*) commonly used by pregnant women in the western part of Nigeria. *Parkistan Journal of Nutrition, 2,* 2007, 665-668.

[2] A.C. Akinmoladun, E.O. Ibukun, E. Afor; E.M.Obuotor, and E.O. Farombi,. Phytochemical Constituent and Antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Sciences Research Essay 2*, 2007,163 – 166.

[3] L. Makhubu.Traditional Medicine: Switzerland. *African Journal of Traditional Complementary and Alternative Medicine, 12(3),* 2006,162-172.

[4] World Health Organization, W.H.O. . The World Health Reports Bridging the Gap WHO, Geneva 1, 1995, 118.

[5] B.S. Fazly – Bazzaz, M. Khajehkaramadin, and H.R. Shokooheizadeh. *In Vitro* Antibacterial Activity of Rheumribes Extract obtained

[6] E.Ernst,The Efficacy of Herbal Medicine:-An Overview. *Fundamental Clininical Pharmocology. 19*, 2005, 405 – 409.

[7] J.Omale and F.Emmanuel,Phytochemical Composition, Bioactivity and wound healing potential of *Euphorbia heterophylla* (Euphorbiaceae) leaf extract. *International Journal on Pharmaceutical and Biomedical Research , 1 (1),* 2010, 54 – 63.

[8] N.D. Prajapati, and T. Prajapati. *Sustainable cultivation of Medicinal Plants, Multitier Agriculture System – A new concept.* Biochemistry(3rd ed.) Benjamin cummings, 2002.

[9] A. Latif, H. Almad, S. Begun, M. Adnan, and W. M. Hussains. Medicinal and other Economic Plant as substitute to Forest logging in Miandam and Sulatat Valleys, Swat. Proceedings of International Workshop on Conservation and Sustainable Use of Medicinal and Aromatic Plants in Pakistan. ww-pak, 2003, pp 101 – 105.

[10] Z.K Shinwari, M. Pehman, T. Watanabe, and T. Yoshikawa . A Pictorial Guide to Medicinal Plants of Pakistan Kohat University of Science and Technology. Pakistan, 2006.

[11] P.C Hoffman, D.K. Combs, and M.D. Caster. Performance of Lactating Dairy Cows Fed Alfalfa Silage or Perennial Ryegrass Silage.*Journal of Daily Science, 81,* 1998, 162 – 168.

[12] C.E. Mathews, K.E,Vanholds and K.G.Ahem, Biochemistry (3rdEd.) Bajamin Cummings, 1999.

[13] S.L. Dingman. Water in Soils: Infiltration and Redistribution, *Physical Hydrology* (2nd Ed.), Upper Saddle River, New Dersey: Prentice-Hall, Inc, 2002, P.646.

[14] M.Adnan and D.Holscher. Medicinal Plant Abundance on Degrade and Reforested Site in Northwest Pakistan. *Mountain Research and Development, 30(1),* 2010, 25-32.

[15] K. Bremer. *Asteraceae Cludistics and Classification*, Timber Press Portland Oregon, 1994.

[16] D.M. Mosango. *Euphorbia heterophylla L*. in: G.H Schumelzer and A.Gurib-Fakim, (Editors). Prota 11(1). *Medicinal Plants PROTA*,Wageninge: Netherlands, 2008.

[17] L. W.Mitich. "Tansy". *Weed Technology 6*, 1992, 242–4

[18] A. Falodun, E.O.P. Agbakwuru, and G.C.Ukoh . Antibacterial Activity of *Euphorbia heterophylla Linn* (Family Euphorbiaceae). *Pakistan Journal of. Scientific Research 46(6)* , 2003, 471 – 472.

[19] O.B. Dokori . *Herbs of Ghana,* (2nd Ed). Ghana University Press ,Accra, 1998, p766.

[20] A. Falodun, and E.O.P. Agbakwuru. Phytochemical Analysis and Laxative Activity of the Leaf Extracts of *Euphorbia heterophylla L.* (Euphorbiacae). *Pakistan Journal of Scientific and Industrial Research 47(5),* 2004 , 345 – 348.

[21] N.G. Rowan, and D.N. Onwukaeme. Deterpnoid Esters of Euphorbiaceae in *Euphorbia hyles*. *Nigeria Journal of Pharmacology, 32*, 2001, 60 – 64.

[22] C.A. Williams, J.R. Hoult, J.B. Harborne, J. Greenham, and J. Eagles. A biologically active lipophilic flavonol from *Tanacetum* ***parthenium. Phytochemistry, 38(1),*** **1995, 267-70 .**

[23] S.Reitman and S.Frankel. A colorimetric method for the determination of serum glutamate-oxaloacetate and pyruvate transaminases. *American Journal of Clinical Pathology 28*, 1957, 56.

[24] D.H. Lee, B.S. Lim, Y.K. Lee and H.C.Yang. Effects of hydrogen peroxide(H_2O_2) on alkaline phosphatase activity and matrix mineralization of odontoblast and osteoblast cell lines.*Cellular Biology and Toxicology,5,* 2006, 22-39.

[25] O.H. Lowry, N.T. Rosebrough, A.L. Fair and R.J. Randall. Protein measurement with the folin-phenol reagent. *Journal of Biological Chemistry, 193*,1951, 265-275.

[26] A. Kaplan and L.L. Tang. *Selected methods of clinical chemistry*, vol 9, Ed by W.R. Foulkner and S. Meites, AACC. Washington, 1982,p357-363.

[27] J.E.C. Gustafsson. Automated serum albumin determination by use of immediate reaction with bromocresol green reagent. *Journal of Cinical Chemistry, 24*, 1978, 369-373.

[28] W.C. Evans . Trease and Evans Pharmacognosy(15th Ed.) .W. B. Saunders: Philadelphia, 2002, p3 – 338.

[29] FAO. Compositional analysis methods. In: manuals of food quality control 7.Food analysis: general techniques, additives, contaminants and composition(Food and Agricultural Organisation of the United Nations Food and Nutrition paper 14/7), 1988,p203-232.

[30] M.T. Yakubu, M.A. Akanji, and A.T. Oladiji . Aphrodisiac potentials of the aqueous extract of fadogia agrest is (schweinf.Exhiem)stem in male albino rats.*Asian Journal of Andrology,7 (4)*, 2005,309-404.

[31] R.I. Okoli, A.A. Turay, J.K. Mensal, and A.O.Aigbe. Phytochemical and Antimicrobial properties of four Herbs from Edo state,Nigeria. *Report and opinion 1(5),* 2009, 67-71.

[32] M.Anilkumer . Ethnomedicinal plants as anti-inflammatory and analgesic agents.*Therapeutics ,2*, 2010,267-293

[33] D.E.Okwu . Evaluation of the Chemical Composition of Indigenous Plant Spices and Flavoring Agents. *Global Journal of Pure and Applied. Sciences, 7(3),* 2001, 455 – 459.

[34] T. Rahila,N. Rukhsandra, A.A. Zaidi, and R. Shamishalia . Phytochemical Screening of Medicinal Plants belonging to *Euphorbiaceae. Pakistan. Veterinary Journal,14*, 1994, 160 – 162.

[35] H. Kheyrodin, and K. Ghazvinian. Assessment of plant extract toxicity in *Euphobial spease*. *Journal of Recent Advances in Agriculture. 1(3),* 2012, 77-83.

[36] J.J. Kaneko. Clinical Biochemistry of Domestic animals. (3rded).orlando: fla, American press, 1980.

[37] J.R. Duncan,K.W. Praise, and E.A. Mahaffe. Veterinary laboratory Medicine (clinical Pathology)3rd ed. Iowa state university press,U.S.A, 1994.

[38] A.A. Adedapo, M.O. Abatan, S.O. Idowu,.and O. lorunsogo. Effects of chromatographic fractions of *Euphorbia hirta* on the rat serum biochemistry. *African Journal of Biomedical Research,8* , 2005, *185 – 189.*

[39] S. Carter, and L.C. Leach . Euphorbiaceae, Subfamily Euphorbioideae, tribe Euphorbieae. In. G.U. Pope, (Editor). *Floral Zambesiaca.*Volume 9, Part 5. Royal Botanic Gardens, Kew, Richmond, United Kingdom , 2001, p339 – 465.

[40] T. Berg, S. DeLanghe, D. AlAlam, S. Utley, J. Estrada, and K.S. Wang, . Beta-catenin regulates mesenchymal progenitor cell differentiation during hepatogenesis. *Journal of Surgical Research*. *16(4)*, 2010, 276-285.