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Effect of *Euphorbia hirta* on Haematological and Biochemical Indices in Albino Rats

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Abstract: This study is designed to evaluate the possible effect of ethanolic extract of *Euphorbia hirta* on haematological and biochemical parameters of wistar albino rats. Forty male wistar albino rats were used for this experiment. The animals were divided randomly into four groups of ten animals each. Group I (control) were given 0.2 ml normal saline to drink. Group II, Group III and Group IV were given 200mg/kg, 400mg/kg and 600mg/kg of *Euphorbia hirta* ethanol extract respectively for 14days. The results of this study with respect to the haematological changes showed that *E. hirta* caused a significant increase ($P<0.05$) in RBC, WBC, PLT, Hb and PCV level while there is a reduction in lymphocytes. *E. hirta* extract caused significant decrease ($P<0.05$) in serum lipid profile (cholesterol, triglyceride, LDL-cholesterol and VLDL) when compared to normal control wistar albino rats. There was a slight increase in the levels ALT and AST activities in the treated group when compared with control. The treated group showed a significant increase in serum urea activity ($p<0.05$) when compared with the control. In conclusion, these plants possess medicinal properties, this study has shown that: *E. hirta* had erythropoiesis and hpyolipidemic activities. It can be suggested based on these reports that the high dose administration of this extract might have been too toxic to the rats to have caused the increase in liver enzymes recorded in the experimental group.

Key words: *Euphorbia hirta* · Haematological Parameters · Liver Enzymes · Lipid Profile and Renal Indices

INTRODUCTION

It is common practice in many parts of the world, particularly in the developing countries to seek out unconventional therapies such as herbal medicine when conventional medicine fails to cure chronic diseases and conditions [1]. Many studies provided useful information on the curing potentials of many medicinal plants [2]. *Euphorbia hirta* is one of such plants that are being used traditionally and has come under serious scientific scrutiny.

Euphorbia hirta is a plant belonging to phylum Angiospermia and family Euphorbiaceae. It is an annual, branched herb with branches up to 50cm long. The parts are hairy with simple leaves and unisexual flower and contain a high amount of latex [3, 4, 5]. The plant is a native to Central Africa and occurs throughout Tropical Africa and South Africa [6]. It is characterized by the presence of white milky latex which is more or less toxic [5]. This group of plants has been a subject of intense phytochemical examination and compounds including, flavanoids, triterpenoids, alkanes, amino acids and alkaloids have been isolated [3].

E. hirta is used in the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, etc.), bronchial and respiratory diseases (asthma, bronchitis, hay fever, etc.) and conjunctivitis. Hypotensive and tonic properties have been also reported. The aqueous extract exhibits analgesic, antipyretic and anti-inflammatory activities. The stem sap is used in the treatment of eye problems and a leaf poultice is used on swelling and boils [5]. *E. hirta* possesses antibacterial, anthelmintic, antiasthmatic, sedative, antispasmodic, antifertility, antifungal and antimalarial properties and have been used for the treatment of such purposes [3, 7, 8]. Haematological, biochemical and lipid profile parameters

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are today being used to assess the state of health of animals and have proved helpful in most clinical diagnosis. Varying degrees of anaemia, cardiovascular diseases and liver functions may be assessed using these parameters. In this light, this study was designed to evaluate the effect of ethanol extract of *Euphorba hirta* on haematological and biochemical parameters in albino rats.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials: Fresh leaves of *Euphorbia hirta* were collected from a local habitat in Umudike, Ikwuano Local Government Area of Abia State, Nigeria. The collected leaves were air dried at room temperature for 7 days and were pulverized to fine powder using a manual blender. 35g of this powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using 95% ethanol as solvent with temperature maintained at 70°C for 48 hours. At the end of the period, the ethanol was evaporated at 40°C in a 400ml beaker placed in an electric oven to obtain a crude extract weighing 11.6g which represented a yield of 33.1%.

Experimental Animals: Forty male wistar albino rats weighing 180-200g were obtained from the Livestock Production Unit of the Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Production, Michael Okpara University of Agriculture, Umudike. The animals were housed under a standard laboratory condition with 12 hours dark/light cycle and with access to standard diet (Guinea feed, Edo State, Nigeria) and water *ad libitum*. The experimental animals were divided randomly into four groups of ten animals each. Group I were given 0.2ml normal saline and served as the control, while groups II, III and IV were given 200, 400 and 600mg/kg of *Euphorbia hirta* ethanol leaves extract respectively. All Treatments were done via the oral route and lasted for 14days. All animal experiments were carried out in accordance with NIH guidelines for care and use of laboratory animals as contained in US guidelines [9].

Collection of Blood Samples: At the end of treatment, all animals were sacrificed and blood was collected by cardiac puncture and used for the determination of haematological parameters, lipid profile and biochemical parameters. Precautions were taken on proper collection of the samples. About 6ml of blood samples were collected into K_3EDTA and plain tubes. The blood collected was used for haematological and biochemical (lipid profile, liver and renal indices) parameters.

Blood Analysis: Haematological parameters were analysed using Coulter® Ac-T™ 5Diff AL, Beckman Coulter, Inc. Port Matilda, Pennsylvania, USA. Electrolytes were estimated with Easylyte® analyzer, Medica Corporation, Bedford, USA. All the other biochemistry were analysed with A25 Biosystem, Barcelona, Spain.

Statistical Analysis: The haematological parameters were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 15.0). Comparisons were made between control and test groups using student's t-test. P-values less than 0.05 were regarded as statistically significant.

RESULTS

Effect of *E. hirta* Leaf Extract on Haematological Parameters: The results obtained following treatment with *E. hirta* showed a dose dependent significant increase ($P<0.05$) in RBC, WBC, PLT, Hb, PCV and Neutrophils values with reduction in lymphocytes counts (Table 1).

Effect of *E. hirta* Leaf Extract on Lipid Profile in Albino Rats: All animals treated with *E. hirta* extract had significant decrease ($P<0.05$) in serum lipid profile (cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and VLDL) when compared to normal control wistar albino rats.

Effect of *E. hirta* Leaf Extract on Renal Indices in Albino Rats: Treatment with *E. hirta* raised urea values in the treated rats with 400mg/kg body weight of EHEE producing the highest effect. Creatinine and electrolytes (sodium, potassium and chloride) were not significantly affected (Table 3).

Effect of *E. hirta* Ethanol Leaf Extract on Liver Function Indices in Albino Rats: All doses of *E. hirta* and 600mg/kg of same significantly ($P< 0.05$) lowered the levels AST and ALT in the treated groups when compared with control. Other parameters were not significantly affected but showed slight alterations (Table 4).

Table 1: Effect of *Euphorbia hirta* ethanol leaf extract (EHEE) on haematological parameters in wistar albino rats

Parameters	Group I Control	Group II 200mg/kg EHEE	Group III 400mg/kg EHEE	Group IV 600mg/kg EHEE
RBC RBC X 10^{12}/l	8.93±0.81[a]	9.24±1.10[b]	9.56±2.30[b]	9.64±1.50[b]
WBC X 10^9/l	21.44±0.92[a]	22.43±1.10[a]	36.15±2.40[b]	38.89±1.30[b]
PLT X 10^9/l	408.4±3.90[a]	442.80±2.80[b]	466.0±0.92[b]	476.55±0.81[b]
Hb (g/dl)	12.00±1.30[a]	12.30±1.10[a]	13.3±1.89[b]	13.54±1.60[b]
PCV (%)	36.0±2.10[a]	36.90±1.80[a]	39.90±1.12[b]	40.62±1.82[b]
MCV (fl)	40.3±0.33[a]	39.94±0.61[a]	41.69±1.01[a]	42.14±0.94[a]
MCH (pg)	13.44±0.52[a]	13.31±0.33[a]	13.90±0.82[a]	14.05±1.02[a]
MCHC (g/dl)	36.0±2.10[a]	36.90±1.80[a]	39.90±1.12[a]	40.62±1.82[a]
Neutrophils (%)	13.0±0.29[a]	20.00±1.05[b]	28.00±1.92[b]	29.71±1.82[b]
Lymphocytes (%)	86.0±0.83[a]	78.00±0.69[c]	71.00±0.83[c]	78.00±0.74[c]
Monocytes (%)	1.0±0.21[a]	2.0±0.13[a]	1.0±0.09[a]	1.0±0.18[a]

Data are mean ± S.E.M. (n = 10). Mean in the same column with different superscript letters are significantly different, P<0.05(one way ANOVA followed by post-hoc LSD).

Table 2: Effect of *Euphorbia hirta* ethanol leaf extract (EHEE) on lipid profile of wistar albino rats

Parameters (mg/dl)	Group I Control	Group II 200mg/kg EHEE	Group III 400mg/kg EHEE	Group IV 600mg/kg EHEE
Cholesterol	184.57±3.40[a]	162.15±2.60[c]	109.50±2.71[c]	98.73±1.86[c]
Triglycerides	132.96±2.30[a]	120.65±1.90[c]	107.31±3.10[c]	89.62±0.96[c]
HDL-Cholesterol	46.56±1.02[a]	34.09±2.80[c]	26.58±0.98[c]	32.33±0.69[c]
LDL-Cholesterol	76.85±2.83[a]	72.56±1.96[c]	40.31±1.05[c]	28.13±1.01[c]

Data are mean ± S.E.M. (n=10). Mean in the same column with different superscript letters are significantly different, P<0.05(one way ANOVA followed by post-hoc LSD).

Table 3: Effect of *Euphorbia hirta* ethanol leaf extract (EHEE) on Electrolyte, Urea and Creatinine (renal indices) values of wistar albino rats

Parameters	Group I Control	Group II 200mg/kg EHEE	Group III 400mg/kg EHEE	Group IV 600mg/kg EHEE
Sodium (mEq/L)	142.30±3.12[a]	138.60±1.72[a]	138.80±2.22[a]	142.60±2.30[a]
Potassium (mEq/L)	5.59±1.01[a]	5.90±0.86[a]	4.92±0.91[a]	6.01±0.96[a]
Chloride (mEq/L)	113.90±2.92[a]	105.80±1.60[a]	107.0±2.01[a]	109.5±1.96[a]
Urea (mg/dl)	17.14±0.25[a]	29.77±1.03[b]	30.00±1.16[b]	32.50±1.09[b]
Creatinine (mg/dl)	1.20±0.09[a]	1.01±0.08[a]	0.75±0.03[a]	0.15±0.03[a]

Data are mean ± S.E.M. (n = 10). Mean in the same column with different superscript letters are significantly different, P<0.05(one way ANOVA followed by post-hoc LSD)

Table 4: Effect of *Euphorbia hirta* ethanol extract (EHEE) on liver function indices of wistar albino rats

Parameters	Group I Control	Group II 200mg/kg EHEE	Group III 400mg/kg EHEE	Group IV 600mg/kg EHEE
BilirubinTotal(mg/dl)	0.96±0.02[a]	1.04±0.12[a]	0.84±0.04[a]	0.47±0.01[a]
Bilirubin Direct (mg/dl)	0.29±0.01[a]	0.17±0.10[a]	0.70±0.09[a]	0.05±0.01[a]
Bilirubin Indirect (mg/dl)	0.67±0.20[a]	0.87±0.09[a]	0.14±0.03[a]	0.42±0.06[a]
AST (IU)	10.82±0.8[a]	15.43±0.21[b]	13.17±0.10[b]	15.16±0.90[b]
ALT (IU)	119.76±3.20[a]	128.80±3.10[b]	123.80±1.14[b]	125.27±2.11[b]
ALP (IU)	186.21±1.23[a]	266.00±2.80[a]	256.77±5.32[a]	224.00±6.01[a]
Total Protein (mg/dl)	7.92±1.10[a]	6.54±1.10[a]	8.1±0.19[a]	14.28±1.25[a]
Albumin (mg/dl)	2.54±0.09[a]	3.36±0.06[a]	2.64±0.71[a]	3.50±0.18[a]
Globulin (mg/dl)	5.38±0.09[a]	3.18±0.20[a]	5.47±0.73[a]	10.78±0.98[a]

Data are mean ± S.E.M. (n = 10). Mean in the same column with different superscript letters are significantly different, P<0.05(one way ANOVA followed by post-hoc LSD)

DISCUSSION

Results obtained from this work revealed a significant ($p<0.05$) dose dependent rise in values of haematological parameters (Red blood cells, Haemoglobin (Hb), Packed Cell Volume (PCV), Platelets, Total White blood cells (WBC) and Neutrophils counts) in all groups treated with *Euphorbia hirta* leaf extract when compared to the control group. Red blood cells, haemoglobin concentration and packed cell volume (PCV) have been used to detect anemia and its severity and to monitor an anemic patient's response to treatment [10]. Studies have shown that over the years, the consumption of leaves and other plant based products have been associated with increase or decrease in RBC, Hb and PCV values [11]. The increase in these parameters after treatment with the plants may be attributed to the rich iron content of most green leafy plants which make them readily available sources of iron required in the process of erythropoiesis [12]. In the current study, the rise in RBC, Hb and PCV values after treatment with the extract suggest that the plant may contain iron and other phytochemicals which favour RBC production. This agrees with [13], who reported that treatment with *Euphorbia hirta* at doses below 1000mg/kg was quite safe and could be of immense benefit in the management of diseases. The white blood cells have been known to play very important roles in improving the immune system via the formation of first line of defense against invading micro organism [10]. The total white blood cells counts of rats used in this experiment increased significantly ($P<0.05$) after 14days of treatment could be attributed to the ability of the extract to stimulate the immune system [14].

Euphorbia hirta extract caused significant decreases ($P<0.05$) in the lipid profile (serum triglyceride, cholesterol and LDL cholesterol) values of the treated rats when compared to control. Reduction of blood cholesterol is known to offer protection against cardiovascular diseases [15]. Reductions of both cholesterol and triglyceride concentrations obtained in this study (Tables 2) could be beneficial in preventing the onset and progression of atherosclerosis, cardiovascular diseases, diabetic complications and could improve lipid metabolism. These benefits have indeed been reported to be associated with the lowering of cholesterol and triglyceride values in animals [16, 17]. Epidemiological studies have also shown that elevated concentrations of total or low density lipoprotein (LDL) cholesterol in the blood are risk factors for coronary disease [18].The blood level of HDL cholesterol in contrast bears an inverse relationship of the risk of atherosclerosis and coronary heart disease [19,20]. Although, extract caused significant decrease ($P<0.05$) in HDL cholesterol when compared to control, however, significant improvement could be noticed but when compared with LDL cholesterol values. The mechanism for the observed lipid lowering effect could be due to reduction in absorption of cholesterol from the gut or by reduction in the biosynthesis of cholesterol [21, 22].

Treatment with the extract did not significantly affect serum Na^+, K^+ Cl^- and creatinine but caused significant rise in the urea concentration at all doses used. This agrees with Patil *et al*. [8] and Otsyina *et al*. [13] who reported that Euphorbia hirta had no significant effect on the biochemical parameters of treated rats.

The results obtained also suggest that the extract did not have adverse effect on proteins, ALP and Bilirubin but caused significant increase in the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), suggesting an inflammation of the liver cells and changes which occurred in the liver leading to an increase in serum liver enzymes concentrations. It has been reported that liver toxicity is associated with increase in various serum liver enzymes resulting from damage to the hepatocytes. Elevation in the extract of AST can be associated with cell necrosis of many tissues [17]. For example, pathology involving the skeletal or cardiac muscle and/or the hepatic parenchyma, allows for the leakage of large amounts of this enzyme into the blood [23]. The elevation in AST produced by these plants is an indication of tissue necrosis. ALT, on the other hand, is present in liver and other cells. It is particularly useful in measuring hepatic necrosis, especially in small animals [24]. Since it is one of the specific assayable liver enzymes, its elevated level in this study may indicate hepatic damage caused by these plants.

In conclusion, these plants possess medicinal properties, this study has shown that: *E. hirta* had erythropoiesis and hpyolipidemic activities. It can be suggested based on these reports that the high dose administration of this extract might have been too toxic to the rats to have caused the increase in liver enzymes recorded in the experimental group. Caution should therefore be exercised in their use for medicinal purposes.

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