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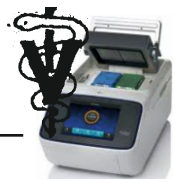

The Effects of Oral Administration of Ethanol Leaf Extract of *Trema Orientalis* on Haematological and Biochemical Parameters in Jamnapari Crossbred Goats

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ABSTRACT

This study was conducted to evaluate the toxic effects of oral administration of ethanolic leaf extract of Trema orientalis (ELETO) on goats fed with commercial goat pellets and Nappier grass twice daily. Meanwhile, water was given ad libitum. The variations in haematological and biochemical parameters were used as indices of the toxicity. A total of 20 goats weighing between 15 and 20 kg were divided into four groups with five goats in each group in a completely randomised design. The test groups I, II, III were given ELETO dissolved in 1% dimethylsulfoxide (DMSO) orally at the dosages of 0.5, 1.0 and 2.0mg/ kg/day respectively, once daily for 14 days. Group IV served as a control and was given 1% DMSO only. There was significant decreased ($P < 0.05$) in the PCV, HB and RBC in the group III with value 15.48 ± 1.89%, 8.06 ± 0.93g/L and 9.27 ± 2.19 (10^6/mm3) respectively compared to the control group with value 22.80 ± 2.97%, 10.10 ± 0.89 g/L, 13.21 ± 1.87 (10^6/mm3). The PCV and RBC in the group III were also significantly decreased ($P < 0.05$) compared to the group I with value 22.68 ± 3.48% and 13.45 ± 2.37 (10^6/mm3) respectively. Meanwhile, the mean corpuscular haemoglobin concentration (MCH), granulocytes, serum alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in the group III with values 7.76 ± 1.58pg, 47.78 ± 13.37%, 52.13 ± 11.92 U/L and 84.40 ± 15.48 U/L were significantly increased ($P < 0.05$) compared to the control group with values 5.25 ± 1.21pg, 24.12 ± 2.22%, 25.02 ± 6.43 and 62.14 ± 5.50 U/L respectively. Goats in group III also had significant elevation ($P < 0.05$) of ALT compare to the group I and II with value 26.50 ± 5.85 and 33.64 ± 8.50 U/L respectively. Meanwhile, the AST value in the group III was also significantly higher ($P < 0.05$) compared to group I with value 60.10 ± 7.43 U/L. Although the number of granulocytes in group III is significantly different; the value, however, was within the normal range. Our studies suggest that oral administration of ELETO daily for 14 days at 2.0mg/ kg of body weight causes anaemia and mild hepatocellular injury in goats.

Keyword: Jamnapari crossbred goats, *Trema orientalis*, haematology, biochemistry, toxicity

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INTRODUCTION

Goats are important meat–producing animals in the tropics [1,2]. The chevon consumption has been increasing around the world [3,4] especially in the developed countries. This is mainly due to its relatively low fat contents compared to the meat of other ruminants [5,6]. Furthermore, animal feed is fast becoming a major constraint due to the tremendous increase in livestock (goats, sheep, pigs, cattle, and buffaloes) population worldwide [7]. In developed countries, livestock accounts for more than half of agricultural production; meanwhile in developing countries, the share is only about one–third [8]. It has been currently reported that the livestock production accounts for about 43% of the gross value of agricultural production [8]. Goats usually feed on grasses or tree leaves [9] which include *Trema orientalis* in most humid tropics; due to its abundance and high palatability. Livestock farmers are thus opted to use alternative plants such as *T. orientalis* which is readily available and palatable with high protein content (18.9%) to feed their animals [10, 11].

Trema orientalis is a species of evergreen flowering plant in the hemp family, Ulmaceae. The genus Trema consists of fifteen species of evergreen trees closely related to the hackberries (Celtis) [12]. Its common names include *menarong, randagong*, *telemukwu, charcoal–tree* and *pigeon wood* [13,14]. *Trema orientalis* most probably originated from low land humid tropics of Asia. It is also found throughout the Southeast Asia and tropical Africa. It is a small to medium size tree with varying heights depending on the climatic conditions and location where it is found. In forest regions, it grows up to 18m high and up to 1.5m tall in the Savannah. Its leaves vary from 2.0 to 20.0cm long, 1.2 to 7.2cm wide and taper from the base to the apex [7]. The leaves margins are finely serrated and frequently unequal. The fruits are small, round, and dark green or purple drupes that change to black when ripe; carried on very short stalks [7]. The matured fruit is 3 to 5mm long [15]. Fruits usually ripen between Decembers to May. *Trema orientalis* grows on a wide range of soils from light sand to heavy clay [16].

Both domestic and industrial uses contribute to the rapid and widespread of the plant in various communities. For instance, the wood is relatively soft and burns quickly when dry and used to make charcoal [17]. The wood is suitable for pulp and paper production with excellent tensile strength and folding endurance [12, 18]. Eckman and Hines [19] reported the ability of *T.orientalis* plant to improve soil fertility through their nitrogen fixing activities. The leaves, seeds, and pods were used as fodder for ruminants, such as goats, cattle and buffaloes in Philippines [12,19]. Game animals browse on the leaves due to palatability while. Various parts of *T. orientalis* is widely used as herbal medicine in a wide range of cultures [13] in treatment of conditions such as pneumonia, pleurisy, tooth ache, hematuria, blood stasis, hyperglycemia, and helminthiasis in dogs [12,20,21,22].

Several studies have reported on the toxic effect of Trema spp following ingestion by animals [23,24,25,26]. Many deaths have been reported in animals such as goats, sheep, cattle, buffalo, rabbits and horses following consumption of either fresh or dried leaves of Trema spp [27]. There was unpublished report of a toxicity study on *T. orientalis* leaf paste orally administered to three goats via stomach tube at 18g/ kg body weight three times daily for seven days. Toxicity signs observed include depression, lowering of the head with frequent recumbency, muscle twitching, shivering, paddling movement when disturbed, and watery diarrhoea. Despite its toxic content; the leaves, seeds, and pods are widely used as fodder for buffaloes, cattle and goats due to the high crude protein content [11,12]. Little research has been conducted on *T. orientalis* and its toxicity effects in goats.

Plant toxicity in animals is usually accidental and most frequently occurs during unfavorable conditions when pastures are poor due to

overstocking, drought, veldt fires, and livestock trampling. The toxicity depends on the condition, part or growth stage of the plant, the amount consumed and the species of the animal involved. Because some of the plants are consumed for several days to weeks or even months, it is necessary to subject them to repeated–dose toxicity tests in order to screen for the potential adverse effects of substances using animal models [28]. Short–term repeated daily dosing tests or exposure to chemical (one to four weeks) are performed to obtain information on the toxicity of a substance.

Haematological Profile Evaluation

Evaluation of haematological parameters is essential in assessing toxicity of exogenous compounds, including plants' extracts in the body system [29,30,31]. Blood is a specialized fluid that makes up the greatest percentage of total body fluid that serve as the major route of drugs, nutrients and oxygen transport to the body cells and waste products away from the cells [31,32,33,34,35]. The formed blood elements of the body are erythrocytes, leukocytes, and thrombocytes. An increase or decrease in haematological variables could be an indicator of toxicity [31]. Assessment of haematological parameters is a pre–requisite to understand the normal functioning of the body system and to confirm the toxic or protective nature of the administered plant extract or drug. The parameters include; red blood cell count (RBC), haemoglobin concentration (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total white blood cell count (WBC), and differential leukocyte count (lymphocytes, neutrophils, basophils, eosinophils, and monocytes). A rise or decline in a haematological variable could suggest excess or suppressed haemopoiesis [31,36].

Biochemical Indices in Toxicity Studies

The measurement of biochemical parameters provides information about the functional status of major organ systems such as the liver, kidney, haematopoietic and immune systems [37]. The most commonly evaluated biochemical indices include enzymes such as aspartate aminotransferase (AST) (serum glutamic oxaloacetic transaminase [SGOT]), alanine aminotransferase (ALT) (serum glutamic pyruvic transaminase [SGPT]), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma–glutamyl transferase (GGT), and Creatine Kinase (CK). Ige *et al.* [38] and Lahon and Das [39] reported that increase in these enzymes concentrations in the plasma is proportional to the extent of tissue damage, loss of functional integrity of tissues cell membranes and consequence leakage into the blood stream. Non–enzymatic parameters mostly investigated in the toxicological survey in plants include; bilirubin, urea, creatinine, total protein, and cholesterol [40,41]. The measurement of levels of substances that may be present in the blood helps in the initial detection toxicity.

The principal objective of this short–term study was to determine adverse effects of ethanolic leaf extract of *T. orientalis* (ELETO) following oral administration on haematology and serum biochemistry in Jamnapari crossbred goats.

MATERIALS AND METHODS

Solvents

Ethanol (95%) and DMSO used in this research were of analytical grade., were obtained from R&M Chemicals Co., Essex, UK.

Collection, Identification and Preparation of crude ELETO

Trema orientalis leaves were obtained from University Sultan Zainal Abidin (UniSZA) Farm, Pasir Akar, Besut Terengganu, Malaysia. The collected leaves sample was photographed and authenticated by a botanist, Prof. Dr. Nashriyah Binti Mat, School of Agricultural Sciences and Biotechnology, Faculty of Bioresources and Food Industry (FBIM), UniSZA, Tembila Campus, Besut Terengganu, Malaysia. The voucher specimen with Voucher No.: 00267 was deposited at the herbarium of School of Agricultural Sciences and Biotechnology, FBIM. The leaf sample were gently pre–washed using tap water to remove impurities and air dried at ambient temperature

until a constant weight (28.6 kg approximately) was obtained. The dried leaves were then crushed and pulverized to obtain homogeneous fine powder using a Laboratory Blender (HGB550, USA). This improved the kinetics of analytic extraction and also increased the contact of sample surface with the solvent system. Proper actions were taken to assure that potential active constituents were not lost, distorted or destroyed during the preparation of the extract [42]. Approximately 500g of powdered leaf sample of *T. orientalis* was placed in 5L conical flask. The sample was completely soaked with 3.5L of 95% ethanol and then covered with aluminum foil. The mixture was allowed to stand at ambient temperature (25°C ± 1) for a period of 72hrs with frequent agitation in order to facilitate dissolution of soluble matter. The mixture was then strained using muslin cloth to remove solid material and pressed. The extraction was repeated by sucking the solid material using 1.5L of ethanol. The strained liquids were then clarified by filtration by gravitation using Smith Filter Papers. The sample to solvent ratio used was 1:10 (w/v). The combined filtrate was concentrated under reduced pressure (180m/bar) at 40°C using Rotary evaporator (BUCHI Rotavapor R–210, Switzerland). The ELETO obtained was dark–green in colour. The dried extracts obtained were then stored in a deep freezer at –20°C (DW–40L508, China) and aliquots of the concentrations were prepared immediately before use and was found to be stable throughout the duration of the experiment.

Determination of Percentage Loss on Drying of the *Trema orientalis*

The percentage loss on drying (%LOD) was determined gravimetrically whereby 5g of accurately weighed air–dried leaf samplewas placed in a previously dried and tared flat weighing bottle. The sample was dried in an oven (Memmert UN 110, Germany) at 105°C until a constant weight was obtained. The percentage loss on drying was calculated using the following equation;

$$\% \text{ LOD} = \frac{\text{Loss in Weight}}{\text{Weight of Dried Sample}} \times 100 \quad \ldots\ldots\ldots (1)$$

Determination of Ethanolic Soluble Extractive Values of the *T. orientalis* Leaf

The extraction was conducted according to the Quality Control Methods for Medicinal Plants Materials, WHO (1998) guidelines and Jain and Argal **[**43**].** Five grams of oven–dried leaf powder of *T. orientalis* was transferred to a conical flask. 100ml of the 95% ethanol (R&M Chemicals, UK) was added, and the flask was covered with aluminum foil. It was then placed on an Orbital shaker (2 Tier, 722–2T, Malaysia) during the first 6hrs and allowed to stand for 18hrs separately. Thereafter, it was filtered rapidly taking precaution to minimize the loss of ethanol. 25ml of the filtrate was collected and transferred to weighed thin porcelain. It was evaporated to dryness on a water bath and dried completely in an oven at 90°C until a constant weight was reached. It was kept in a desiccator to cool and the percentage of alcohol soluble extractive yield was calculated with reference to oven–dried leaf [44,45] using the following formula;

$$\% \text{ Extractive value} = \frac{\text{Initial Weight} - \text{FinalWeight}}{\text{Initial Weight}} \times 100 \quad \ldots\ldots\ldots (2)$$

Ethical Clearance, Animal Management and Experimental Design

The experimental protocol and procedures used in this study were approved by the Animal Ethics Committee (AEC) UniSZA, Tembila campus, Terengganu with Permit Number: UniSZA/AEC/14/002 in conformance with the Guide and Care of the Use of Animals in Research and Teaching of the University (published by the AEC of UniSZA). A total of 20 Jamnapari crossbred goats weighing between 15 and 20 kg were randomly selected from a flock of 260 goats in UniSZA farm, Pasir Akar, Besut, Terengganu. The goats were divided into four groups with each group consisting of five goats. The experimental groups consist of group I, II and III whereas, the control made up of group IV. The goats were fed twice daily at 10 am and 6 pm with commercial goat pellet and Pennisetum purpureum (Napier

grass). Water was given *ad libitum.* The animals were allowed to acclimatize under ambient temperature and humidity for two weeks. All experimental goats were kept in hygienic and well–ventilated pens in the goat shed. Determination of toxicity of the ELETO was carried out by repeated dose toxicity testing. The test groups I, II, III were given ELETO dissolved in 1% DMSO orally at the dosages 0.5, 1.0 and 2.0g/ kg/day respectively. Group IV served as a control and was given 1% DMSO. Administration of ELETO was aided by the use of a stomach tube and carried out between the hours of 8 am, and 10 am daily for 14 days.

Blood Samples Collection and Laboratory Analysis

Blood samples were collected via jugular venipuncture on day 14 of the experiment. 10ml sterile plain and 5ml sterile EDTA coated Vacutainer tubes were used for the biochemical and haematological analysis respectively. Blood samples were stored under chilled condition (8°C) before transported to the laboratory for further analysis. Haematological parameters which include HB, PCV, RBC, WBC, differential leukocyte counts (lymphocytes and granulocytes) and erythrocytes indices (MCV, MCH, MCHC), were assessed using automated analyzer (Medonic CA 530, Japan). The supernatant sera were then harvested and kept at −20°C for subsequent biochemical analysis using Automated Chemistry Analyzer (Olympus AU 400, Shinjuku, Japan). Alanine aminotransferase, AST, ALP, total bilirubin, total protein, urea, Na, K, and Cl activities were assessed.

Statistical Analysis

Quantitative results of haematological and biochemical indices were analyzed using the Shapiro–WilkTest for normality, the Levene Median Test for an anequal variance, and by one–way Analysis of Variance (ANOVA) using SPSS version 20. Differences between each group were compared by using the Tukey's HSD Multiple Comparison Tests. If either the normality or equal variance test failed, then the analysis was continued using the non–parametric Kruskal–Wallis on rank–transformed data. If the Kruskal–Wallis indicated statistical significance among experimental groups, then the Mann–Whitney U Test was used to delineate which groups (if any) differed from the control. The probability value (P) < 0.05 (two–tailed) was used as the critical level of significance.

RESULTS

Haematology

The effect of oral administration of ELETO on mean haematological values of Jamnapari crossbred goats is shown in Figure 4.1, Table 4.2 and Table 4.3. The MCHC, WBC and lymphocyte values were comparable (P > 0.05) in all the test groups than that of the control group. The MCV values in groups I, II and III, were significantly decreased (P < 0.05) compared to the control group. There was significant decreased (P < 0.05) in the PCV, HB and RBC in the group III compared to the control group. The PCV and RBC in the group III were also significantly decreased (P < 0.05) compared to the test group I. The PCV, HB and RBC in group III were above the normal upper limit of the reference value (normal reference value ranges of the PCV, HB and RBC of Jamnapari goat age 6 to 24 months). Meanwhile, MCH and granulocytes in the group III were significantly increased (P < 0.05) compared to the control group. Although the granulocytes count was significantly higher than the control group, however, the value was within the normal range.

Table 1: Effect oral administration of ELETO on mean haematology of Jamnapari crossbred goats, measured at day 14.

Variables	Group I (0.5g/kg/day)	Group II (1.0 g/kg/day)	Group III (2.0 g/kg/day)	Group IV (Control)	F (df)	X^2 (df)	*P* value*	Normal ranges
PCV	22.68 ± 3.48^{b}	20.24 ± 4.17^{ab}	15.48 ± 1.89^{a}	22.80 ± 2.97^{b}	5.607 (3)	-	< 0.05	18.0 – 27.2
HB	9.62 ± 1.45^{ab}	9.26 ± 0.78^{ab}	8.06 ± 0.93^{a}	10.10 ± 0.89^{b}	3.465 (3)	-	< 0.05	3.6 – 6.6
RBC	13.45 ± 2.37^{b}	12.44 ± 1.76^{ab}	9.27 ± 2.19^{a}	13.21 ± 1.87^{b}	4.386 (3)	-	< 0.05	8.02 – 17.05
MCV	16.5 ± 2.85^{a}	17.3 ± 1.45^{a}	21.9 ± 4.20^{a}	16.1 ± 2.48^{a}	-	9.713 (3)	< 0.05	16.0 – 25.0
MCHC	30.94 ± 4.86	34.04 ± 5.36	39.20 ± 7.15	31.62 ± 4.60	2.252 (3)	-	> 0.05	30.0 – 36.0
MCH	6.46 ± 1.00^{ab}	6.32 ± 1.04^{ab}	7.76 ± 1.58^{a}	5.25 ± 1.21^{b}	3.502 (3)	-	< 0.05	5.2 – 8.0
WBC	10.46 ± 3.96	11.12 ± 3.98	15.36 ± 5.56	10.41 ± 3.58	1.494 (3)	-	> 0.05	4.0 – 13.0
LYM	65.02 ± 6.57	56.20 ± 8.74	53.06 ± 12.95	66.92 ± 6.43	2.745 (3)		> 0.05	0.0 – 99.9
GRAN	31.90 ± 11.03^{ab}	42.62 ± 17.75^{ab}	47.78 ± 13.37^{a}	24.12 ± 2.22^{b}	3.643 (3)		< 0.05	0.0 – 99.9

* One–Way ANOVA , * Kruskal–Wallis Test, a different superscript (a, b) within each haematological variable indicates the significant differences (P <0.05), df = Degree of freedom, F = Fisher's exact test, X^2 = Chi–Square, PCV = Packed cell volume (%), HB = Haemoglobin concentration (g/L), RBC = Red blood cell count (106/mm^3), MCV = mean corpuscular volume (µm^3), MCHC = Mean corpuscular haemoglobin concentration (g/L), MCH = Mean corpuscular haemoglobin (pg), WBC = Total white blood cell count (10^3/mm^3), LYM = lymphocytes (%), and GRAN = granulocytes (%). Values are presented as Mean ± SD, n = 5.

Biochemistry

The effect of oral administration of ELETO on mean serum biochemical values of Jamnapari crossbred goats is shown in Figure 4.2, Table 4.4 and Table 4.5. The ALP, total bilirubin, urea, total protein, Na, K, and Cl were comparable (P > 0.05) in all groups. The ALT and AST levels in group III and were significantly decreased (P < 0.05) compared to the control group. Meanwhile, the AST value in group III was significantly higher (P < 0.05) compared to the test–group I.

Table 2: Effect oral administration of ELETO on mean serum biochemistry of Jamnapari

Variables	Group I (0.5g/kg/day)	Group II (1.0 g/kg/day)	Group III (2.0 g/kg/day)	Group IV (Control)	X^2(df)	F (df)	P value*	Normal ranges
ALT	26.50 ± 5.85^{b}	33.64 ± 8.50^{b}	52.13 ± 11.92^{a}	25.02 ± 6.43^{b}	-	10.707 (3)	< 0.05	18.7– 48.2
AST	60.10 ± 7.43^{b}	69.06 ± 10.99^{ab}	84.40 ± 15.48^{a}	62.14 ± 5.50^{b}	-	5.432 (3)	< 0.05	55.5 – 72.2
ALP	150.52 ± 47.34	166.74 ± 43.14	189.66 ± 42.46	140.68 ± 36.52	-	1.265 (3)	> 0.05	20.0 – 120.0
TP	57.68 ± 8.75	60.00 ± 7.48	54.36 ± 5.41	57.86 ± 5.67	-	0.559 (3)	> 0.05	62.9 – 100.5
TBIL	2.88 ± 0.38	2.86 ± 0.42	3.40 ± 0.92	2.54 ± 0.40	-	1.922 (3)	> 0.05	2.0 – 12.0
Urea	5.60 ± 1.01	5.60 ± 1.15	5.40 ± 1.90	5.60 ± 1.15	0.037 (3)	-	> 0.05	2.8 – 7.2
Na	134.54 ± 13.48	137.14 ± 3.65	139.32 ± 6.86	137.58 ± 6.02	-	0.280 (3)	> 0.05	136.0 – 150.0
K	4.64 ± 0.52	4.88 ± 0.28	4.66 ± 0.31	4.96 ± 0.41	-	0.839 (3)	> 0.05	3.5 – 5.1
Cl	100.14 ± 9.33	101.96 ± 2.84	101.74 ± 5.55	103.44 ± 4.40		0.251 (3)	> 0.05	98.0 – 108.0

crossbred goats, measured at day 14.

* One–Way ANOVA , * Kruskal–Wallis Test, a different superscript (a , b) within each haematological variable indicates the significant differences (P < 0.05), df = Degree of freedom, F = Fisher's exact test, X^2= Chi–Square. ALT = Alanin aminotransferase (U/L), AST= Aspartate aminotransferase (U/L), ALP = Alkaline phosphatas e (U/L), TP = total protein (µmol/L), TBIL = Total bilirubin (µmol/L) , Na = Sodium, K = Potassium, Cl = Chloride. Findings are presented as Mean ± SD, n = 5.

DISCUSSION

Haematology

In the present study, the decrease in PCV indicates anaemia which is a reduction in the number of erythrocytes, HB, or both, in the circulating blood. It could be the results from excessive RBC destruction, RBC loss, or decreased in RBC production and is a manifestation of an underlying disease process [46,47,48]. Adedapo *et al.* [49] reported that reduction in RBC, HB and PCV can be an indicator of either destruction of RBC or their decreased production which may lead to anaemia. Therefore, a significant reduction of PCV, RBC and HB following administration of ELETO for 14 days in group III goats, is suggestive of anaemia. Similarly, significant decrease ($P < 0.05$) in PCV, HB and RBC were obtained for the extracts of the species *Hibiscus sinensis* [50] and *Moringa oleifera* [51]. Olson *et al.,* [52] reported that excessive ingestion of a wide variety of plants or their products caused hypoproliferative or non–regenerative anaemia, which is a stem cell disorder characterized by reduced bone marrow production of all blood components in the absence of a primary disease process infiltrating the bone marrow or suppressing haematopoiesis. However, extract of plant such as *Sorghum bicolor* [53] caused an increased ($P < 0.05$) in these parameters compared to control; whereas, no changes were observed in rats treated with extract of *Cryptolepis sanguinolenta* [54].

Jaime and Howlett [55] reported that increase in MCV may indicate anaemia due to the liver disease, bone marrow abnormalities, nutritional deficiencies, chronic lung disease, or treatment with certain regimes. Therefore, increased in MCV of the test groups in the current study could be attributed to the macrocytic form of anaemia.Increase in MCH indicates that the RBC is abnormally enlarged and probably followed by bursting of the RBC. Adebayo *et al.* [56] and Ashafa *et al.* [57] reported that if MCHC, MCH and MCV were not affected by the administered extract, it means that neither the incorporation of HB into RBC, nor the morphology and osmotic fragile of RBC is altered. Abnormally high RBC suggests lung diseases, congenital heart disease, polycythaemia Vera, kidney disease, or dehydration. A mean corpuscular volume could be defined as the average volume of individual RBC and is use in determining the type of anaemia an animal might have. When the RBC is smaller than normal size (i.e., microcytic), the MCV value will be below the normal range. Low MCV may indicate chronic disease, iron deficiency, pregnancy, and thalassemia. When the RBCs appeared larger than normal size (i.e., macrocytic), the MCV value is elevated above the normal range. High MCV may indicate anaemia due to liver disease, bone marrow abnormalities, nutritional deficiencies, chronic lung disease, or treatment with certain regimes [55]. Normal size RBC is termed normocytic.

Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are further guides to the investigation of anaemia. The MCH is the HB content of the average RBC. The MCH may be low in types of anaemia where the RBC is abnormally small, or high in types of anaemia where the RBC is abnormally enlarged (e.g. vitamin B12 deficiency). Mean corpuscular haemoglobin concentration is a measure of the average HB in a given PCV. The causes of low MCHC include; blood loss, forum deficiency, chronic disease, and pregnancy.

It has been reported that increased WBC supposed to be helpful in boosting immune system [49,58], whereas a decreased in WBC shows the suppression of leukocytes and their production from bone marrow [59,60,61]. Swenson and Reece [62] stated that toxic plants do not causing a direct effect on WBC. This also supports the current finding where the total WBC counts of the test groups showed comparable result to the control group.

Serum Biochemistry

Alanine aminotransferase and AST have been established as markers of hepatocellular injury while Alkaline phosphatase is a marker of cholestasis [63,64,65]. Increase in these enzymes is proportional to the extent of

damage to cytosolic and/or mitochondrial membranes consequent leakage into the blood stream [38,39,66,67]. The non–significant alteration (P > 0 .05) in the levels of the ALP, total bilirubin, urea, and total protein, Na, K, and the Cl suggests that the extract, even though might have increased or decreased these biochemical parameters in animals, but may not interfere significantly with the metabolism of these biochemical parameters within the duration of the study. Hence, the result suggests that the biliary duct and the synthetic function of the liver were preserved; also, showed an absence of nephrotoxicity and muscular involvement. It also indicates an absence of electrolytic disturbance, cell membrane degeneration, and cardiac arrhythmia following administration of the ELETO. Meanwhile, screening of possible involvement of other organs with ALT and AST significantly elevated in the systemic circulation (enzymes which are normally present at low levels in the blood), is suggestive of hepatocellular injury [68].

Aminotransferase enzymes (ALT and AST) are involved in the transfer of an amino group from a 2–amino acid to a 2–oxoacid with the aid of pyridoxal phosphate as a cofactor for optimal activity [67]. These enzymes play important roles in diagnostic enzymology established as markers of hepatocellular injury especially in the assessment of hepatotoxicity [63,65,69]. The ALT and AST leak into circulation when liver cells or their membranes are damaged and their elevation is proportionateto the number of hepatocytes affected [70]. Alanin aminotransferase is widely accepted as more specific marker of hepatocellular damage, because it occurs in the cytosol primarily in the liver, with lower amounts in kidney, heart and skeletal muscle; meanwhile, AST has cytosolic and mitochondrial forms and is present in various tissues including the heart, kidneys, brain, skeletal muscle, pancreas and erythrocytes, hence damage to any of these tissues may increase plasma AST levels [31,38,67,71].

Therefore, significant elevation in the activities of ALT and AST in the serum of test animals suggests that ELETO might induce hepatocellular injury in the goats. These increases of ALT and AST in serum levels may be due to the leakage from hepatocytes through peroxidative damage of their membranes leading to increased membrane permeability [72]. These enzymes are usually raised in acute hepatotoxicity or mild hepatocellular injury, but tend to decrease with prolonged intoxication, as a result of severe damage to the liver [73,74]. Similar results were obtained for oraladministrations of extracts of the species *Euphorbia balsamifera* [61], *Khaya senegalensis* [75], *Pyrenacantha staudti* [76], *Moringa oleifera* [77], *Mangifera indica* [78], and *Rhodiola imbricata* rhizome in rats [79], providing a biochemical evidence of significant liver damage.The ratio of AST to ALT may help to determine whether the liver or another organ has been damaged [80] though, both ALT and AST levels indicating liver damage. AST is similar to ALT in that both enzymes are associated with liver parenchymal cells as observed with ELETO. However, the level cannot be used to predict the type of lesion; whether cell damage is reversible (leakage) or irreversible (necrosis).

Plasma ALP is hydrolyzed organic phosphates at high pH, is primarily a marker of hepatobiliary effect (cholestasis) [63,64,65]. It is found in the biliary poles of the hepatocytes, epithelial of the bile duct, kidney, osteoblasts, lung, intestine wall and the placenta [67,81]. Wright and Vandenberg [82] reported the elevation of ALP due toinhibited bile excretion as a result of liver damage (cholestasis). Thus, an isolated increase in ALP is seen in the third trimester of pregnancy, during growth, following ingestion of a fat–rich meal and anti–epileptic drugs (phenytoin, carbamazepine) [83,84]. However, bilirubin excretion usually becomes impaired in cholestasis, causing a rise in the plasma bilirubin concentration [85]. Increase in conjugated bilirubin indicates cholestasis but is not a specific marker. Increases can occur secondary to hemolysis, by the parenchymal liver condition that interferes with biliary excretion by sepsis, bile sludging, and physical obstructions to bile flow in the biliary system.

Therefore, similar result of test goats and control goats suggests lack of significant hepatobilliary injury (cholestasis) as a result of ELETO administration for 14 days.
Plasma proteins, urea, and bile acids are synthesized in the liver [86]; therefore, the synthetic functions of the liver are not compromised as result of the oral administration of ELETO at different dosages. Hence, determination of serum protein is one method of assessing liver function. Estimation of plasma urea is a screening test for renal function. These metabolites are usually eliminated from the body through glomerular filtration. An increased plasma urea implies the impairment of renal function. The serum level of these metabolites is usually parallel to the severity of renal malfunction. In the present study, all groups showed normal values for plasma urea. This probably reflects that oral administration of ELETO at 0.5 to2.0g/ kg/day for 14 days does not affect the renal function of the animals.

Increased levels of Na in the cells and decrease inter cellular level of K leading to an elevated level of K in the plasma; can cause deactivation of the cell membrane Na–K ATPase in affected tissues by glycosides. The most important physiological role of K is in the regulation of muscle and nerve excitability, especially on muscle and nervous tissue excitability. During periods of K imbalance, the cardiovascular system is of principal concern. Cardiac muscle cells depend on their ability to change their electrical potentials, with accompanying K flux when exposed to the proper stimulus, to result in muscle contraction and nerve conduction. Between 60% and 75% of the total body K is found within muscle cells, with the remainder in bone. Only 5% of K is located in the ECF, therefore K concentration in blood is not always an indication of total body K levels. Plasma (ECF) K1 concentration is tightly regulated; fairly small changes can have marked effects on organ function. Serum Cl^- values are used as confirmatory tests to identify fluid balance and acid–base abnormalities [87]. Like Na^+, a change in the serum Cl^- concentration does not necessarily reflect a change in the total body content. Rather, it indicates an alteration in fluid status and acid–base balance.

The lack of significant alteration ($P > 0.05$) in ALP, total bilirubin, total protein, urea, and electrolytes suggest; the biliary duct and the synthetic function of the liver were preserved and also absence of nephrotoxicity, muscular involvement, electrolytic disturbance, cell membrane degeneration, and cardiac arrhythmia following administration of the ELETO. Though, screening out of possible involvement of other organs, significant increase in ALT and AST reaffirmed the involvement of hepatocellular damage. The presence of systemic toxicity in ELETO can be justified by the significant differences observed for the PCV, HB, RBC, MCH, granulocytes.

Although hepatocytes contain more AST than ALT, ALT is confined to the cytoplasm in which its concentration is higher than AST [31,67,88]. Therefore, elevated ALT is more specific for hepatic damage while AST is more sensitive to skeletal muscle and other organs damage [67,80]. Aminotransferases have a short half–life (one to two days). Evaluation of the serum transaminases' activity is the most useful tool for the assessment of liver toxicity [68,89].

Conclusion

This study has shown from the preliminary data that increased dosage of ELETO upto 2.0g/ kg/day for 14 days can promote haematolgical and biochemical changes characterized by decreasd in PCV, HB and RBC indicating anaemia and increased in ALT and AST suggesting hepatocellular injury, respectively. The oral administration of ELETO causes haemato–biochemical changes ($P < 0.05$) in goats at 2.0g/ kg/day for 14 days pointing towards liver damage. Clinical toxicity observed include decrease appetite, decrease body weight, bilateral reddening and lacrimation of the eyes, apathy and rectal tenesmus. The toxic effect in the blood was anaemia following a decreased in RBC and HB, and elevated transaminase enzymes (ALT and AST) which are the pointer to hepatocellular injury. Another important finding of the study is

that *T. orientalis* leaves, if only given as a supplement, were safe for goats considering dosage < 2.0g/ kg/day or equivalent fresh leaves for two weeks. However, at high dosages, the alterations observed on the PCV, HB, MCH, granulocytes, ALT, and the AST suggest selective dose toxicity of ELETO in goats for repeated dosing for 14 days. Therefore, special caution should be taken when keeping goats in areas with T.orientalis.

Limitations

Multiple samples at regular intervals for the vital parameters, haematology and serum biochemistry were not considered for logistic reasons. Emphasis in research of the major bioactive components and their mechanisms of actions.

Recommendation

To reduce the use of animal in toxicity studies, there is a need for a long–term in vitro system which was not conducted in the course of the present study.

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