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Effect of *Chrysanthemum indicum* Aqueous Extract on Some Biochemical and Haematological Parameters in Albino Rats

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Authors' contributions

This work was carried out in collaboration of all authors. Author BH designed the study, wrote the protocols and the first draft of the manuscript. Author MYT corrected the manuscript and performed statistical analysis. Authors GAO, GGM and UIN managed literature searches and analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was carried out to investigate the acute and sub-acute toxicity of *Chrysanthemum indicum* on albino rats.
Study Design: *In-vivo* acute toxicity, haematological and Biochemical effect of *Chrysanthemum indicum.*
Place and Duration of the Study: Department of Chemical Science Technology, Federal Polytechnic Mubi, Adamawa State, between October to December 2017.
Methodology: For acute toxicity, four groups of 3 male rats each were dosed orally with *Chrysanthemum indicum* aqueous extract at 500, 1000, 2000 and 4000 mg/kg body weight, the animals were observed for mortality, clinical sign and gross pathological changes for a period of 14 days. For sub-acute toxicity and other parameters, four groups of five albino rats were equally

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dosed orally with 0 (control), 75, 150 and 300 mg/kg (experimental group) body weight of an aqueous extract of *Chrysanthemum indicum* for six (6) weeks. After, complete administration, the biochemical and haemological indices were investigated and determined appropriately.

Results: The aqueous extract of *Chrysanthemum indicum* was found to be safe at 4000mg/kg body weight in acute toxicity study. Results further showed that there are changes compared to control and group administered the extract in sub-acute toxicity study. In RBC, from 5.40 to 5.10, and MCV, from 78.00 to 82.30 which were significantly different ($p<0.05$) but non-significant decrease ($p<0.05$) was noticed in AST, albumin, and urea. However, the level Protein, (13.00 to 10.00) ALT, (7.00 to 5.00) ALP (15.00 to 10.25) bilirubin (13.00 to 10.00) and creatinine (88.00 to 84.00) significantly reduced ($p<0.05$) in the experimental groups when compared to the control group.

Conclusion: Although the acute toxicity of *Chrysanthemum indicum* suggests its safety at 4000 mg/kg body weight, however, the findings of sub-acute toxicity suggest that the therapeutic and herbal tea use of *Chrysanthemum indicum* plant is not safe especially when taken orally in high dose for a prolonged period of time.

Keywords: Haematology; biochemical parameters; Chrysanthemum indicum; toxicity.

1. INTRODUCTION

Literally, a plant is a storehouse of hundreds of chemicals of diverse biological activities. Most of the chemicals present in the plants, are harmless and are necessary for the survival of both the plants and animals. Man and animals mostly depend on vegetables for their food. Plants by their metabolic activities besides being the source of feeds and fodder also elaborate other substance viz; alkaloid, glycosides, albumins, essential oils, resins, bitter principles etc., which are essential from the medicinal and toxicological point of view. Many plants are categorized as poisonous plants. Plants toxins may refer to as secondary metabolites which are toxic. Most of these secondary metabolites do not have any apparent function in the plant except for defense mechanisms or survival adaptations [1].

The applications of herbs and medicinal plant on traditional medicine to diagnose prevent or treat diseases dates back to many centuries, among rural communities throughout the world [1,2]. A medicinal plant possesses therapeutic properties or exerts beneficial pharmacological effects on the human and animals body [3]. Plants are the source of about 25% of prescribed drugs in the. World Health Organization estimated that perhaps eighty percent of the inhabitants of the world rely chiefly on traditional medicines [1].

The wide range of medicinal plants parts like flower, leaves, stem, bark, fruit and root extracts are used as powerful raw drugs possessing a variety of pharmacological and non-pharmacological way of activities. The used of medicinal plants as an alternative is encouraged especially in rural areas, and it has been used because of economic reason as well. Nowadays, there is wide spread interest in drugs derived from plants which reflect its recognition of the validity of many traditional claims regarding the value of natural products in health care [4]. Thus, in order to determine the potential use of any medicinal plants, it is essential to intensify the toxicological study of a medicinal plant that has found a place in folklore.

There are large numbers of medicinal plants used as herbal preparation, and only a small amount of them was investigated for their toxicity. However, recent scientific reports demonstrated that several medicinal plants used in phytomedicine are potentially toxic and some are even mutagenic and carcinogenic. The toxicity of herbal drugs, therefore, depends on their purity, herbal combinations, absorption, bio-availability and reported adverse effects. Toxicity in phytomedicine may also result in the botanical identification, accidental ingestion of cardio-tonic plants [5,6,7].

The plant *Chrysanthemum indicum* is the subspecies of *Afroamericanium* commonly known as a wild daisy in English, is a natural herbaceous plant which is part of *Asteraceae* family. It is widely consumed in North central and North eastern part of Nigeria as herbal tea that helps in indigestion and gastrointestinal tract disturbance [8]. *Chrysanthemum indicum* extract has been used for a number of medicinal conditions including recovery from influenza, for treating yellow fever. According to folklore

medicine, *Chrysanthemum* tea is used for various veins and atherosclerosis treatment. It was also said to be effective for inhibiting the agglutination of blood platelets and promote the myocardial blood circulation and white blood phagocytosis and it is used in treating many diseases such as furuncle. This plant has been also used as an herbal medicine used as anti-inflammatory, analgesic, anti-pyretic purposes and the treatment of eye diseases. It is also known to showed inhibitory activity against rats lens aldose reductase and against nitric oxide production in lipopolysaccharides activates macrophages [3]. *Chrysanthemum indicum* flower has a strong aroma and many of previous studies focused on the essential oil of this plant.

Since *Chrysanthemum indicum* plant is widely consumed in the northern part of Nigeria to date and little or no work has been done on its acute and sub-acute toxicity. Therefore, this study intends to elucidate the effect of the said plant on some hematological and biochemical parameters on albino rats.

2. MATERIALS AND METHODS

2.1 Plants Materials

Fresh matured plant of *Chrysanthemum indicum* was collected from Gashala Pubba Hong Local Government of Adamawa State Nigeria. The plant was identified at the Federal College of Forestry Jos Nigeria and further authenticated by Mr Azila of the Herbarium unit of the same Institution.

2.2 Plant Extraction

The plant was shade dried and pulverized to dry powder using wood mortar and pestle with continual pounding and sieving. About 100 g of the powder plant material was macerated in 400 ml of distilled water for 24 hours and was filtered using Whatman No 1 filter paper. The filtrate was evaporated at 40-50^{o}C on a water bath to get a solid extract and was kept in the refrigerator until when the need arises.

2.3 Animals Treatment

Male albino rats weighing 140-150 g was obtained from Modibbo Adama University of Technology Yola (MAUTECH). The rats were allowed to acclimatize for a week. They were kept in plastic cages at room temperature and fed with pelleted diet (Grand cereal Limited Jos Nigeria) and water at *libitum* throughout the experimental period.

2.4 Ethical Approval

The research was also approved by the Institution's Research Committee. Animal care and handling were in compliance with the international guidelines [9].

2.5 Acute Toxicity

Acute toxicity of the extract was determined using the method described previously [10,11]. Briefly, four groups of 3 male rats were dosed orally with *Chrysanthemum indicum* aqueous extract at 500, 1000, 2000 and 4000 mg/kg body weight, the animals were observed for mortality, clinical sign and gross pathological changes for period of 14 days.

2.6 Sub-acute Toxicity

Twenty male rats were randomly divided into four groups of 5 rats each and administered with aqueous extract of *Chrysanthemum indicum* at 0 (control), 75, 150 and 300 mg/kg body weight. Administration of the extracts commenced after 10 days (period to acclimatized) and lasted for the period four weeks on daily bases. After four weeks, 2 ml of blood sample was collected through cardiac puncture and were dispensed into EDTA container for haematological analyses while another 4ml for Biochemical studies. Serum AST, ALT, ALP was determined using Randox Reitman and Frankel Level 2 control (Cat. No SC.2643) total bilirubin, total protein, albumin, urea, and creatinine were determined using GOD/PAP Manual RX MONZA kits purchased from Randox laboratories Co. Antrium UK. Plasma samples were obtained for the determination of pack cell volume, haemaglobin concentration and red blood cells. In addition, mean corpuscular values (MCV) mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) values were also computed using the standard formulae [12].

2.7 Statistical Analysis

All the result was expressed as the mean standard error for five replicates. Statically, analysis of variance was carried out using one way ANOVA (SPSS16.0). A level of $p<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

Traditional medicines in any form (decoction, infusion, concoction) are widely used in rural, peri-urban and urban areas of most African countries. This is because most pharmaceutical-based medicines are rather too expensive and the majority of individuals in these communities are low-income earners. Moreover, the efficacies of most of these plants on infectious diseases are awesome. However, the fact still remains that some plants that are used in traditional medicine may have a toxic effect. Toxicity study is necessary for newly developed drug and decoction that is taken either intraperitoneal or orally [1].

The LD_{50} result showed that *Chrysanthemum indicum* aqueous extract was found to be safe at 4000 mg/kg body weight because there was no mortality, clinical sign and gross pathological changes for period of 14 days which is an indication that the plant is save in terms of acute toxicity up to 4000 mg/kg body weight. Although there was no previous report on the acute toxicity of the same plant to verify our claim, previous other studies of acute toxicity on other herbal plants either concurred with our findings [11,13] or are contrary to our findings [14,15].

Assessment of hematological and biochemical parameters can be used to determine the extent of deleterious effects of foreign compounds including plant extracts in the blood constituents and liver enzymes of an animals.

Assessment of hematological parameters are not only used to determine the extent of the deleterious effect of extracts on the blood of animals, but it can also be used to explain blood relating functions of a plant extract or its products [1,16]. The various hematological parameters investigated in this study are useful parameters in evaluating the toxicity of plant extract in animals [1,17]. This type of study is significant in determining risk evaluation because changes in the haematological parameters have higher predictive indices for human toxicity, when data are translated from animal studies [18].

The mean corpuscular values (MCV), mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) computed from RBC, Hb and PCV are usually useful in elucidating and classifying anaemia. Morphologically, they represent an estimation of the alterations in the size and hemoglobin content of individual red blood cells [12]. In this study, the absence of observable significant effect of the extracts on some of these parameters may be an indication that neither the incorporation of haemoglobin into the red blood cells nor the morphology and osmotic fragility of the red blood cells was altered. This observation was quite similar to previous studies on medicinal plants other than *Chrysanthemum indicum* [19,20]. Contrary to this however, the findings of our study also showed that the MCV of the treated animals varies significantly (P=0.032) with that of the control animals (Table 1).

Erythrocytes indices on red blood cells (RBC) packed cell volume (PCV) and hemoglobin concentration (Hb) is important indicators of the functional state of the red blood cells. Red blood cells count reflects the total number of red blood cells per unit volume of circulating blood, while haemaglobin concentration determination indicates the oxygen carry capacity of blood; PCV determination on the other hand shows the proportion of blood that is made up of cellular element and the proportion that is plasma [7].

Packed cell volume (PCV) results showed that there was a significant decrease in the group administered 150 mg/kg body weight with value of 37.00 compared to the control group value of 42.00 (Table 1). The RBC has also noticed significant decrease in the group administered with all the concentrations compared to that of the control (P=0.001) (Table 1). This could mean that the balance between the rate of production and destruction of blood corpuscles (erythropoiesis) was affected negatively [20]. The haemaglobin concentration has noticed significant decrease in the group administered with 150 mg/kg body weight with value 12.50 compared to 14.00. The RBC, PCV and Hb values decreased was not consistently with dosage i.e. without any specified pattern in the variation among respective dosage (Table 1). These findings showed that there was either reduction in size or number of the red cells per unit volume of the blood; which may reflect the reduction of the oxygen-carrying capacity of those rats in those respective groups.

Administration of aqueous extract of *Chrysanthemum indicum* has also shown a significant decrease (P=0.032) in plasma protein

from 70.00 (control) to 68.00 (test group) administered with 300 mg/kg body weight. The observed decrease in plasma protein seems to be consistent, which may be due to low albumin level cause by several hemorrhage or increase in protein breakdown or decrease in the immune system [21].

The result of ALT, AST and ALP consistently declined (Table 2); Alkaline phosphatase (ALP) indices values decrease from 15.00 (control group) to 10.25 in the test group administered 300 mg/kg body weight; Alanine transaminase (ALT) indices values decrease from 7.00 (control group) to 5.00 in the test group administered 300 mg/kg body weight; Aspartate transaminase (AST) indices values decrease from 5.00 (control group) to 4.00 in the test group administered 300 mg/kg body weight; Changes in plasma level enzymes are attributed to damage of hepatic cells because they are located in the cytoplasm and are released as a result of cellular damage. So, difference in the level of the enzymes between the control and the test group signifies that administration of the extract has toxic indications especially on alkaline phosphatase and alanine transaminase [22].

Total bilirubin indices values also decrease consistently from 13.00 (control group) to 10.00 in the test group administered 300 mg/kg body weight (Table 2); the changes in bilirubin, reflect the physicochemical properties of the erythrocyte surface and the plasma [12]. This is an essential index for evaluating the response of an animal or human body to necrotic processes [23].

Urea and creatinine indices are parameters used for indication of kidney function. Normally, blood contains a small amount of urea and creatinine which varies with the amount of protein in the diet. Administration of the extract of *Chrysanthemum indicum* showed a significant decrease (P=0.025) (Table 2) which was due to poor perfusion of the kidney resulting in diminished glomerular filteration. This can be as a result of reduced blood flow to the kidney or increased protein catabolism. The observed difference in serum creatinine level in the group administered with *Chrysanthemum indicum* is attributed to the renal causes ureamia, the creative level tends to plateau.

A previous report showed that the fraction of *Chrysanthemum indicum* contain several classes of biologically active compounds which includes terpenoids, phenolic acids, essential oils and flavonoids. Among the nonvolatile oil, the major active components include flavonoids of luteolin-7-*O*-β-D-glucoside and linarin, as well as phenolic acids of chlorogenic acid, 3,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid [24]. According to them, phenolic acids have antibacterial, antiphlogistic, antimutagenic, antioxidant and other biological activities. Linarin and luteolin-7-*O*-β-D-glucoside are used as remedies because of their antiphlogistic, spasmolytic, good antioxidant and free radical scavenging properties. Other chemical components reported to be present in extracts of *Chrysanthemum indicum* includes α-pinene, 1,8-cineole, α-thujone, camphor, terpinen-4-ol, bornyl acetate, borneol, *cis*-chrysanthenol, β-caryophyllene, germacrene D and α-cadinol [25].

Table 1. Effect of aqueous extract of *Chrysanthemum indicum* on some haematological parameters in rats

Groups	Hb (g/l)	PCV (L/L)	RBC ($x10^{12}$/L)	MCV (fl)	MCHC (%)	MCH (pg)
Normal	14.00^{a}±0.57	42.00^{a}±0.40	5.40^{a}±0.04	78.00^{a}±0.40	33.30^{a}±0.05	27.00^{a}±0.40
75 mg/kg body weight	13.00^{a}±0.57	39.00^{b}±0.91	5.20^{b}±0.09	75.00^{b}±1.29	33.00^{a}±0.04	25.00^{b}±0.91
150 mg/kg body weight	12.50^{a}±0.20	37.00^{b}±0.57	4.90^{b}±0.04	75.00^{b}±0.05	33.70^{a}±0.04	25.50^{a}±0.09
300 mg/kg body weight	14.00^{a}±0.40	42.00^{a}±0.57	5.10^{b}±0.05	82.30^{c}±0.08	33.30^{a}±0.04	27.40^{a}±0.00

Legend: The superscripts indicate the level of significant difference from the control group (a= p>0.05; b or c = p<0.05). PCV= Packed cell volume, RBC= Red blood count, Hb= Hemoglobin, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin (MCH), MCHC= Mean corpuscular haemoglobin concentration

Table 2. Effect of aqueous extract of *Chrysanthemum indicum* on some biochemical parameters in rats

Groups	Protein (g/L)	Albumin	ALP (UI/L)	ALT (UI/L)	AST (UI/L)	Total Bilirubin	Urea (g/L)	Creatinine (mg/L)
Normal	70.00^{a}±0.91	42.00^{a}±0.91	15.00^{a}±091	7.00^{a}±0.90	5.00^{a}±0.40	13.00^{a}±0.00	4.00 ±0.71	88.00^{a}±0.91
75 mg/kg body weight	68.00^{a} ±091	43.00^{a}±0.90	13.00^{b}±0.14	5.00^{b}±0.41	5.00^{a}±0.00	11.00^{b}±0.58	4.10±0.04	85.00^{b}±0.91
150 mg/kg body weight	69.00^{a}±0.58	41.00^{a}±0.90	10.00^{c}±0.40	6.00 ^{b}±0.40	4.00^{b}±0.17	11.00^{b}±0.58	4.00±0.06	90.00^{a}±0.82
300 mg/kg body weight	68.00^{b}±0.58	40.00^{b}±0.58	10.25^{c}±0.63	5.00 ^{b}±0.41	4.00^{b}±0.58	10.00^{b}±0.82	3.90±0.04	84.00^{b}±0.58

Legend: Values represent means ± SE. The superscripts indicate the level of significant difference from the control group (a= p>0.05; b or c = p<0.05).
ALP = Alkaline phosphatase, AST= Aspartate transaminase,
ALT= Alanine transaminase

4. CONCLUSION

Although the acute toxicity of the plant under study showed its safety at a higher dose, the findings also revealed that the plant can be toxic when consumed for an extended period of time even at low dose.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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