

The effect of *Chrysanthellium indicum* aqueous extract on Biochemical and Haematological parameters in Albino rats.

Abstract

Aims: to carry out acute and sub-acute toxicity including biochemical and haematological effect of *Chrysanthellium indicum* on albino rats. Acute and sub-chronic toxicity study was carried out using albino rats.

Study design: in-vivo acute toxicity, haematological and *Chrysanthellium 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 were dosed orally with *Chrysanthellium indicum* aqueous extract at 500, 1000, 2000 and 4000mg/kg body weight, the animals were observed for mortality, clinical sign and gross pathological changes for period of 14days. For sub-acute toxicity and other parameters, four groups of five albino rats were equally dosed orally with 0 (control), 75, 150 and 300mg/kg (experimental group) body weight of aqueous extract of *Chrysanthellium indicum* for six (6) weeks. After, complete administration, the biochemical and haematological indices were investigated and determined appropriately.

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

Keywords: Haematology, Biochemical parameters, Rats, Aqueous extract, *Chrysanthellium indicum*, Toxicity.

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 important from 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.

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 alternative is encourage especially in rural areas and it has been used because of economic reason as well. Nowadays, there is wide spread interest of 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 medicinal plant that has found place in folkare.

There are large numbers of medicinal plants used as herbal preparation and only small amount of them were 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 botanical identification, accidental ingestion of cardio-tonic plants.

The plant *Chrysanthellium indicum* is the sub species of *Afroamericanium* commonly known as wild daisy in English, is a natural herbaceous plant which is part of *Asterace* 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 [5]. *Crysanthellium indicum* extract has been used for a number of medicinal conditions including recovery from influenza, for treating yellow fever. According to folkare medicine, *Crysanthellium* 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]. *Chrysanthellium indicum* flower has a strong aroma and many of previous studies focused on the essential oil of this plant.

Since *Chrysanthellium indicum* plant is widely consumed in the northern part of Nigeria to date, little or no work has been done on its acute and sub-chronic toxicity. Likewise, its effects on biochemical and haematological parameters that mediates this study to ascertain the effects of the plant aqueous extract on the later parameters in rats. The relevant haematological and biochemical parameters determinations were carried out on the blood samples immediately upon collection.

Materials and Methods.

Plants materials:- Fresh matured plant of *Chrysanthellium 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.

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

Animals: - Male albino rats weighing 140-150g 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.

Acute toxicity: - Acute toxicity of the extract was determined using the method described by Lorke [6]. Briefly, four groups of 3 male rats were dosed orally with *Chrysathellium indicum* aqueous extract at 500, 1000, 2000 and 4000mg/kg body weight, the animals were observed for mortality, clinical sign and gross pathological changes for period of 14days.

Sub-acute toxicity: twenty male rats were randomly divided into four groups of 5 rats each and administered with aqueous extract of *Chrysathellium indicum* at 0 (control), 75, 150 and 300mg/kg body weight. Administration of the extracts commenced after 10days (period to acclimatized) and lasted for the period four weeks on daily bases. After four weeks, blood sample were collected through cardiac puncture and were dispensed into EDTA container for biochemical and haematological analyses. Serum AST, ALT, ALP were 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 [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 used the level of significance

RESULTS

TABLE 1: Effect of aqueous extract of *Chrysathellium indicum* on haematological indices in rats.

GROUPS	Hb	PCV	RBC	MCV	MCHC	MCH
Normal	14.00	42.00 ^a	5.40 ^a	78.00 ^a	33.30 ^a	27.00 ^a
	±	±	±	±	±	±
	0.57	0.40	0.40	0.40	0.05	0.40
75mg/kg	13.00	39.00 ^b	5.20 ^b	75.00 ^b	33.00 ^a	25.00 ^c
body	±	±	±	±	±	±
weight	0.57	0.91	0.09	1.29	0.04	0.91
150mg/kg	12.50	37.00 ^b	4.90 ^b	75.00 ^b	33.70 ^a	25.50 ^c
body	±	±	±	±	±	±
weight	0.20	0.57	0.04	0.05	0.04	0.09

300mg/kg	14.00	42.00 ^a	5.10 ^b	82.30 ^c	33.30 ^a	27.40 ^a
body	±	±	±	±	±	±
weight	0.40	0.57	0.57	0.08	0.04	0.00

The superscripts indicate the level of significant difference (p<0.05)

TABLE 2: Effect of aqueous extract of *Chrysanthellium indicum* on biochemical parameters in rats.

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

The superscripts indicate the level of significant difference (p<0.05)

RESULT 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 medicine are rather too expensive and 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 toxic effect. Toxicity study is necessary in newly developed drug and decoction that is taken either intraperitoneal or orally.

The LD₅₀ result showed that *Chrysanthellium indicum* aqueous extract was found to be safe at 4000mg/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 4000mg/kg body weight.

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 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, 8]. The various hematological parameters investigated in this study are useful indices of evaluating the toxicity of plant extract in animals [1, 9].

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 size and hemoglobin content of individual red blood cells [7].

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 haemoglobin 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 150mg/kg body weight with value of 37.00 compared to the control group value of 42.00. The RBC has also noticed significant decrease in the group administered with 150mg/kg body weight with value 5.40 compared to that control 4.90. The haemoglobin concentration has noticed significant decrease in the group administered with 150mg/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 (Table1). 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. Results of this study showed that the MCV, is highest in the group administered with 300mg/kg body weight, with an MCV of 82.30 which is significantly different ($p<0.05$) compared to control value 78.00. The MCHC of both test and control were not found to vary significantly (Table1).

Administration of aqueous extract of *Chysanthellium indicum* has also showed a significant decrease ($p<0.05$) in plasma protein from 70.00 (control) to 68.00 (test group) administered with 300mg/kg body weight. The observed decrease in plasma protein seem to be consistent, which may be due to low albumin level cause by several hemorrhage or increase in protein breakdown or decrease in immune system [10].

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 300mg/kg body weight; Alanine transaminase (ALT) indices values decrease from 7.00 (control group) to 5.00 in the test group administered 300mg/kg body weight; Aspartate transaminase (AST) indices values decrease from 5.00 (control group) to 4.00 in the test group administered 300mg/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 enzymes level between the control and the test group signify that administration of the extract has toxic indications especially on alkaline phosphatase and alanine transaminase which were parameters found to be significant different ($p<0.05$) compared to control group [11].

Total bilirubin indices values decrease also consistently from 13.00 (control group) to 10.00 in the test group administered 300mg/kg body weight (Table 2); the changes in bilirubin, reflect the physicochemical properties of the erythrocyte surface and the plasma [7]. This is an important index for evaluating the response of animal or human body to necrotic processes [12].

Urea and creatinine indices are parameters used for indication of kidney function. Normally, blood contains small amount of urea and creatinine which varies with the amount of protein in the diet. Administration of the extract of *Chysanthellium indicum* showed significant decrease ($p<0.05$) (Table 2) which was due to poor perfusion of the kidney resulting in diminished glomerular filtration. 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 *Chysanthellium indicum* is attributed to the renal causes ureamia, the creative level tends to plateau.

CONCLUSION

In conclusion this research tends to suggest that the therapeutic and herbal tea use of *Chysanthellium indicum* plant is not safe especially when taken orally in high dose for prolonged period of time. That is to say there were some changes related to the administration of *Chysanthellium indicum* aqueous extract in rats.

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