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Acute Toxicity and Lethality of *Gladiolous psittacinus*

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Abstract:*The use of Gladiolouspsittacinusplant in traditional medicine is drastically increasing across the country Nigeria. This study was therefore conducted to evaluate the safety level of G. psittacinus using acute toxicity test (determination of median Lethal Dose; LD50) and the Brine shrimp lethality assay (BSLA). Histopathological analysis of the liver of rats orally administered with different dosages of G. psittacinus (1600, 2800 and 5000 mg/kg body weight) was also conducted. Results revealed amedian lethal dose (LD50) of the aqueous extract of G. psitticanus as 2116.60 mg/kg body weight. Similarly, the lethality concentration (LC_{50}) of methanolic extract of G. psitticanus (16.950µg/ml) was higher than the test standard, $K_2Cr_2O_7$ (5.653 µg/ml). The degree of lethality was also observed to be directly proportional to the concentration of the extracts. Histological studies also revealed that oral administration of G. psittacinus at 1600, 2800 and 5000 mg/kg body weight has the potential to cause some damages on the liver cells. Although G. psittacinus is a potential ethno-medicinal plant, its oral consumption is slightly toxic.*

Keywords:*Gladiolous psittacinus,brine shrimp, toxicity, medicinal plant,LD50, oral dose*

Introduction

Herbs or plant parts such as leaves, stem, root, bulb, fruit, flowers and bark have been used in traditional medicine to assist the healing process during illness and diseases. The use of herbs in the history of man has been reported to date back to the time of the early men who had the crudest tools as implements (Kafaru, 1999) and continues to date by different cultures (Odhiambo*et al.*, 2014). According to WHO (1993), about 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant materials. These plant materials have been used in many domains including medicine, nutrition, flavouring, beverages, dyeing, repellents, fragrances, cosmetics, smoking, and other industrial purposes (Dahanukar *et al.*, 2000; Exarchou *et al.*, 2002).As reported by Farombi(2003), the biodiversity and presence of a wide array of bioactive phytochemicals and secondary metabolites in plants have made them a source of potential therapeutic agents against various diseases.

Several plants have been used worldwide for the treatment of numerous diseases.Some of such diseases include hypertension(Adjanohoun *et al.*, 1985), cardiovasculardiseases (Ouedraogo*et al.*,2004), hepatic illness (Phillipson and Wright,1991), arthritis (Dongmo *et al.*, 2003), diabetes(Al-Ghaithi*et al.*, 2004) and malaria(Traore*et al.*, 2000). This surge in the useof herbal medicines was suggested by Okpuzor*et al.* (2009) to be due to theperceived failure of synthetic drugs in thetreatment of some chronic diseases, theside effects associated with most drugs andthe incidence of drug resistance especiallyamong the antibiotics family.In the south-western Nigeria alone, Soladoye *et al.* (2012) identified 132 plant species whichhave been tested and used by the herbalists in the treatment ofdiabetics. Similarly, Makinde *et al.* (2015) identified 107 plant species of medicinal plants used by the Badagry people of Lagos State Nigeria for thetreatment of obesity, asthma, diabetes and fertility. In Osun state, a total of 45 plant species was found to be useful in the treatment of diabetes (Mustafa *et al.*, 2014).With the wide array of plant and plant parts used in traditional medicine by the people today, most of them are yet tobe documented and evaluated for safety and efficacy (Moshi et al., 2010). One of these plants is *Gladiolous psittacinus*. *G. Psittacinus*is an herbaceous and bulbous plant belonging to the family Iridaceous whichcomprises of about 1800 species grouped in 88 genuses (Francois*et al.*, 2013). According toAmeh*et al.*(2011), *G. Psittacinus*has been used for various purposes in different parts of Nigeria.In the South West, the corms wereused in treating gonorrhoea, dysentery and otherinfectious conditions. In Hausa land a preparation madefrom Gladiolus corms called "rumanandoki" is used totreat dysentery in humans and horses.Gladiolus corms are also used in Benue State for the production of "Enyi" or "Umu" – a pleasantly sweet and very slightly sour, non-alcoholic beverage made from cereals (Ameh *et al.*, 2011).

With the increasing use of *G. Psittacinus*among various ethnic groups of Nigeria, there is the need to evaluate and document the safety and efficacy of the plant. This study was therefore conducted to evaluate the

safety level of *G. Psittacinus*using acute toxicity test (determination of median Lethal Dose; LD50) and the Brine shrimp lethality assay (BSLA).

Materials and Method

Plant Material

Samples of fresh *Gladiolouspsittacinus* were purchased from the market at Ibadan, Oyo state and were authenticated at the Obafemi Awolowo University, Ile- Ife, Osun state. The bulbs were sliced, air dried and grinded into powder.

Preparation of Crude Extract

Two hundred grams of the bulb powder were macerated in 2000ml of 80% methanol. The mixture was stirred for four days using magnetic stirrer. The extract was filtered, concentrated to dryness using rotary evaporator at a temperature of 40^{o}C. Theextract yield was 2.65% w/w.

Experimental Animals

Thirty six albino rats, weighing 180-200g were purchased from faculty of Veterinary Medicine, University of Ibadan and used for the studies. The rats were housed in metal cages in the animal holdings of Biochemistry Department, Crescent University and allowed to acclimatize for two weeks. The animals were kept and maintained under standard conditions (12h light and dark cycle and room temperature at 25^{o}C) The rats were maintained on standard rats feed (Neimeth Livestock feed) *ad libitum* and allowed freeaccess to drinking water. Experimental procedure involving animals andtheir care were employed in conformity with guidelines for care and use of laboratoryanimals and the procedure approved by the Ethical Committee of Crescent University, Nigeria.

Acute toxicity study (Determination of LD50)

Determination of the median lethal dose (LD50) of the plant extract was done in two phases. In the first phase, nine rats were divided into three groups of three rats each and were treated with the methanol extract of the plant at dosages of 10, 100 and 1000mg/kg body weight orally. They were observed for 24 hours for signs of toxicity. In the second phase, nine rats were again divided into three groups of three rats each and were also treated with aqueous extract of *Gladiolouspsittacinus*at dosages of 1600, 2800 and 5000 mg/kg body weight orally. The median lethal dose (LD50) was calculated as follows:

LD50 = √D0 X D100

Where D0 = Dosage of 0 per cent mortality

D100 = Dosage of 100 per cent mortality

Brine Shrimp Lethality Assay (BSLA)

The protocol of Miller and Tainter was adopted as described by Lilybeth and Olga (2013). Brine shrimp eggs were obtained from the ocean Nutrition, Belgium for this research work. Filtered, seawater was obtained from the bar beach Lagos state Nigeria for hatching the shrimp eggs. The seawater was put in a small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) will attract the hatched shrimp. Two days were allowed for the shrimp to hatch and mature as nauplii (larva). After two days, when the shrimp larvae are ready, 4 mL of the seawater was added to each test tube and 10 brine shrimps were introduced into each tube. Thus, there were a total of 30 shrimps per dilution. Then the volume was adjusted with seawater up to 5 mL per test tube. The test tubes were left uncovered under the lamp. The number of surviving shrimps were counted and recorded after 24 hours. Using probit analysis, the lethality concentration (LC50) was assessed at 95% confidence intervals. LC50 value of less than 1000 µg/mL is toxic while LC50 value of greater than 1000 µg/mL is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number, and then multiplied by 100%.

Histological evaluation of the liver

The rats were sacrificed and the liver harvested for histological studies using standards methods. Briefly, a section of the organ was excised and fixed in 10% neutral formal saline for twenty – four (24) hours. This was followed by dehydration of tissues in series of graded alcohol 70%, 90% and 3 changes of absolute alcohol. The tissues were then cleared in chloroform. Impregnation and embedding of tissues using molten paraffin wax was followed by sectioning in a rotary microtome. Sections were

prepared, stained with haematoxylin and eosin (H&E) stain, and then mounted using DPX (Distrene 80 dibutylphthalate Xylene) for light microscopy and photomicrography.

Results

Acute toxicity study and Determination of LD50

Photomicrographs of hepatic tissue obtained from the histopathological evaluation of the rats used in the acute toxicity study of aqueous extract of *Gladiolous psitticanus* is shown in Plate 1. At the administration of 1600 mg/kg body weight of aqueous extract of *G. psitticanus*, moderate infiltration of inflammatory cells into the sinusoids and mild infiltration of inflammatory cells into the portal triads was observed. Administration of 2800 mg/kg body weight of aqueous extract of *G. psitticanus* revealed moderate congestions of vessels, dilation and congestion of sinusoids, moderate infiltration of inflammatory cells into the portal triads and mild infiltration into the sinusoids. However, mild infiltration of inflammatory cells into the portal triad and the sinusoids was evident in the hepatic tissues of rats administered with 5000 mg/kg body weight of aqueous extract of *G. psitticanus*.

The median lethal dose (LD50) of the aqueous extract of *G. psitticanus* was calculated as 2116.60mg/kg body weight.

Brine Shrimp Lethality Assay (BSLA)

The results of the Brine Shrimp Lethality Assay, the percentage mortality and LC_{50} value are represented in Table 6. The lethality concentration (LC_{50}) of methanol extract of *G. psitticanus* (16.950µg/ml) was higher than the test standard, $K_2Cr_2O_7$ (5.653µg/ml). The degree of lethality was observed to be directly proportional to the concentration of the extracts with highest mortality recorded at 100 µg/ml for both methanolic extract of *G. psitticanus* (85% mortality) and the test standard, $K_2Cr_2O_7$ (100% mortality). However, percentage (%) mortality was lower in the methanolic extract of *G. psitticanus* than the test standard at the different levels of concentration used (6.25, 12.5, 25, 50 and 100 µg/ml).

Discussions

Although *Gladiolouspsittacinus*has been used for various medicinal purposes in different parts of Nigeria (Ameh *et al.*, 2011), this study evaluated its safety and toxic effects.In this study, the median lethal dose (LD50) of the aqueous extract of *G. psitticanus* was calculated as 2116.60mg/kg body weight. This LD50 value was described as slightly toxic according to Hodge and Sterner toxicity scale (Hodge and Sterner, 2005).Similarly, the histopathological evaluation of the liver revealed that the integrity of the liver cells may be compromised when administered with oral dose of aqueous *G. psitticanus* at toxic level. Therefore there is the need to carefully monitor the dose rate of*G.psitticanus* for man, especially in the form of locally produced medicinal drinks.

Brine shrimp lethality bioassay (BSLA) was described as the simple bioassayuseful for screening large number of extractsin the drug discovery process from medicinal plants (Alluri et al., 2006).BSLA has beenused routinely in the primary screening of the crude extracts toassess the toxicity towards the brine shrimp, which could alsoprovide possible indication of toxicity of the test materials (Urmi et al., 2012). It was used to predict cytotoxicity properties of plant extracts and also possible presence of compounds with potential anticancer activity (Moshi at el, 2010) and anti-tumor (Ganatra et al., 2012). According to Kumar et al.(2011), a number of novel antitumor and pesticidal natural products have been isolated using this method. In this present study, the Brine Shrimp lethality concentration (LC_{50}) ofmethanolic extract of *G. psitticanus* was 16.950µg/ml. According to Meyer et al. (1982), LC_{50} value of less than 1000 µg/mL istoxic while LC50 value of greater than 1000 µg/mL is non-toxic. Therefore, methanolic extract of *G. psitticanus* could be considered as containing active or potent components since its LC50 value is less than 1000 µg/mL (Lilybeth and Olga, 2013).The LC_{50}value of methanolic extract of *G. psitticanus* which is less than 20 µg/ml could make it a potential plant in the treatment of cancer. Plant extracts with LC_{50} values below 20 µg/ml have earlier been reported to have alikelihood of yielding anticancer compounds(Moshi et al., 2006; 2004). The LC_{50}value of methanolic extract of *G. psitticanus* (16.950µg/ml) recorded in this study was however lower than those reported for some other plants such as *Antiaris toxicaria* (38.2 µg/ml), *Bidensshimperi* (46.9 µg/ml), *Brideliamicrantha* (32.0 µg/ml), *Lantanatrifolia* (32.3µg/ml), *Picralimanitida* (18.3 µg/ml), *Rubus rigidus*(19.8 µg/ml), *Moringa oleifera*(26.639 µg/ml and 36. 485 µg/ml) and *Picralimanitida* (29 - 317µg/ml) (Nkya et al., 2014; Moshi et al., 2010; Owolarafe et al., 2014).

Conclusion

This study has shown that *G. psitticanus* is a potential plant for use in ethno-medicine and consumption by man and livestock. However, oral consumption of the extract of this plant is slightly toxic.

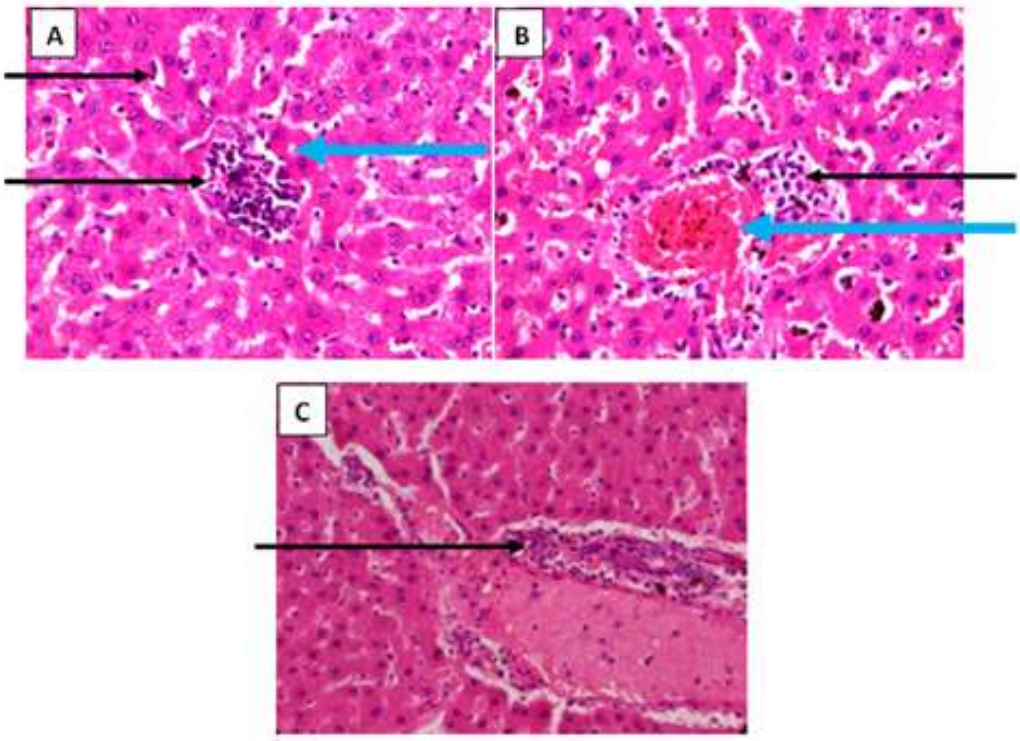

Plate 1: Photomicrographs of the hepatic tissues of rats used in Acute toxicity study of aqueous extract of *Gladiolous psitticanus*(H&E X400); A = 1600 mg/kg body weight; moderate infiltration of inflammatory cells into the sinusoids (Zone 2) (black arrow) and mild infiltration of inflammatory cells into the portal triads (blue arrow). B = 2800mg/kg body weight; moderate congestions of vessels (blue arrow), moderate infiltration of inflammatory cells into the portal triads (black arrow) and mild infiltration into the sinusoids.C = 5000mg/kg body weight; mild infiltration of inflammatory cells into the portal triad (black arrow).

Table 6: The number of shrimp nauplii that survived after treatment with the extract of *G. psitticanus*, the percentage mortality and LC_{50} Value

Plant Methanolic Extract	**Concentration µg/ml**	**Number of Surviving Nauplii (after 24 hrs)**		**Total Number of Nauplii survivors**	**% Mortality**	**LC_{50} Value (µg/ml)**
		T_1	**T_2**			
Control	Distilled water	10	8	18	10	
Standard $K_2Cr_2O_7$	6.25	4	4	8	60	
	12.5	3	2	5	75	
	25	1	2	3	85	5.653
	50	1	0	1	95	
	100	0	0	0	100	
Methanolic Extract of Baaka	6.25	7	8	15	25	
	12.5	5	6	11	45	
	25	3	3	6	70	16.950
	50	2	3	5	75	
	100	2	1	3	85	

T= number of trials for each concentration.

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