PHYTOCHEMICAL AND ACUTE TOXICITY STUDIES OF THE AQUEOUS AND METHANOL EXTRACTS OF *EMILIA COCCINEA* (SIMS) G. DON

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Abstract: *Emilia coccinea* serves various indigenous medicinal uses in traditional settings without much consideration about the possible adverse effects of the consumption of its crude extracts. The present study examined the possible acute toxic effects of the oral administration of the aqueous and methanol extracts of *E. coccinea* leaves in mice. Graded doses (1, 2, 4, 6 and 8 g/kg) of the extracts were administered to 6 groups of mice and their responses observed for 2 hrs and 24 hrs for behavioural changes and mortality respectively. The results showed that the administration of a single dose of the extracts did not produce any harmful effect or death in the animals and the mice had no negative behavioural changes. The LD_{50} was found to be greater than 8 g/kg since up to this dose no death was recorded. There was no significant change ($P>0.05$) in the mean body weight of the test and control mice. The phytochemical screening using qualitative standards revealed the presence of alkaloids, flavonoids, cardiac glycosides and terpenoids in both extracts. The results of this study suggest that the aqueous and methanol extracts of the leaf of *E. coccinea* can be considered safe within the administered doses.

Keywords: Acute toxicity, Aqueous, Methanol extracts, *Emilia coccinea*, Ethnomedicine and Phytochemistry

INTRODUCTION

Over the years, plants have been used medicinally. A large and increasing number of patients use medicinal herbs or seek their use ('O' Hara *et al*., 1998). The great economic value of medicinal plants in the African continent including Nigeria can not be overemphasized (Iwu, 1993). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga and Gomina, 2000). Plant secondary metabolites have been implicated for most plant therapeutic activities (Cowan, 1999; Ogunleye and Ibitoye, 2003). The most important of these bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds (Okwu,, 2001).

Again , increasing interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decisions about their use use ('O' Hara *et al*., 1998). However, it is pertinent to note that one undesirable property of any chemical (natural or synthetic) drug capable of producing injurious or detrimental effects on a living organism is its toxicity. Whether or not these injuries occur depend on the amount of chemical absorbed (Betram, 1998). There is therefore the need for proper scientific investigation of both beneficial and harmful effects of any medicinal plant (De Smet, 1991; Idu *et al.*, 2006).

The toxic effects caused by drugs in man have been reported to be similar to that of some other animals, a premise why animal models are used in toxicological studies (Range *et al*., 1995). Toxicity testing of new drugs on animal models can be extrapolated to identify the relative potential hazard it may have on man (Fabricant and Farnsworth, 2001). It also helps to determine the upper limits of administration of effective therapy (Sofowora, 1993). Most toxic effects of drugs occur at a predictable (usually short) time after administration; a basis for acute toxicity analysis (Curtis, 2001).

Emilia coccinea (Asteraceae) commonly called yellow or red tassel flower. is a weed of roadsides, waste places and fallow land. It is also a common weed of arable crops. It is widespread in West-Africa, especially in the forest zone. It grows to a height of 50-120 cm and reproduces from the seeds. *Emilia coccinea* serves various indigenous medicinal uses in many countries. In Nigeria, The leaf decoction was reported to be used as a febrifuge and

has a mild laxative effect (Ainslie, 1937). *E. coccinea is* said to be effective in treating, ulcers, lice, ringworm, gonorrhea, measles, cough and convulsion in children (Edeoga *et al.,*2005; Odugbemi and Akinsulire, 2006). In Tanzania, the leaves are mixed with those of *Ipomoea eriocarps*, pounded, soaked in water and the liquid used as eye drop for eye infections. The crushed green leaves can be used to treat wounds, sores and sinusitis while the dried powered leaves can also be applied to sore (Bosch, 2004). The use of *E. coccinea* as a vegetable has been reported from East Africa (Kenya, Tanzania and Malawi) and the West tropical region (Nigeria, Ivory Coast and Mali) (Burkill, 1985; Bosch, 2004). some of its bioactivities have been confirmed in the laboratory. These include antidiarrhoeal, antimicrobial and antifungal activity (Ogbebor and Adekunle, 2005; Teke *et al*., 2007; Okiei *et al.*, 2009).

Moreover, due to the folkloric usage of the plant to manage /treat diseases, and for the preparation of local medicine, it is necessary to evaluate possible risks that the consumption of the crude preparation of the leaf of this plant may pose to the health of the local people.

The present study was aimed at evaluating the immediate effect (LD50) of the oral administration of the aqueous and methanol extracts of the leaves as well as reassesses its phytochemical constituents.

MATERIALS AND METHODS

Plant Collection and extract preparation

The leaves of *Emilia coccinea* were obtained from within and around the University of Benin, Benin City, Nigeria an the plant identified by Professor M. Idu of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. The fresh leaves were air-dried under room temperature and transferred to the oven set at 40^0C for 5-10 minutes before being reduced to fine powder with the aid of a mechanical grinder and stored in a tightly covered glass jars. The methanol extraction was done by macerating 500g of the powdered leaf in 2.5L of methanol with the resultant solution filtered using Whatman filter paper No 1 after 48hours under room temperature.

700g of the powdered material was boiled in 1000L of water after which the resultant solution was filtered using filter paper to obtain the aqueous extract. Both extracts were concentrated via evaporation process using an oven set at 40^0C and the extracts stored in a refrigerator until required for use.

Animals

Forty-eight (48) Swiss albino mice of both sexes weighing between 20-25g, obtained from the Animal House of the Nigerian Institute for Medical Research (NIMR), Yaba, Lagos were used for the study. They were maintained at the Experiment Animal House of the Department of Microbiology, University of Benin, Benin City. They were kept in rat cages and fed on Top Feed Grower's Mash bought from an Animal feed store in Benin City, Nigeria and allowed free access to clean tap water. They were allowed to acclimatize for 21 days prior to the experiment after which they were thoroughly examined physically to establish their state of health and suitability for the experiments.

Administration of extract

The animals were randomly divided into 6 groups of four mice per group for the two separate treatments. The mice were fasted for 16 hours prior to the administration of the two extracts (Aqueous and methanol). Graded doses of the extracts (1, 2, 4, 6 and 8 g/kg) corresponding to groups B, C, D, E, and F were separately administered to the mice in each of the 'test' groups by means of bulbed steel needle. The control groups representing group A for each treatment was orally administered distilled water (2 ml/kg) only. The animals in all the groups were observed during the first 2 hrs after the single oral administration of the extracts for behavioural changes (i.e. locomotion, reaction to noise, tail activities and the appearance of the faeces). The mice were said to be active (locomotion) when they are roaming in the cage; have normal reaction to noise when they are unsettled on hearing a noise and the tail were taken to be normal if flexible (i.e. not rigid) because a rigid tail is a sign of anger. After the first 2 hrs of observation, all the animals were allowed free access to food and water. The mortalities were counted within the first 24 hrs and the Lethal Dose (LD_{50}) was determined. The surviving animals were further observed for two weeks for any sign of delayed toxicity. All the

experimental mice were weighted before treatment and on the last day of the experiment.

PHYTOCHEMICAL ANALYSIS

The phytochemical tests were carried out on the aqueous and methanolic extracts using standard procedures as described by Trease and Evans (1996) and Edeoga *et al.* (2005).

STATISTICAL ANALYSIS

Results were expressed as mean ± standard error of mean (S.E.M) and the level f significance between means were computed by student's t – test using SPSS 10.0 computer software package. The level of significance was determined at 0.05.

RESULTS

The acute toxicity study in mice after 24hrs – 2wks of administration of the aqueous and methanolic extracts of the leaf of *E. coccinea* are presented in Table 1 and 2. It was observed that the aqueous and methanol extracts showed no visible signs of toxicity in the animals after 24hrs. No death was recorded within the dose range used. Within the 14 days period of observation for delayed toxicity, no toxic symptom was noted.

Table 1: Acute toxicity study in mice after 24hrs – 2wks of administration of aqueous extract of the leaf of *E. coccinea*

Group	Dose (g/kg)	Initial mean weight (g) ± SEM on day 1	Final mean weight (g) ± SEM on day 14	Mortality
A	Control	21.75±0.85	23.75±1.11	Nil
B	1	21.00±0.71	24.75±0.85	Nil
C	2	20.75±0.48	25.25±0.85	Nil
D	4	21.75±1.11	24.50±1.19	Nil
E	6	21.50±0.65	24.00±0.91	Nil
F	8	22.00±1.22	25.00±1.58	Nil

Control = 2 ml/kg (distilled water) Mean ± SEM = values of 4 animals

Table 2. Acute toxicity study in mice after 24hrs – 2wks of administration of the methanolic extract of the leaf of *E. coccinea.*

Group	Dose (g/kg)	Initial mean weight (g) ± SEM on day 1	Final mean weight (g) ± SEM on day 14	Mortality
A	Control	22.50±0.65	23.75±0.48	Nil
B	1	21.50±0.65	21.75±0.48	Nil
C	2	22.00±0.71	23.25±0.65	Nil
D	4	23.25±0.85	25.00±0.91	Nil
E	6	21.00±0.71	22.50±0.97	Nil
F	8	21.00±0.71	21.50±0.87	Nil

Control = 2 ml/kg (distilled water) Mean ± SEM = values of 4 animals

Table 3 and 4 shows the behavioural changes observed during the acute toxicity study. The activities of the mice administered 1, 2 and 4 g/kg of the aqueous extract were normal, while those administered 6 and 8 g/kg, showed reduced activities. The reaction to noise, state of tail and faeces of the mice in all groups for the aqueous extract was observed to be normal when compared with control. For the methanolic extract, the activities of the mice in groups administered 1 and 2 g/kg were normal and reduced for groups given 4, 6 and 8 g/kg of the extract. The reaction to noise was reduced for the mice in the group that received 8g/kg and normal for the other groups. The mice in all the groups had normal tail (flexible) and faeces (granular excrement).

Table 3. Effect of aqueous extract of the leaf of *E. coccinea* on the behaviour of mice within 2 hrs of observation in acute toxicity study

Behavioural parameters	Dose (g/kg) and behaviour of mice					
	Control	1	2	4	6	8
Locomotion	+	+	+	+	-	-
Reaction to noise	+	+	+	+	+	+
State of tail	+	+	+	+	+	+
State of faeces	g	g	g	g	g	g

+: Normal; - : Reduce; g: Granular

Table 4. Effect of methanolic extract of the leaf of *E. coccinea* on the behaviour of mice within 2hrs of observation in acute toxicity study

Behavioural parameters	Dose (g/kg) and behaviour of mice					
	Control	1	2	4	6	8
Locomotion	+	+	+	-	-	-
Reaction to noise	+	+	+	+	+	-
State of tail	+	+	+	+	+	+
State of faeces	g	g	g	g	g	g

+: Normal; - : Reduce; g: Granular

The results of the phytochemical analysis of the aqueous and methanolic leaf extracts of *Emilia coccinea* revealed the presence of alkaloids, flavonoids, cardiac glycosides and terpenoids in both extracts (Table 5).

Table 5. Phytochemical screening of the aqueous and methanolic leaf extracts of *E. coccinea*

Chemical components	Extracts	
	Aqueous	Methanol
Alkaloids	+	+
Flavonoids	+	+
Anthraquinones	-	-
Saponins	+	-
Tannins	+	-
Cardiac glycoside	+	+
Steroid	-	+
Terpenoids	+	+
Phlobatannins	-	-

Key: + = Presence of chemical component
– = Absence of chemical component

DISCUSSION

The acute toxicity study in mice within the administered dose (1, 2, 4, 6 and 8 g/kg body weight) did not show any sign of acute toxic effect. No mortality was recorded in all the groups that were administered doses up to 8 g/kg (Table 1 and 2). This suggests a high tolerance and safety of the extract within the administered doses. It can be deduced therefore, that the lethal dose (LD_{50}) for both extracts is greater than 8 g/kg, since up to this dose no death was recorded. Similar report by Gatsing *et al.* (2010)

shows a zero percent (0 %) mortality of the aqueous leaf extract of *Alchornea cordifolia* even at much higher doses of 8, 16 and 32 gkg^{-1}. It can also be deduced from the acute toxicity results (Table 3 and 4) that no negative behavioural change was recorded within the administered doses. Generally the treated groups showed no reduction in food and water intakes throughout the period of study using their body weights when compared with the control group. No significant change was observed in the mean body weight ($P > 0.05$) (Table 1 and 2), suggesting a normal appetite for all the animals.

The phytochemical tests carried out on the aqueous and methanolic leaf extracts of *E. coccinea* which revealed the presence of alkaloid, flavonoid, cardiac glycoside, saponins, tannins, and terpenoid was in conformity with the work done by Edeoga *et al.* (2005). The absence of anthraquinones and phlobatannins in both extracts was observed. Steroid which was found present in the methanolic extract was absent in the aqueous extract which contradicts earlier report by Edeoga, *et al.* (2005) that steroid was present in the leaf of *E.coccinea.* These bioactive compounds have been known to show medicinal activity as well as exhibit physiological activity (Sofowora, 1993). Stephen *et al.* (2009) reported that tannins have antioxidant properties while Steroids which are of important interest in pharmacy have been implicated as potent starting material in the synthesis of sex hormones (Okwu, 2001). The presence of these phytochemicals in the leaf suggests that the plant is pharmacologically active which supports claims from the ethnomedical uses.

CONCLUSION

Low toxicity is one property that is vital in drug formulation and administration. This study has shown that the aqueous and methanol extracts of the leaves of *E. coccinea* have low toxicity ($LD_{50} > 8$ gkg^{-1}). The extracts of the leaves may be relatively safe when taken orally for a short period of time. The presence of secondary metabolites in the leaf of *E. coccinea* is thought to be probably responsible for its medicinal properties. However, further studies should be carried out to evaluate the toxicity of *Emilia coccinea* using long-term study protocol as well as determining the actual biochemicals present in the plant.

ACKNOWLEDEGEMENTS

We are grateful to Mr Dickson O. Uwaya , Mr Emma Ikpefan, Mrs Adesina of Pharmacognosy Department, University of Benin, Nigeria and Late Mr Igboke for their technical assistance.

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