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Full Length Research Paper

Acute toxicity studies of the aqueous root extract of *Lawsonia inermis* Linn. in rats

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The study evaluated the lethal dose LD $_{50}$ of the aqueous root extract of *Lawsonia inermis*; a potent uterine vaso constrictor, usually taken by pregnant women in the first two months of pregnancy to induce spontaneous abortion. The study was carried out to determine the lethal dose of the aqueous root extract to ascertain its safety using Wister rats. Five groups of rats (4 rats per group; including two pregnant females) were administered intraperitoneally (i.p.) doses of the stock aqueous root extract (0.3 g/ml) volumes corresponding to 200,400, 800, 1200 and 1600 mg/Kg Body Weight (BW). The control group was given 0.5 ml of distilled water and observed for 7 to10 days. Various clinical symptoms (physiological changes) like dizziness, loss of appetite, partial paralysis, temporary amnesia and spontaneous abortion in the included pregnant females; were visibly observed in groups treated with 800 to 1600 mg/kg, while groups with 200 to 400 mg/Kg BW and the control remain active and healthy. No mortality was recorded in any of the groups. Conclusions from the result indicate delayed toxicity after ip route administration of the extract at various concentrations. It demonstrated that the aqueous root extract of the *L. inermis* although, it is active in inducing spontaneous abortion; is slightly toxic and is safe for therapeutic purposes within the dose range. This activity could be related to the various phytochemical compounds proven to be present in the aqueous extract in preliminary phytochemical analysis.

Key words: LD_{50}, spontaneous abortion, Wister rats, intraperitoneally, temporary amnesia, mortality, toxicity.

INTRODUCTION

The use of natural products together with their therapeutic properties is as ancient as human civilization (Hernedez et al., 2002). For long time, natural plant and animal products were the main source of drugs. In recent years there has been growing interest in alternative therapies and therapeutic use of natural products, especially those derived from plant. It is estimated that about 64% of the total world population depend on traditional medicine and nearly 8000 species of plants have been recognized as of ethno-botanical importance (Rates, 2001). However, the potential use of plant as source of new drugs is still poorly explored. Of the estimated 250,000 to 500,000 plant species, only a small percentage has been investigated phytochemically and an even smaller percentage has been properly studies in terms of their pharmacological properties. The level of toxicity of particular plant species is achieved through the determination of its lethal dose (LD). A LD is an indication of the lethality (ability to kill) of a given substance (Fulder, 1988). The research is aimed specifically to determine the LD_{50} value (level of toxicity) of the aqueous root extract of *L. inermis* and the screening of phytochemicals

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Abbreviations: i.p, Intraperitoneally; **BW,** body weight; **LD,** lethal dose; **AGEs,** advanced glycated end products; **HFS,** hand-foot syndrome.

Table 1. Results of phytochemical analysis.

2^0 Metabolite group	Inference
Reducing sugar	+
Tannins	+
Flavanoids	+
Saponins	+
Alkaloids	+

Key: (+) Present, (-) Absent.

presents in the extracts.

MATERIALS AND METHODS

Sample collection and extraction

Roots of the plant material (*L. inermis*) were collected, air dried and pounded into powder. The pounded roots (250 g) were macerated in distilled water (800 ml) and soaked for 7days; decanted and filtered.

The filtrate was evaporated using water bath and dried in an oven at 40°C. It was then weighed and stored in sterile bottles prior to use (Wannag and Bichi, 2004).

The animals

Male and pregnant female Albino rats (100 to110 g) kept at the animal house of the department of biological science, Bayero University, Kano Nigeria were used.

The animals were maintained under standard environmental condition and had free access to feed (Pfizer products, Lagos, Nigeria) and water.

Phytochemical screening

The aqueous extract was phytochemically screened for the qualitative detection of reducing sugars, tannins, alkaloids, flavonoids; using standard techniques (Sofowora, 1984; Ciulei, 1994; Brain and Turner, 1975).

Lethal dose (LD_{50}) determinations

An initial study was conducted to determine the dose range of the extract to be used for the study. Five groups of rats (4 rats per group consisting 2 males and 2 pregnant females) were administered i.p. doses of the stock aqueous root extract (0.3 g/ml) volumes corresponding to 200,400, 800, 1200 and 1600 mg/Kg BW. The control group were given 0.5 ml of distilled water and observed for 7 to10 days. Any sign of change in mental or physical activities were recorded and the number of death in each group (if any) within 24 h was recorded for 10 (ten) days. The groups that do not record any mortality within 7 days are further observed for 3 more days for any sign of delayed toxicity (Wannang and Bichi, 2004).

RESULTS

Aqueous extraction

Using 250 g of the powdered roots of *L. inermis*, the aqueous extract obtained was 11.3 g, corresponding 4.52% yield. This clearly indicates that most the organic components in the plant's root are not water soluble or strongly polar compounds

Phytochemical screening

Phytochemical analysis of the aqueous root extract of the plant indicates the presence of alkaloids, tannins, reducing sugars, saponins and flavonoids. The results are shown in Table 1.

Lethal Dose (LD_{50})

Rats administered with doses ranges from (200 to 1600 mg) are observed for any clinical symptoms changes in physical activities, mortality rates or spontaneous abortion (in the case of the pregnant female rats) and recorded as shown in Table 2.

DISCUSSION

Phytochemical analysis

Result of phytochemical analysis from Table 1 indicates the presence of reducing sugar, flavonoids, tannins, saponins, and alkaloid in the aqueous root extract of the plant. This goes in conformity with the work of Ibrahim et al. (2010), which proved the presence alkaloids, saponins, steroids, cardiac glycosides, flavonoids, tannins and reducing sugars in the aqueous root extract of *L. inermis.* Certain other phytochemical are also present but do not show their presence due to the fact that not all organic molecules are soluble in water (Jallad and Espada-jallad, 2008). Therefore, the activity of this plant against all the test animals is probably due to these secondary metabolite groups in addition to the probable contribution of other bioactive components which is conformity with the work of Fatope and Takeda (1988). Their investigation revealed biochemical components; lawsoniaside and laliodise isolated from the ethanol extract of the leaves of *L. inermis* as new phenolic glucosides but no bio activity was reported. Nevertheless, the plant is used in the treatment of skin diseases such as an anti helminthic and astringent and in the control of perspiration and partly menstrual cycles. The extract have also been found to contain lawsone and gallic acid, and showed significant inhibition of Advanced Glycated

Table 2. Results of LD_{50} analysis.

No. of days		1	2	3	4	5	6	7	8	9	10
Group	**Dose (mg/Kg)**	**Clinical symptoms**									
1	200	No	No	No	No	No	No	No	No	No	No
2	400	No	No	No	No	No	No	No	No	No	No
3	800	No	L,W	L,W	L,W	P,TW,S	R,W	R	R	R	R
4	1200	No	L,W	L,W	P,TW,S	P,TW	R,W	R,W	R	R	R
5	1600	No	L,W	P,TW,S	P,TW	P,TW	C,P	R,W	R,W	R	R
control	-	No	No	No	No	No	No	No	No	No	No

Key: No = No visible abnormal change in health or behaviour, L, W = Laziness and Weakness, P, TW, S = Paralysed and totally weaken, Spontaneous abortion, C, P = collapsed and paralysed, R,W = Regaining consciousness but still weak, R = Fully recovered and Normal behaviour returns.

End products (AGEs) formation (Sultana et al., 2009). Structural determination of the dye molecule, Lawsone; have shown it to be 2-hydroxy-1, 4 naphthoquinone (Polat et al., 2009). This molecule has an affinity for bonding with protein and thus has been used to dye skin, hair, finger nails, leather, silk and wool. It also has the ability to cool down the human body temperature. It aids in healing acne, boils, burns bruises, fever, heat rashes, skin rashes warts and athletes foots (Polat et al., 2009; Jovanovich and Slavkovic-jovanovic, 2009). *L. inermis* is widely used traditionally in Arabic and Hindu cultures as a hair colorant and as a dye for decorating the nail or making temporary skin tattoos (Sauriasari et al., 2007). In traditional Hausa society, in the villages, an aqueous decoction of the roots of *L. inermis* is taken by pregnant women in the first two months of the pregnancy to procure spontaneous abortion of an unwanted pregnancy (Dalziel and Hutchinson, 1958). Other reported activities of the plant that could be attributed to the observed phyto compounds includes the wound healing activity by Nayak et al. (2007), and preventive drug against Hand-Foot Syndrome (HFS) caused by Capecitabine; by Yucel and Guzin (2008).

LD_{50} toxicity test

The result obtained from the study (Table 2) show that the aqueous root extract of *L. inermis* is slightly toxic; causes delayed toxicity (with no death) which is accompanied by clinical symptoms in the body system of the rats for more than 7 days after i.p injection. The Clinical symptoms include paralysis, total collapse of the body, weakness, laziness and loss of appetite. Most importantly, spontaneous abortion of the foetus in the pregnant female administered doses ranging from 800 to 1600 mg/Kg BW, even though, no death in the pregnant females was observed, and they later regained their composure and normalcy. Previous study of the methanol extract was found to be most effective in inducing abortion in mice, rats and guinea (Munday et al., 1999a; Munday et al., 1999b). The effect is approximately dosage dependent. This confirms its use in ethno medicine for procurement of abortion in humans in some part of Nigeria. The results of the animal experiments support the methanol extract effectiveness as an abortant, due to its maternal toxic effect.

According to Munday et al. (1991), Lawsone is considered to be the active component in henna, responsible for its haermotoxicty (Sauriasori et al., 2007). In support of this view when administered to rats as evidenced by decreased haematocrits; reduces level of haemoglobin, and increased spleen/liver weight ratio. On the basis of these observations, Lawsone can be suggested as responsible for the observed physiological changes. It has been shown to induce an oxidant stress related to haemolytic anaemia when administered to rats (Munday et al., 1991). However unlike other 1, 4 napthoquinones, Lawsone does not appear to elicit oxidant stress type responses when incubated directly with rat red cells (Saldanha, 1995), suggesting a distinctly different mode of action.

Conclusion

The LD_{50} (acute toxicity) study showed the extract to be slightly toxic due to fact that it shows delayed toxicity. It was also observed that the loss of body balance, paralysis and other physiological changes are due to oxidative injury within the red cell of the rat that induces haemolytic anemia. In conclusion, although, it proves to be an abortant; that is, induces spontaneous abortion at therapeutic doses, it is safe as no record of mortality is recorded. Asuch, more work is in progress to isolate, purify, characterize and structurally elucidate the bioactives from the aqueous root extracts of the plant.

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