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Original Article

FETAL TOXICITY OF HYDROALCOHOLIC EXTRACT OF *AGERATUM CONYZOIDES* L. LEAVES (ASTERACEAE) IN RATS

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ABSTRACT

Objective: *Ageratum conyzoides* is known to possess pharmacological and therapeutic pro perties in Africa. Some pyrrolizidine alkaloids, chemicals known to induce fetuses toxicity, have been identified in *A. conyzoides.* This study aims to evaluate the fetal toxicity of *A. conyzoides.*

Methods: Mated females were randomly assigned to three experimental groups of 8 animals each. Pregnant rats received orally 500 or 1000 mg/kg of 80% hydroalcoholic extract of *A. conyzoides*, daily from the 17th to the 20th day of gestation. On day 21 of pregnancy, the females were sacrificed. Laparotomy was performed and uterine horns were removed. The number of implants, resorptions, and dead and live fetuses was then recorded. The ovaries were also observed and the corpora lutea were counted.

Results: No visible signs of toxicity were observed in females and their pups throughout the study period. However, *A. conyzoides* (500 and 1000 mg/kg) caused a significant decrease ($p<0.01$) of fetal weight compared with the control. For the implantation, resorption and morta-lity there was no significant difference between groups.

Conclusion: The administration of hydroalcoholic extract of *A. conyzoides* to female rats in late pregnancy is toxic to the fetuses. This fetal toxicity can be due to the oxidative stress induced by pyrrolizidine alkaloids present in this plant.

Keywords: *Ageratum conyzoides*, Pyrrolizidine alkaloids, Fetal toxicity.

INTRODUCTION

Pyrrolizidine alkaloids (PAs) are a class of hepatotoxic, carcinogenic, genotoxic, teratogenic and sometimes pneumotoxic phytochemicals. Several reports in the literature prove the fact that PA-containing plants are hazardous for livestock [1]. For a long time, it has also been well established that humans can be affected by toxic PA [2-5]. It has been reported that about 3% of the world flowering plants contain toxic pyrrolizidine alkaloids. They are found in more than twelve higher plant families, among which three families, Boraginaceae, Asteraceae, and Fabaceae, contain most toxic pyrrolizidine alkaloids [5].

Ageratum conyzoides is an annual herbaceous plant belongs to Asteraceae family. Some pyrrolizidine alkaloids have been identified in this plant [6-9]. *A. conyzoides* is known to possess a broad spectrum of medicinal, pharmacological and therapeutic properties [10, 11]. In African traditional medicine, *A. conyzoides* has been used as purgative, febrifuge, anti-ulcer and wound dressing [12, 13].

Traditional communities in India use this plant as a bactericide, antidysenteric, and antilithic [14, 15]. In Togo, *A. conyzoides* is reported to treat fever, measles and snake bites [12]. In addition to its popular use for skin diseases, wound healing, diarrhoea, pain associated with navel in children; in Nigeria [14] it was reported in the treatment of HIV/AIDS [11]. Our previous studies have shown that the limit dose of 5,000 mg/kg did not cause any mortality or any signs of acute toxicity in rats tested [12].

In 28 days subchronic test, the result did not show any treatment–related abnormalities except the relative weight of the liver where there was a significant increase [12]. This increase's in the relative weight of the liver could be attributed to a toxic action or not. It's could be due to an inflammation or an increase of enzymes or peroxysomes synthesis [16-18]. We have shown also the genotoxicity of total alkaloids of *A. conyzoides* with Alkaline Comet Assay [19]. Pyrrolizidine alkaloids are also known to induce fetal toxicity [20, 21].

Several authors believe that pyrrolizidine alkaloids are toxic to fetus [20, 21]. Therefore, we aim in this study to evaluate the fetal toxicity of the 80 % hydro alcoholic extract of *A. conyzoides* leaves.

MATERIALS AND METHODS

The study was conducted following an approved animal use protocol at from the institutional Ethical Committee for Teaching and Research (ref no. CNCB-CEER 2801/2010). Animal care and handling are conducted as conformed to accepted guidelines [22, 23].

Chemicals

All chemicals were purchased from Sigma Chemicals Co., Hymedia (India).

Collection and extraction of plant materials

A. conyzoides was collected from Djagblé (Togo) in July 2012. It was identified by Prof Kouami Kokou from the Botany department of University of Lome (Togo) and a voucher specimen was kept in the herbarium of the Laboratory of Botany and Plant Ecology (Faculty of Science/University of Lome) under the reference N° 10553 of Tchala.

Preparation of hydroalcoholic extract

A. conyzoides leaves were washed in running water, then dried and ground to a powder. The powder was soaked in ethanol-water (80-20: v/v) for 72 h with manual discontinue agitation. The solution was filtered and evaporated using a rotary evaporator (yield: 12.34). The study was conducted in the Department of Animal Physiology, Faculty of Sciences and in the Department of Toxicology, Faculty of Health Sciences, University of Lome, Togo.

Experimental procedure

Animals

Female Wistar rat (150-200 g) provided by the department of Animal Physiology of University of Lome (Togo) was used. They were

housed in a standard environmental condition and fed with rodent standard diets and water ad libitum.

Fetal toxicity test

Female rats were mated with fertile males and the presence of spermatozoa in the vagina or seminal plug was considered as day 1 of pregnancy. The mated females were randomly assigned to three experimental groups of 8 animals each [24]. Group 1 received 10 ml/kg of distilled water and served as control. Group 2 and 3 received *A. conyzoides* 80% hydroalcoholic extract at 500 mg/kg body wt. and 1000 mg/kg body wt. respectively from the 17th to the 20th day of gestation. Doses of 500 and 1000 mg/kg are therapeutic doses used in our previous study [25].

Animals were observed at least twice daily for morbidity and mortality. Body weight of animals was evaluated daily. On day 21 of pregnancy, the females were sacrificed under ether anesthesia. Laparotomy was performed and uterine horns were removed. The number of implants, resorptions, and dead and live fetuses was then recorded. The ovaries were also observed and the corpora lutea were counted. A longitudinal section was made in order to record the number of dead and live fetuses (those that responded with movement, when touched with tweezers), according to Gleich and Frohberg [24].

To study the reproductive capacity of the female rats the implantation (number of implantations/number of corpora lutea x 100), resorption (number of resorptions/number of implantations x 100) and mortality indexes (number of dead fetuses/number of fetuses x 100) were calculated. The fetuses were weighed and examined for macroscopic external malformations.

Statistical analysis

The results are expressed as mean±standard error of the mean (SEM). Statistical analysis was performed by one way analyse of variance (ANOVA) with Tukey test to evaluate significant differences between groups. Values of $p<0.05$ were considered significant. All statistical analysis was carried out using the Instat Statistical package (Graph Pad software, Inc. USA).

RESULTS

All rats from control and treated group survived throughout the study period. No visible signs of treatment such as changes in respiratory, circulatory, autonomic and central nervous system, behavioral pattern were observed in females and their pups throughout the study period. However, *A. conyzoides* (500 and 1000 mg/kg) caused a significant decrease ($p<0.01$) in fetal weight compared with the control (table 1). For the implantation, resorption and mortality there was no significant difference between groups.

Table 1: Effect of 80% hydroalcoholic extract of *A. conyzoides* L. leaves on some fetal toxicity parameters

Parameter	Dose (mg/kg/day)		
	0	500	1000
Body weight on 17th day (g)	172±12	167±13	174±20
Body weight on 21th day (g)	181±14	178±13	191±13
Corpora lutea (no.)	10±1	8±1	11±1
Ovary weight (mg)	0.12±0.01	0.10±0.01	0.14±0.02
Live fetuses (no.)	8±1	8±1	8±1
Dead fetuses (no.)	0	0	0
Fetuses weight (g)	2.9±0.02	2.36±0.05 **	2.42±0.13 **
Placenta weight (g)	0.33±0.02	0.30±0.02	0.29±0.01

Values were expressed as mean±SEM; N.= number; Data were analyzed by one way ANOVA followed by Tukey multiple comparison test; **P<0.01 (control group versus extract group).

Table 2: Effect of 80% hydroalcoholic extract of *A. conyzoides* L. leaves on some reproductive toxicity indexes.

Parameter	Dose (mg/kg/day)		
	0	500	1000
Implantation	80±0.02	100±0.00	72±0.02
Resorption	0±0.00	0±0.00	0±0.00
Mortality	0±0.00	0±0.00	0±0.00

Values were expressed as mean±SEM; N.= number; Data were analyzed by one way ANOVA followed by Tukey multiple comparison test. There was no significant difference between groups.

DISCUSSION

When testing possible fetal toxic effects of a specific substance, it is necessary to establish if these effects are due to direct action on the fetus or an indirect action through the maternal organism that could secondarily interfere with the fetus. There are many ways of valuating maternal toxicity; among them, the clinical criteria suggested by Khera [26] and by Mason and Kang [27] are food intake, body weight, piloerection, locomotor activity, diarrhoea and vaginal bleeding.

A. conyzoides induced feto toxicity at 500 and 1000 mg/kg. Fetal toxicity may be due to direct or indirect action. For example, a decrease in blood sugar or anorexia can decrease fetal weight [28]. Indeed, the fetus requires energy and nutrients for their development. A decrease of these elements can logically lead to reduced fetal weight. In this study, we have evaluated the hypoglycemic effect of 80% hydroalcoholic extract of *A. conyzoides* at 500 mg/kg and 1000 mg/kg. The results have shown that *A. conyzoides* do not decrease the blood glucose level (data not shown). Our previous study has shown that, the administration of *A. conyzoides* for 28 days does not induce a decrease of the blood glucose and the weight of the rats [29]. Then the decrease of the fetal weights cannot then be explained by anorexia. Our previous study has also shown that *A. conyzoides* hydroalcoholic is cytotoxic and can induced oxidative stress due to its contain in pyrrolizidine alkaloids [6, 29]. Pyrrolizidine alkaloids are known to induced oxidative stress [29] and several authors believe that oxidative stress may cause fetal toxicity. Oxygen radicals or reactive oxygen species (ROS) act as primary or secondary messengers to promote cell growth or death. Many instances demonstrate an important direct role of ROS in the development because redox status regulates key transcription factors that influence cell signaling pathways involved in proliferation, differentiation, and apoptosis. Therefore, oxidative stress can alter many important reactions that affect embryonic or fetus development both positively and negatively [30-32]. It is known that the implantation index, that correlates the corpora lutea with the number of implantations in the uterine horn, is an indicator of the reproductive

capacity success. The data obtained in this study indicate that the number of blastocysts implanted was similar between control and treated groups. The resorptions index indicates the failure in the progress of the embryo development. As the resorption index was similar in all experimental groups, it may be assumed that experimental group failures in the progress of embryo development are similar.

CONCLUSION

The result of this study concluded that the administration of 80% hydro alcoholic extract of *A. conyzoides* to female rats in late pregnancy is toxic to the fetuses, as it has caused a significant ($p<0.01$) reduction of the weight of fetuses. This fetal toxicity can be due to the oxidative stress induced by pyrrolizidine alkaloids present in this plant.

CONFLICT OF INTERESTS

Declared None

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