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Subchronic Toxicological Evaluation of *Eupatorium Aschenbornianum* in Wistar Rats

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Abstract: *Eupatorium aschenbornianum is a plant used for the treatment of gastric ulcer, therefore, the present study aimed to evaluate the safety of this dehydrated plant in order to find the appropriate doses for further human studies. Male Wistar rats were fed with different incremental doses dehydrated plant from 200 to 1000 mg/kg bw in a subchronic test during 45 days. Animals were sacrificed, then the clinical, hematological and biochemical changes were assessed and compared with the control group. The behavior, weight and feed consumption of the rats was normal. Also the biochemical parameters were normal except the uric acid and glutamic oxaloacetic transaminase showing elevated levels in the group treated with the highest dose. The dose of 800 mg/kg bw could be considered as no observed adverse effect level and could be used in further clinical trials in a safe manner.*

Keywords: *Subchronic toxicity, Eupatorium aschenbornianum, Toxicology, Rats.*

I. Introduction

In Mexican culture, there is a traditional use of medicinal plants. The genus *Eupatorium* belongs to the Eupatorieae family, one of the 13 families of Asteraceae, comprising around 1,200 species that are mainly distributed in regions with a tropical climate [1]. *Eupatorium aschenbornianum* commonly named axihuitl is used for gastrointestinal illness like gastric ulcer [2]. Other researchers have shown its antimicrobial and fungicidal activity in several strains [3]. Plant-based products that have been used in traditional medicine have little evidence available on their potential toxicity; recent studies have indicated that many medicinal plants applied in traditional medicine showed adverse effects [4]. Before evaluating the possible therapeutic effects of *E. aschenbornianum* in a clinical study, it is necessary to conduct preclinic toxicological studies to determine optimal doses for the study in humans. Therefore, the aim of the present study was investigate the subchronic (45 days) toxicity of *E. aschenbornianum* in Wistar rats.

II. Material And Methods

2.1. Plant material

E. aschenbornianum plants were obtained from Morelos state in Mexico. The branches were broken off and dried at room temperature until the moisture content reached ~7%. Then they were chopped and pulverized with the aid of a blender. Finally it was sieved through 30-mesh and vacuum packaged until use.

2.2 Animals

Male Wistar strain rats of 7-8 eight weeks of age and 180-200 g weight were purchased from the Zooterio of the University of Guadalajara. They were housed under standard conditions at 23 ± 2°C and 44–55% relative humidity with light and dark cycles of 10 and 14 h, respectively. The experiments were conducted in accordance with the National Insitute of Health "Guide for the Care and Use of Laboratory Animals" (National Institute of Health, 1985) and they also were handled following the animal care guidelines in accordance with regulations enacted by the Federal Government of Mexico (NOM-062-ZOO-1999).

2.3 Subchronic toxicity study

Animals were divided into six groups of ten animals each. The *E. aschenbornianum* flour was mixed with standard rodent diet to prepare doses of 200, 400, 600, 800, 1000 mg/kg bw. The sixth group received the standard rodent diet without *E. aschenbornianum* flour as control. The food consumption, water intake, behavior, body weight gain as well as general toxicity signs were observed daily for 45 days.

2.4 Biochemical parameters

All rats after the last observation were sacrificed and blood samples were obtained by intracardiac puncture for biochemical analyses [5, 6], including urea, creatinine, uric acid, cholesterol, low-density lipoprotein, triglycerides, total protein, albumin, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase, gamma glutamil transpeptidasa, and bilirubin.

III. Results and Discussion

3.1 Subchronic study

In the assessment of long-term effects of *E. aschenbornianum* consumption in rats. For the five animal groups treated a different doses (200, 400, 600, 800, 1000 mg/kg bw), no alteration of behavior such as immobility, vocalization, or lethargy were observed regarding control group throughout the study. There was no difference between the water and food consumption of *E. aschenbornianum*-treated groups and control group. The average weight gain in the animals of all groups treated with *E. aschenbornianum* was 35.47 g, similar to the control (35.1 g). No death was observed in any of the groups at the end of the experimental period, and no abnormal gross findings were observed in any of the treated animals.

3.2 Biochemical study

Treated groups with 200, 400, 600, and 800 mg/kg bw of *E. aschenbornianum* no showed changes in biochemical parameters, such as uric acid, GOT, GPT, bilirubin, alkaline phosphatase, and/or urea regarding control group (Table1). However, the biochemical analysis showed a significant increases in uric acid and GOT levels, and a significant decreases in the total bilirubin and GPT values at dose of 1000 mg/ kg regarding control group (Table 1).

Table 1. Biochemical parameters of Wistar rats after 45 days treatment with *E. aschenbornianum*.

Parameter	Dose group					
	200 (mg/kg)	400 (mg/kg)	600 (mg/kg)	800 (mg/kg)	1000 (mg/kg)	Control
Urea (mg/dL)	57.03	60.28	56.4	47	45.14	51.04
Creatinine (mg/dL)	0.967	0.829	0.873	0.75	0.757	0.79
Uric acid (mg/dL)	1.5	1.2	1.3	1.4	1.8	1.07
Cholesterol (mg/dL)	81.92	67.53	82.03	64.09	61.47	68.82
Triglycerides (mg/dL)	130.5	129.2	80.8	128.7	115.2	93.4
Total protein	7.22	6.03	6.59	6.23	5.59	5.96
Albumin (g/dL)	4.4	3.69	4.15	3.64	3.33	3.32
GOT (IU/L)	129.4	120.46	128.6	125.39	131.89	116
GPT (IU/L)	70.6	71	69	61.9	59.8	70
Alcaline phosphatase (IU/L	475	409	355	521	452	356
Gama Glutamil Transpeptidasa (IU/L)	2.5	1.889	1.7	3	3	2.2
Total bilirubin (mg/dL)	0.16	0.19	0.26	0.26	0.1	0.23
Direct bilirubin (mg/dL)	0.03	0.05	0.03	0.04	0.04	0.04
Indirect bilirubin (mg/dL)	0.13	0.14	0.22	0.21	0.1	0.05
LDL (mg/dL)	5.32	4.97	5.2	5.7	4.38	4.6

Many traditional medicine plants are used, however only in few plants have been tested their safety. Before developing a product based on plants used in complementary and alternative medicine is required thorough safety and efficacy evaluation due to their use around the world. *E. aschenbornianum* commonly called as axihuitl belongs to the genus *Eupatorium*. Its chemical constituents include mainly sesquiterpene compounds, monoterpene derivatives, diterpenes, triterpenes, flavonoids and pyrrolizidine alkaloids [7, 8]. This genus has showed antimicrobial activity [9]. *E. aschenbornianum* is used for gastrointestinal illness in traditional medicine, and it has recently been reported its antimicrobial and antifungal activity, but this is the first report on subchronic toxicity for this plant.

The present study after 45 days of treatment did not show toxicity signs for any *E. aschenbornianum*-treated group. Subchronic toxicity did not show changes in the biochemical parameters in the treated groups at doses of 200, 400, 600, and 800 mg/kg bw, but in the treated group at dose of 1000 mg/kg bw it was observed elevated levels of glutamic oxaloacetic (GOT) and uric acid, and decreased levels of total bilirubin and glutamic pyruvic transaminase (GPT). The GOT increase indicates that *E. aschenbornianum* components at dose of 1000 mg/kg may be acting on the kidney causing accumulation of uric acid. Meanwhile the reduction in GPT and bilirubin are indicative of hepatic damage [10].Although traditional and herbal medicines are perceived as safe, they can cause severe consequences such as liver damage [11, 12, 13]. It has been reported that other species belong to Eupatorium genus cause pneumotoxic and hepatotoxic effects on different species of animals [14]. Recently, He et al. [2015] showed that *E. adenophorum* induces splenocyte apoptosis via the activation of the mitochondrial apoptosis pathway in goat splenocytes [15].

IV. Conclusion

E. aschenbornianum branches showed low oral toxicity since no animal death was detected at doses from 200 to 1000 mg/kg. However, the highest dose promoted biochemical alterations, which indicates that caution is required regarding its use. Based on evidence of this study, the no observed adverse effect level of *E. aschenbornianum* is considered to be 800 mg/kg/day for male rats.

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