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Acute Toxicity Study of *Anvillea Radiata* Aqueous Extract in Albino Rats

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ABSTRACT

Despite the popular use and the biological effects of *Anvillea radiata*, there are no studies or data about its safety. The aim of the present study was to assess the acute toxicity of *A. radiata* aqueous extract in vivo. A single dose of 0.25, 0.5, 1, 1.5, 2.5 or 5 g/kg was administered to female rats by gavage. Body weight gain, general behavior and mortality were monitored for up to 2 weeks. Selected biochemical parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and blood creatinine levels were determined, as well as, liver and kidney histology. Results showed no significant changes in body weight gain and organ indexes with no mortality during the experimentation period. A significant increase in AST and ALT levels were observed in 2.5 and 5 g/kg extract treated groups, and a significant decrease in BUN and creatinine levels in 1, 1.5, 2.5 or 5g/kg extract treated groups compared to control. Microscope examination of liver sections showed several anomalies in rats exposed to high concentrations (1.5, 2.5 and 5 g/kg) including fatty changes, glycogen accumulation and ballooning degeneration hepatocytes. Renal parenchyma anomalies were also observed in rats exposed to 2.5 and 5g / kg of plant extract including shrunken renal corpuscles with marked hypo-cellularity and atrophied glomeruli, large interstitial space, and renal tubules with dilated lumina which appear completely distorted. From this study, it can be concluded that *Anvillea radiata* aqueous extract at high concentration (higher than 1 g /kg b.w.) may be toxic and affect sensitive organs function such liver and kidney.

Keywords: *Anvillea radiata*, Acute toxicity, Biochemical parameters, Histology.

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INTRODUCTION

Anvillea radiata is a plant belonging to the Asteraceae family that grows in northern Africa and particularly in the two Maghreb countries, Algeria and Morocco[1]. *A. radiata* is a small woody shrub, densely branched, 20-50 cm high. The leaves are green-gray, small, and roughly triangular, with a large petiole and strongly toothed limb. The big solitary capitules have a diameter of 3-5 cm, with long ligules. The flowers are all yellow-orange, the outside one 25 mm long. It usually flowers in spring, but can flower throughout the year. It is widely used in traditional medicine for the treatment of dysentery, gastric-intestinal disorders, and chest cold[2]. It is used by populations and traditional healers in infusions or decoctions of the leaves and flowers to treat a variety of ailments such as chest cold, gastrointestinal, liver and pulmonary disorders, and postprandial hyperglycemia[3]. It has been reported to have antifungal activity[4], as well as antitumor activity[5]. Anti-inflammatory and anti-tumor activities of *A. radiata* are attributed largely to the presence of Germacranolides. Six germacranolides have been isolated from *anvillea.* They have been identified as 9α-hydroxyparthenolide, 9β-hydroxyparthenolide, 9β-hydroxy-1β, 10 α-epoxyparthenolide, 9α-hydroxy-1β,10α-epoxyparthenolide , 8 α,9 α-epoxyparthenolide , parthenolid-

9-one, and cis-parthenolid-9-one[2]. Phytochemical analysis revealed also, the presence of some chemical groups such as volatile oils, fatty acids, aglycones, glycosides, tannins, flavonoids, anthracénosides, emodols, saponins, free quinones, anthraquinones, alkaloids, sterols and triterpenes[4]. These compounds are well known for their beneficial effects on human health and their ability to limit damage due to oxidative stress due to radical species[6, 7]. However, despite the traditional use and the reported benefic effects of *Anvillea radiata*, there are no reports regarding its safety or toxicity. Therefore, this study aimed to assess the effects of acute administration of *A. Radiata* aqueous extract, at different concentrations (0.25, 0.5, 1, 1.5, 2.5 or 5 g/kg bw.), on the function of sensitive organs such as liver and kidneys.

MATERIALS AND METHODS

Preparation of *Anvillea radiata* aqueous extract

The aerial part of *Anvillea radiata* was washed in running water, dried and powdered. 10 g of the powder was boiled in 100 mL (100 °C) water bath reflux system for 15 min and then filtered with N°1 Whatman Millipore filter paper. The filtrate is combined, centrifuged at 4000 rpm for 20 min, the supernatant is concentrated to dryness using a rotary evaporator and the residue is stored at 4 ° C until use. This procedure was repeated weekly throughout the study[8].

Animal material

Experiments were carried out on 21 albino female rats, aged 12 weeks and weighing 192.55±06.93 g. The animals were housed in a room with a 12/12-hour light/ dark cycle, at 22 ± 2°C, and had access to *ad libitum* to water and special rodent pellet diet.

Acute toxicity study

Rats were randomly allocated into 7 groups, with 3 rats in each one. Groups 1, 2, 3, 4, 5 and 6 received respectively: 0.25, 0.5, 1, 1.5, 2.5 or 5g/kg of *Anvillea radiata* aqueous extract in a single dose by gavage compared to the control group with no treatments. The animals' body weight and general behavior were continuously monitored during the 2 weeks of experimentation. At the end of the experiment, the animals were sacrificed in the morning after fasting for 12 hours and anesthetizing with diethyl ether in a large desiccator[9]. After incision of the abdomen, blood is collected from the inferior cava vena in heparin tubes for biochemical analysis. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and blood creatinine levels were determined by colorimetric methods using Chronolab kit. Livers and kidneys are carefully removed, separated from their fat tissues, rinsed with saline solution, and weighed in order to calculate the corresponding index (organ weight / body weight ratio)[10], before being used in histological study according to standard techniques: after formalin fixation, paraffin embedding, and hematoxylin-eosin staining [11].

Statistical Analysis

Results were represented as mean ± SE. The data were analyzed by using one-way analysis of variance (ANOVA) followed by the Tukey-test using SigmaPlot version 11.0. P values <0.05 were considered significant.

RESULTS

The acute administration of *Anvillea radiata* aqueous extract to albino female rats showed no significant changes in body weight and body organs, and no mortality during the whole experimentation. However, the animals exposed to higher doses showed some side effects like Anxiety and diarrhea (Table 1). Table 2 showed that no significant changes were observed in either body weight gain or organ index during the experimental period. Figure 1 shows dose-dependent changes in blood biochemical parameters of aqueous extract exposed animals compared to control. Liver function biomarkers show a significant increase in AST and ALT levels in groups 5 and 6 receiving respectively 2.5 and 5 g/kg *Anvillea radiata* aqueous extract. While, biomarkers of renal function indicate a significant decrease in BUN and creatinine levels in groups 3, 4, 5 and 6 receiving respectively 1, 1.5, 2.5 and 5 g/kg of plant extract as compared to control animals. Histological study in liver reveals a normal architecture in control group with normal appearance of hepatocytes and kupffer cells (Figures 2-a). While the observation of histological sections of *Anvillea radiata* aqueous extract treated groups (Figure 2-b, c and d) shows several anomalies namely: fatty change (Steatosis), glycogen accumulation and ballooning degeneration of hepatocytes. Microscopic examination of control rats' kidney sections showed normal renal parenchyma architecture of consisting of renal corpuscle, composed of glomeruli, Bowman's capsule and Bowman's space (BS), and a normal tubular system composed of proximal convoluted tubule and distal convoluted tubule, with a thin interstitial tissue and blood vessels (Figure 3-a and b). However, several abnormalities were observed in renal cortex and medulla of kidney sections of the group 5 and 6, exposed to 2.5 and 5g / kg of plant extract. Among the detected anomalies we noticed: shrunken renal corpuscles with marked hypo-cellularity and atrophied glomeruli, large interstitial space, and renal tubules with dilated lumina which appear completely distorted (Figure 3-c and d).

Table 1: The behavioral monitoring, mortality and other signs.

Doses (g/kg)	Behaviour	Mortality	Further signs
0	None	None	None
0.25	None	None	None
0.5	None	None	None
1	None	None	None
1.5	None	None	Diarrhea
2.5	Anxiety	None	Diarrhea
5	Anxiety	None	Diarrhea

Table 2: Effect of *Anvillea radiata* aqueous extract on body weight and organ index.

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Liver index (%)	Right kidney weight (g)	Left kidney weight (g)
Control	159.50±62.40	181.00±67.87	22.00±04.86[a]	4.29±0.38[a]	0.43±0.03[a]	0.44±0.04[a]
G1	178.00±06,24	191.33±07.75	13.33±01.76[a]	3.34±0.47[a]	0.28±0.06[a]	0.27±0.03[a]
G2	194.00±05.86	210.67±04.33	16.67±03.76[a]	3.60±0.49[a]	0.25±0.02[a]	0.27±0.02[a]
G3	179.00±08.14	195.33±12.44	16.33±04.70[a]	3.41±0.50[a]	0.27±0.05[a]	0.27±0.04[a]
G4	178.00±14.36	204.66±11.10	17.66±03.28[a]	4.02±0.36[a]	0.25±0.03[a]	0.26±0.02[a]
G5	203.00±25.81	222.00±22.68	19.00±04.00[a]	3.86±0.73[a]	0.36±0.05[a]	0.33±0.04[a]
G6	236.33±20.76	251.00±18.03	20.00±14.67[a]	3.18±0.78[a]	0.26±0.07[a]	0.29±0.12[a]

Data are expressed as means ± SE (n=3). A comparison between groups was made using the Tukey-test. Column not sharing a common letter (a–g) differ significantly at $p < 0.05$.

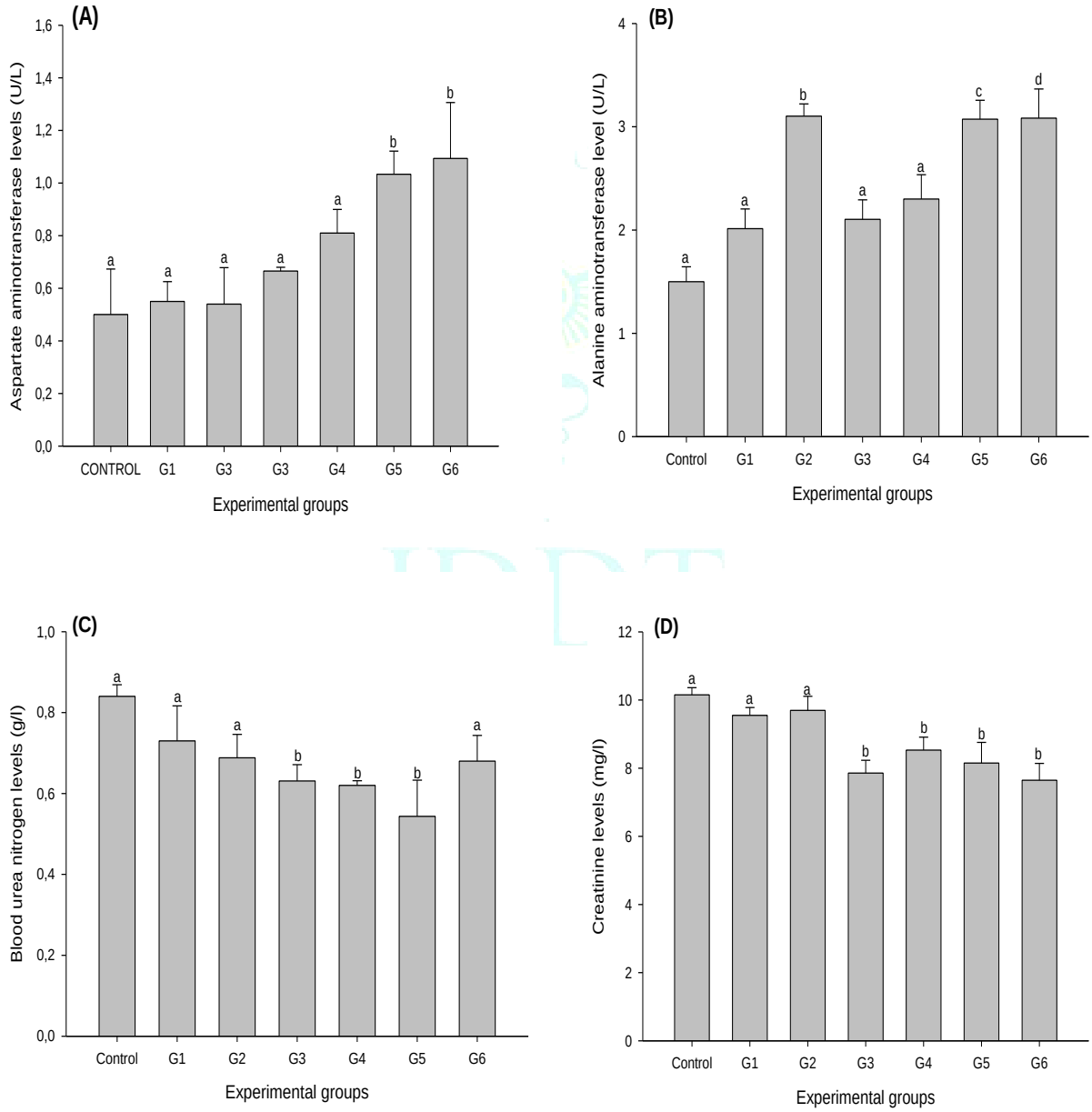
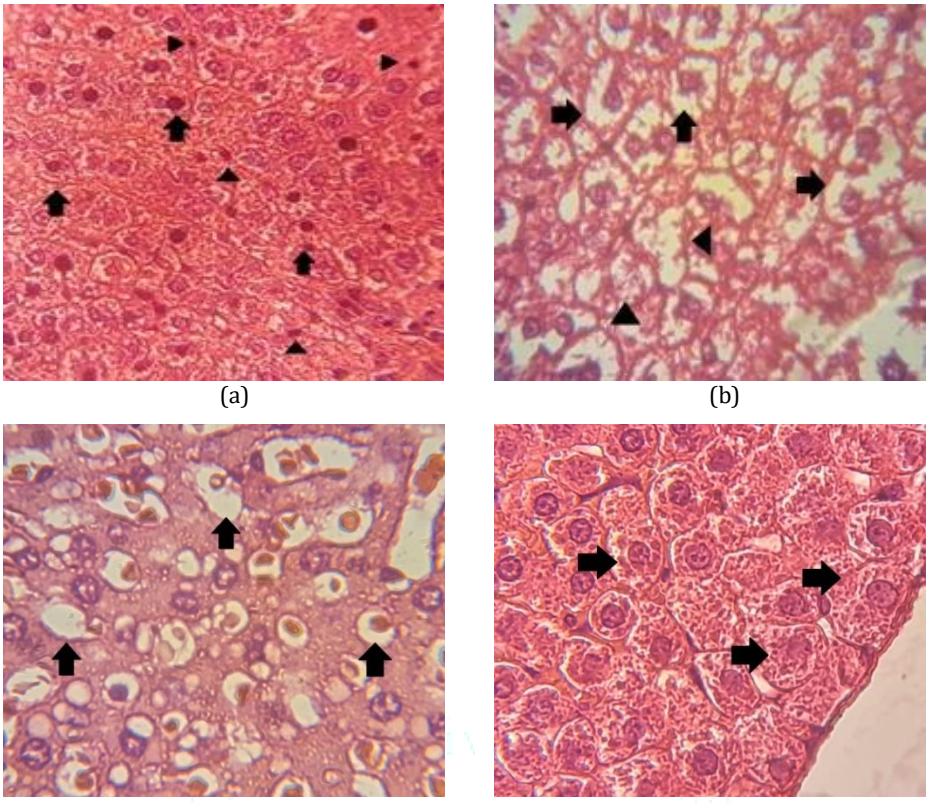


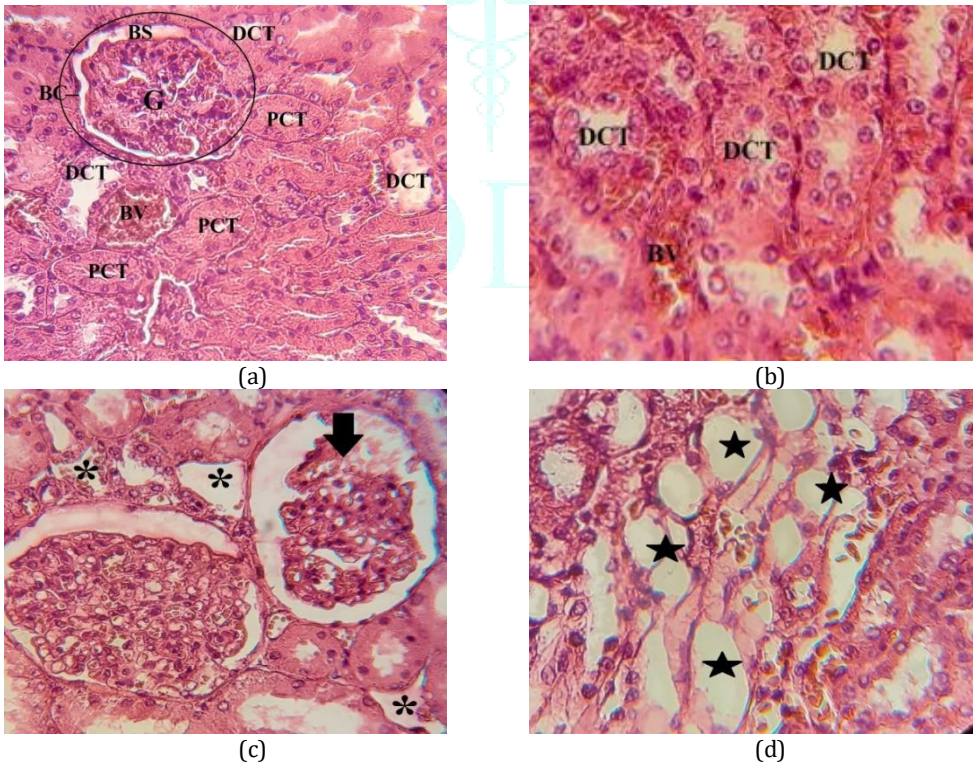
Figure 1: Effect of *Anvillea radiata* aqueous extract on rats' blood serum selected biochemical parameters. A: Aspartate aminotransferase (AST) level; B: alanine aminotransferase (ALT) level; C: Blood urea nitrogen (BUN) level; D: Blood creatinine levels; Data are expressed as means ± SE (n=3). A comparison between groups was made using the Tukey-test. Bars not sharing a common letter (a–g) differ significantly at $p < 0.05$.



(a) (b)

(c) (d)

Figure 2: Effect of *Anvillea radiata* aqueous extract on histological morphology of rat liver. a: Control group showing a normal architecture of liver with normal appearance of hepatocytes (arrows) and kupffer cells (arrowheads). b: Rat treated with a dose of 1.5g/Kg B.W. showing a mix of fatty change or Steatosis, (arrowhead) and glycogen accumulation (arrows); c: Rat treated with a dose of 2.5 mg/Kg B.W. showing fatty change (arrows); d: Rat treated with a dose of 5g/ Kg B.W. showing ballooning degeneration hepatocytes (arrows). Hematoxylin and eosin staining (×100).



(a) (b)

(c) (d)

Figure 3: Effect of *Anvillea radiata* aqueous extract on histological morphology of rat kidney. a-b: Renal cortex and medulla respectively showing normal renal parenchyma architecture consisting of renal corpuscle (black circle) composed of glomeruli (G), Bowman's capsule (BC) and Bowman's space (BS); a tubular system composed of proximal convoluted tubule (PCT) and distal convoluted tubule (DCT); a thin interstitial tissue (IT) and blood vessels (BV). c-d: Renal cortex and medulla respectively showing the abnormalities detected in group 5 and 6 exposed to 2.5 and 5g / kg of extract; among the detected anomalies: shrunken renal corpuscles with marked hypo-cellularity and atrophied glomeruli (arrows), large interstitial space (asterisk) and renal tubules with dilated lumina and appear completely distorted (black stars). Hematoxylin and eosin staining (×100).

DISCUSSION

The current study revealed that the acute administration of *Anvillea radiata* aqueous extract at single dose of 0.25, 0.5, 1, 1.5, 2.5 or 5 g/kg, to albino female rats, showed no significant changes in body weight and body organs, and no mortality during the whole experimentation. It is well known that increasing or decreasing body weight can indicate significant physiological changes such as hormonal changes, and decreased absorption of proteins, amino acids, and other nutrients[12]. However, in our study, none of those parameters were reached which explains why the aqueous extract has no effect on body weight and organ weight. Moreover, the animals exposed to higher doses showed some side effects like anxiety and diarrhea. Babulka, (2004)[13] indicates that the laxative effect of plants is linked to the different anthraquinones, but above all because of the ratio between the level of anthraquinones and tannins. Phytochemical analysis of *A. radiata* revealed the presence of some chemical groups such as volatile oils, fatty acids, tannins, flavonoids, anthracénosides, emodols, saponins, free quinones, anthraquinones, alkaloids, sterols and triterpenes[4]. It is known that several toxic compounds accumulate in liver where the detoxification occurs. Liver damage is usually assessed by the determination of serum transaminases (ALT and AST) and histology[14]. Our study indicates that high doses of *A. radiata* aqueous extract induced significant increase in liver function biomarkers while no adverse effect was recorded at doses below than 1g / kg bw. Our results are in agreement with those of Perveen *et al.,* (2018)[15], who tested the effect of ethanol extract of *A. garcinii* leaves, as well as the chloroform and n-butanol fractions, for their hepatoprotective effect using the carbon tetrachloride liver toxicity model, and the chloroform fraction, at a dose of 400 mg/kg, demonstrated a significant hepatoprotective effect. Renal function was evaluated by serum levels of blood urea nitrogen, creatinine level and by histological analysis. Our finding, indicate a significant decrease in BUN and creatinine levels in groups of animals receiving high plant extract. Burtis and Bruns, (2015)[16] explain that BUN and creatinine serum levels change when the cortex and/or the glomeruli are damaged. This finding corroborates with our histological observations of the kidneys, showing several cortical and glomerular abnormalities. In this context, *Anvillea radiata* demonstrated a toxic potential in acute administration, especially regarding sensitive organs function such as liver and kidney. Since this plant is already used in Algeria as medicine to treat diabetes[3, 17, 18] and to regulate the blood sugar level[19], these results provided valuable and primary data on the toxicity profile of *A. radiata* aqueous extracts and will serve as a guide to future studies. However, further studies are necessary to fully determine the toxic profile of this plant on other organs functions over short and long periods including genotoxicity, mutagenicity, and carcinogenicity evaluations.

CONCLUSION

Despite the benefic effect of *Anvillea radiata*, this plant can be harmful at high doses (higher than 1 g /kg b.w.) and may affects sensitive organs function such as liver and kidney.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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