

Research Article

Acute Toxicity Study of *Abies numidica* Extracts in Broiler ChickensOUMAIMA NAILI^{1,2*}, ZINEB BELHAMRA², NAILA CHAABNA², DAOUD HARZALLAH²¹ Faculty of Nature and Life Sciences, University Abbes Laghrour Khenchela, 40004, Algeria² Laboratory of Applied Microbiology, Faculty of Nature and Life Sciences, University Ferhat Abbas Setif 19000, Algeria.**ARTICLE DETAILS***Article history:*

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*Keywords:**A. numidica*,
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The aim of this study was to assess *in vivo* acute toxicity of hydroalcoholic and aqueous extracts of Algerian fir needles (*Abies numidica*) in broiler chickens. Total 18 one-day-old broiler chicks were obtained from hatchery. At three weeks of age chickens were weighed and separated into 3 groups. The control group received only distilled water, while treatment groups were orally administered 2000 mg.kg⁻¹b.wt. of hydroalcoholic and aqueous extracts of *Abies numidica*. No mortality or significant changes were observed in body weight, feed intake and feed conversion. Also, Blood parameters and relative organ weights were normal. Microscopic observations of liver and kidneys tissues of treatment and control group were similar. In conclusion, acute toxicity test indicated that extracts were not toxic when administered by the oral route to broiler chickens at 2000 mg.kg⁻¹b.wt.

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INTRODUCTION

Removal of antimicrobial growth promoters in poultry diets has increased the incidence of pathogenic infections, this lead to a negative effect on poultry output. In recent years, searching for available alternatives has increased; among possible alternatives, phytobiotic feed additives [1-4]. These phytobiotic includes a wide range of plant-derived product such as, essential oils, herbs, and spices. However, it is important to establish the safety of herbal supplements before use in poultry diet.

The fir of Numidia (*Abies numidica* De Lannoyex Carrière) is an endemic plant growing in Algeria, and is often used in folk medicines. *A. numidica* appears only on the top of mountains of Tababort and Babor from Algeria [5]. This tree has a pyramidal port becoming conical with age. The needles are dense, short, 1 to 2cm long and rounded at the apex [6].

As no information exists in the alternative, the objective of the present research was to evaluate the safety of *A. numidica* extracts as a dietary additive in poultry diets.

MATERIAL AND METHODS**Plant material:**

Abies numidica was collected from Setif, Algeria, during the month of November (Fig.1). The plant needles were shade dried at room temperature and powdered.

**Figure 1:** *Abies numidica* (Setif, Algeria)**Plant Extracts Preparation:**

Water extract: 10g of *A. numidica* needles powder was extracted with 250mL of boiling water for 10 minutes. The extract remained in the warm water for 15 minutes then it was filtered [7, 8].

***Author for Correspondence:**

Email: nailioumama@yahoo.fr

Hydroalcoholic Extract: Needles powder was macerated in 80% methanol for 24h, 48, and 72h at room temperature. After maceration, the resulted extracts were collected, filtered, and evaporated [9].

Acute Toxicity:

Acute oral toxicity test of both extracts was performed according to the Organization of Economic Co-operation and Development (OECD) guideline for testing chemicals [10, 11]. Total 18 one-day-old broiler chicks (ISA HUBBARD F15) vaccinated against Newcastle disease and infectious bronchitis were obtained from hatchery. At three weeks of age chickens were weighed and separated into 3 groups of 6 birds each.

The first group served as control and received only distilled water while the second and the third groups were administered a single oral dose of 2000 mg.kg⁻¹ b.wt. of hydroalcoholic and aqueous extracts of *Abies numidica*.

Animals were maintained at temperature of 32°C during the first week of age and it was reduced by 2°C/week until the birds were 3 weeks old. Feed and water were provided *ad libitum*. They were fed starter diet from 1 to 20 days and growth diet from 20 days to 35 days (Table 1).

Table 1: Nutrient composition of diets

Ingredients	Amount in diet (g/kg)	
	Starter	Growth
Maize (corn)	628	648
Soybean meal	290	270
Wheat bran	50	50
Di-calcium phosphate	10	10
Lime stone	12	12
Vitamin-mineral permix	10	10

Vitamin premix per kg of premix: 1000000 IU Vitamin A. 180000 IU Vitamin D3. 3295 mg Vitamin E. 200 mg Vitamin K. 120 mg Vitamin B1. 450mg Vitamin B2, 900mg Vitamin-B3 240mg Vitamin B6. 1.5mg Vitamin B12, 60g Folic Acid, 6mg Biotin, 2000 mg Vitamin PP. 35000 mg Choline Chloride.

Mineral premix per kg: 9590 mg manganese, 4920mg iron. 7500mg zinc. 2250mg cuivre, 132620mg calcium, 120mg iode, 36mg selenium, 332000mg sodium chloride,

Other: 180000 mg/ kg DL méthionine; 2500mg/kg anti-oxydant.

The animals were observed for general behavioral changes, signs toxicity, and mortality

continuously for 24 h after treatment, and thereafter over a period 14 days.

Performance Parameters:

The chickens were weighed individually on days 21, 28 and 35. Feed intake for each group was recorded weekly and feed conversion ratio (FCR) was calculated.

Blood Samples and Measurement of Hematological and Biochemical Parameters:

Blood samples were collected from the jugular vein and were placed into EDTA tubes for hematological assay and heparin tubes for biochemical assay.

The blood for hematological parameters (white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), was measured using hematological analyzer (Coulter Beckman -USA-).

Serum creatinine, urea, aspartate aminotransferase (AST), alanine aminotrasferase (ALT) and gamma-glutamyltransferase (GGT) were measured by specific commercial kits using spectrophotometer.

Organs Weight:

On day 35, all chickens were killed and their livers, kidneys, spleens, and hearts were removed and weighed to calculate relative organ weights.

Histopathological Studies:

Liver and kidneys were fixed in formalin solution (10%), dehydrated with alcohol, cleared in xylene, and embedded in paraffin wax. Slices of 5µm were prepared using Microtome. Then, these slices were de-paraffinated in xylene, passed through alcohol, and stained with hematoxylin/eosin. The slides prepared by this process were observed under light microscopy [12].

Statistical Analysis:

Data were expressed as means±SEM using Graph pad. Analysis of variance was determined by one-way ANOVA. The Tukey test was used to determine the significance of differences between the mean values of the treatment groups at the level of P<0.05.

RESULTS

Oral administration of both extracts did not cause behavioral changes in the chickens of three groups. Also, no treatment-related mortality was recorded.

Body Weight, Feed Intake, and Feed Conversion:

During all period, there was no significant difference between growth parameters of all groups ($P < 0.05$) (Table 2).

Hematological and Biochemical Parameters:

Oral administration of aqueous and hydroalcoholic extracts did not cause any

significant difference ($P < 0.05$) on hematological values compared to the control (Table 3).

The biochemical results do not reveal any significant effect of both treatments on renal (Urea, Creatinine) and liver (AST, ALT and GGT) markers (Fig. 2).

Relative Organ Weights:

Relative organ weights of control and treated groups are shown in Fig. 3. There was any significant difference ($P < 0.05$) compared to control group.

Table 2: Effect of *A. Numidica* extracts on body weight gain, feed intake and feed conversion in broiler

Parameters		CG	AE	HE	P
Initial body weight, g	21 days	296.6± 25.05	315.17±20.50	315.33±24.21	ns
Body weight gain, g	21- 28 days	163.4± 25.51	117.33±18.37	136.33±15.49	ns
	28-35 days	191.6± 37.21	143.33±10.73	184.33±26.36	ns
Feed intake, g	21- 28 days	395.00	327.83	330.00	ns
	28-35 days	586.00	575.00	556.67	
Feed conversion	21- 28 days	2.42	2.79	2.42	ns
	28-35 days	3.06	4.01	3.02	

C: Control group; AE: group treated with aqueous extract; HE: group treated with Hydroalcoholic extract; ns: not significant

Table 3: Hematological values of control and treated groups measured during acute toxicity.

Parameters	C	AE	HE	P
WBC ($10^9/l$)	36.95± 4.81	32.40± 2.94	30.30± 4.76	ns
RBC ($10^{12}/l$)	3.18± 0.11	3.47± 0.04	3.14± 0.15	ns
HGB (g/dl)	12.08± 2.80	15.68± 0.15	14.97± 0.88	ns
HCT (%)	1.40± 0.24	2.50± 0.34	2.33± 0.33	ns
MCV (fl)	137.05± 2,80	133.22± 1.65	137.15± 2.47	ns
MCHC (g/dl)	33.97± 0.77	33.97± 0.29	34.72± 0.27	ns
MCH(pg)	46.425± 0.75	45.23± 0.73	47.57± 0.60	ns

WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular; MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin. All data are expressed as means± SEM. C: Control group; AE: group treated with aqueous extract; HE: group treated with Hydroalcoholic extract; ns: not significant

Histopathology of Liver and Kidneys Tissues:

Histological sections were made at the anatomic pathology laboratory of the University Hospital of Ferhat Abbas University, Setif. Microscopic examination of liver and kidneys tissues of control and treated groups showed a normal histological structure of hepatic and renal parenchyma with presence of vascular congestion.

DISCUSSION

The purpose of the present study was to evaluate acute toxicity of *A. numidica* extracts in broiler

chickens. Oral administration of both extracts at 2000 mg.kg⁻¹b.wt. showed no mortality or behavioral changes in animals.

Changes in feed intake and body weight gain have been used as indicators of general health status of experimental animals [13]. The pattern of body weight and feed intake was not altered significantly by administration of *A. numidica* extracts, suggesting that this extracts did not induce changes in the appetite and did not exert an adverse effect on growth and the general health status of broiler chickens.

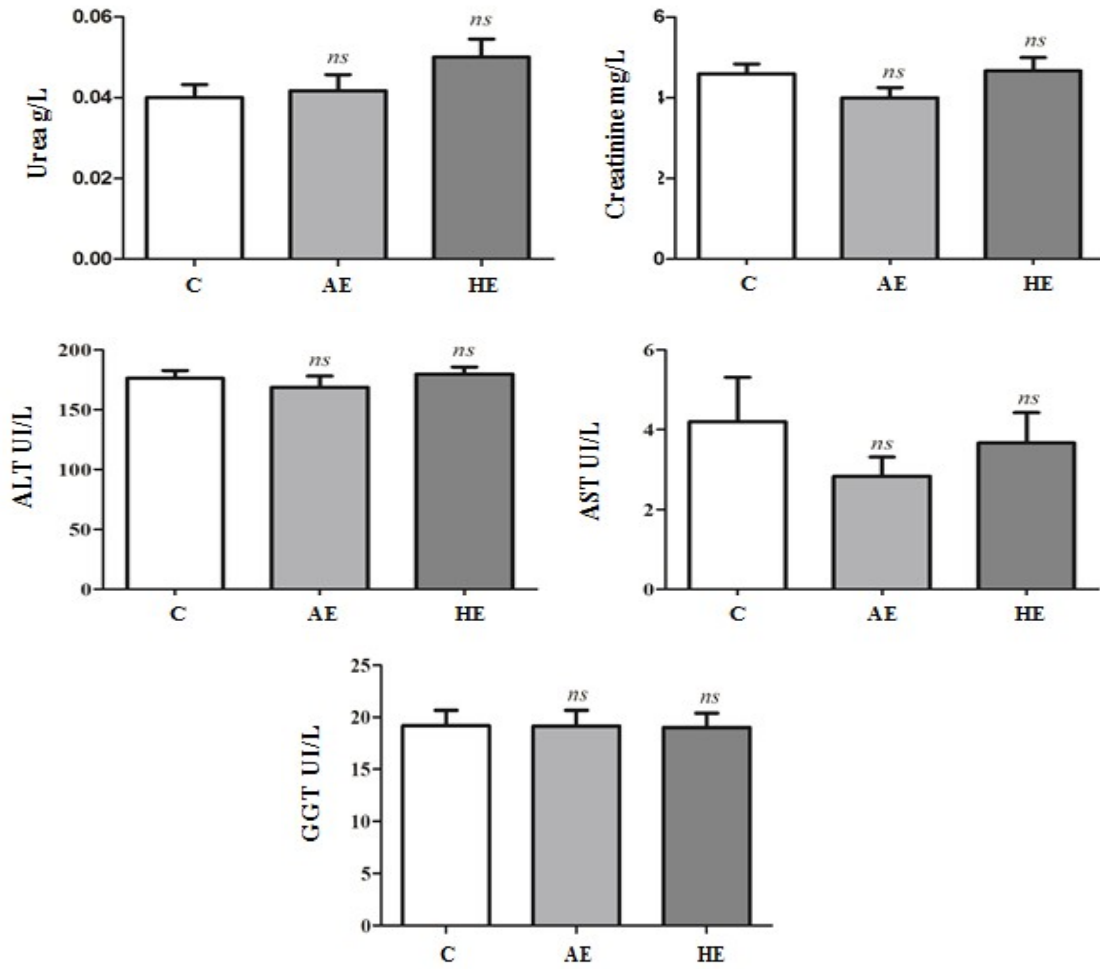


Figure 2: Assessment of renal and hepatic functions. All data are expressed as means± SEM. C: Control group; AE: group treated with aqueous extract; HE: group treated with Hydroalcoholic extract; ns: not significant

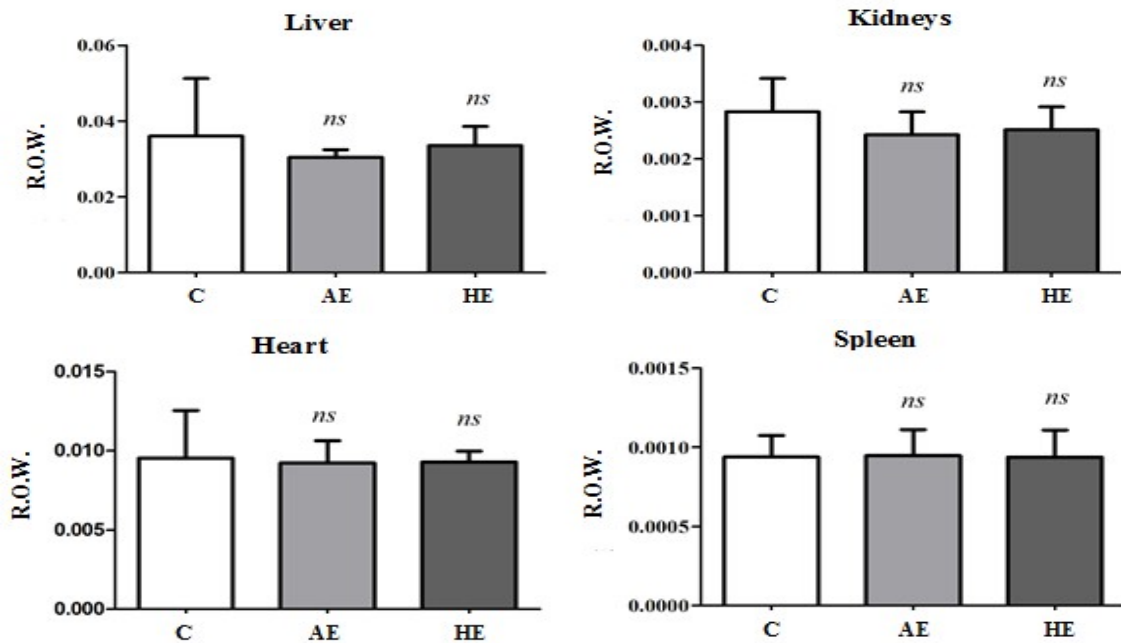


Figure 3: Effect of extracts on relative organ weights. All data are expressed as means± SEM. R.O.M: Relative organ weight; C: Control group; AE: group treated with aqueous extract; HE: group treated with Hydroalcoholic extract; ns: not significant

Blood profiles are important indices of the physiological state of animals [14]. Hematological components are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of animals [15]. Biochemical tests are important indicators for the assessment of renal and liver function.

Liver and kidneys function analysis is very important in the toxicity assessment of plant extracts and they are necessary for the survival of an organism [16]. In this study, two serum renal biochemical parameters (urea and creatinine) and three liver enzyme markers were analyzed. There were no significant differences between all hematological and biochemical parameters of the control and treated groups.

The relative organ weight in toxicity tests is a sensitive indicator for particular organs and it correlates well with histopathological changes in those organs [17, 18]. No significant changes in relative organ weight of liver, kidneys, spleen, and heart were observed. In addition, Macroscopic and microscopic observations of liver and kidneys tissues of all groups were similar, which indicated that the *A. numidica* needles extracts have no effect on normal growth of chickens.

CONCLUSION

In conclusion, acute toxicity study of aqueous and hydroalcoholic *A. numidica* extracts administered orally at 2000 mg.kg⁻¹b.wt. did not have any negative effects on the health status of broiler chickens. This suggests that *A. numidica* extracts can be used safely in poultry feed.

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