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Safety assessment of a traditionally used extract from leaves of *Boldoa purpurascens*

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Abstract

Ethnopharmacological relevance: *Boldoa purpurascens* Cav. (Nyctaginaceae) is a plant species used in traditional medicine in Cuba as a diuretic.

Aim of the study: The aim of the present investigation was to evaluate the safety profile of a hydroalcoholic extract from leaves of *Boldoa purpurascens*.

Materials and Methods: First, an experimental study to assess the oral acute toxicity at a dose of 2000 mg/kg body weight of the extract was carried out. Potential genotoxicity of the extract was evaluated using the Ames test and the micronucleus induction assay in mouse bone marrow. In the Ames test a concentration range of 50, 100, 150, 300 and 500 µg/plate was tested. In the micronucleus induction assay, doses of 500, 1000 and 2000 mg/kg of body weight were tested. For completeness, since the extract contains saponins, the evaluation of the hemolytic activity, ocular and skin irritation were included.

Results: No signs or symptoms of toxicity were observed in the oral acute toxicity test (body weight at baseline, seven days and end of the experiment of 236.41 ± 20.07 , 256.81 ± 30.44 and 240.02 ± 26.16 for the treated group). The hydroalcoholic extract from the leaves was not mutagenic in the Ames test, and no genotoxicity was observed in the micronucleus assay. A hemolysis test at concentration of 1 mg/mL confirmed hemolytic activity, which is not a safety concern since saponins are not absorbed after oral administration. In order to evaluate the percentage of protein denaturation, the ocular irritability index was calculated. The extract was found to be irritating. Finally, skin irritability was evaluated and the irritation index was equal to zero.

Conclusions: Based on the toxicological evaluation of a traditionally used hydroalcoholic extract from the leaves of *Boldoa purpurascens* we can confirm the safety of its oral use.

Key words: *Boldoa purpurascens*, Nyctaginaceae, safety assessment; oral acute toxicity, genotoxicity

1. Introduction

Boldoa purpurascens Cav. (Nyctaginaceae) is a plant growing in the Western and Central regions of Cuba, where many people use it traditionally as a diuretic (Roig, 1988). According to the Chinese traditional medicine, the species promotes urination, is antitoxic, is used to treat edema, swelling of feet, red urine and sweating blood deficit heart. In addition, it is used as a tonic (Li, 1993). Phytochemical analysis has shown the presence of biologically active compounds such as fatty acids, flavonoids, nitrogen compounds and saponins (González, D., 2006). In a previous study we have reported the isolation and characterization of three new methylenedioxyflavonols from the species (González et al., 2008) with an *in vivo* anti-inflammatory activity (González et al., 2011). Aqueous extracts of this plant have been shown to possess antibacterial activity (Rojas et al., 2004).

Although traditional usage supports the safety of medicinal plants it is very important to evaluate their toxicity. Safety assessments are crucial to validate the continuous use of medicinal plant and phytotherapy (Sponchiado et al., 2016). The aim of the present investigation was to evaluate the safety of the hydroalcoholic extract obtained from leaves of *Boldoa purpurascens*. Next to the assessment of the oral acute toxicity, the genotoxicity was investigated including the Ames test and the micronucleus assay in bone marrow cells (Sponchiado et al., 2016; EMEA, 2008a; EMEA, 2008b). Since phytochemical investigations revealed the presence of saponins, toxicity tests typically related to this type of secondary compounds, such as hemolytic activity and ocular and skin irritation were included for completeness of the assessment.

2. Materials and methods

2.1 Plant material

Leaves of *Boldoa purpurascens* were collected in January – February 2014 from its natural habitats in the experimental station Las Antillas at the Central University “Marta Abreu” of Las Villas, Cuba. The plant material was identified and authenticated by Dr. C. Cristobal Albuerne, a specialist of the Botanical Garden of the University. The voucher specimen No. 3012 is kept at the herbarium of the Botanical Garden for further reference.

2.2 Extraction and fractionation

Dried and pulverized leaves (1.0 kg) of *Boldoa purpurascens* were defatted with CHCl_3 (2.5 liters) and then extracted with 80% methanol (5 liters). The extract was concentrated under reduced pressure to complete dryness and resuspended in water.

2.3 Safety assessment of the extract

2.3.1 Evaluation of the oral acute toxicity

The evaluation of acute toxicity in a limit dose test was carried out using the procedures described by the Organization for Economical Cooperation and Development (OECD, 1987).

Sprague-Dawley rats of approximately the same age (9 weeks), weighing about 200-280 g, were housed in polypropylene cages at 23 ± 2 °C with 45-55% of relative humidity and were exposed to alternate cycles of 12 h light and darkness. Rats were fed with a diet consisting of standard pellets. Food and water were available *ad libitum*. Animals and commercial pellet diet were provided by the National Center of Production of Animals Laboratory (CENPALB). The experimental protocol was approved by the Institutional Ethic Committee of the Experimental Toxicological Unit of Villa Clara Medical School in accordance with institutional guidelines.

Experimental group (group 1) consisted of ten rats (five males and five females) selected at random. The extract was administered orally using an intragastric cannula 16G, after prior fasting of 16 to 18 h. A single dose of 2000 mg/kg body weight, divided into four administrations every three hours, was administered. Group 2 (five males and five females) was used as control (distilled water). Animals were observed individually during the first 30 min, with particular care during the first 4 hours, daily until day 14 of the experiment, in order to collect signs and symptoms of toxicity. The observations were aimed at determining: death and time of occurrence of the signs and symptoms of toxicity including its onset and duration, changes in the skin, mucosal and eyes membranes; respiratory and circulatory systems, central and autonomic nervous system in somatomotor activity and behavior. Particular attention to the potential occurrence of tremors, convulsions, salivation, diarrhea, lethargy and drowsiness was paid. Live weight of the animals was monitored on days 1, 7 and 14 of the experiment as one of the toxicity parameters. At the end of the experiment the animals were sacrificed by inhalation of ethyl ether for subsequent macroscopic pathological examination of the brain, stomach, liver, spleen, lungs, kidneys, muscles, esophagus and small intestine.

2.3.2 Genotoxicity studies

The genotoxic potential of the extract was evaluated in two short-term assays: the *in vitro* Salmonella / microsome assay (Ames test) and the *in vivo* micronucleus induction assay in mouse bone marrow.

2.3.2.1 Ames test

Salmonella typhimurium strains TA 98 and TA 100 (histidine-demanding auxotrophs) were used to determine frame shift mutations and base pair substitutions, respectively (Anderson et al., 1997). 2-Acetylaminofluorene (10 µg for TA 98) and sodium azide (5 µg for TA 100) were used as positive controls and distilled water as negative control. The Ames test was performed as a standard plate incorporation assay. Doses of 50, 100, 150, 300 and 500 µg/plate were used. Phenotypic characteristics of the strains were evaluated before the experiments. Each strain was cultured for 16 h, without stirring. After incubation the bacteria were seeded in E. Vogel - Bonner medium for a plate incorporation assay. 0.1 mL of each dose was added to a top agar tube containing 0.1 mL of *Salmonella typhimurium* culture, as well as 0.5 mL of S9 mix (crude preparation of enzymes obtained from the homogenized liver of rats). Each tube was vortexed vigorously before preparing the plate, which were incubated at 37 °C for 66 h.

Mean number of revertants and the relationship between the number of revertant colonies of treated plates and controls (MR, mutagenicity ratio) were calculated. The possibility that the evaluated compound produced a positive response in any of the strains with and without metabolic activation was assessed. The dose response relationship was analyzed by the statistical program Salmonel (Myers et al., 1987), which fits this relationship in at least four mathematical models:

Constant $Y = b$

Simple linear $Y = ax + b$

Lintox I (linear, attenuated by single exponential toxicity) $Y = (ax + b) 4x$

Lintox II (linear, exponentially attenuated by the square of the toxicity) $Y = (ax + b) T^2$

2.3.2.2 Micronucleus assay in bone marrow cells

25 male Balb/c mice of approximately the same age (8 - 12 weeks), weighing about 22 ± 3 g, were used. Animals were maintained under controlled conditions of temperature and light (light: dark, 10h: 14h). They received standard mice feed (Ratonin, EM 1001) provided by the National Center of Production of Animals Laboratory (CENPALB). Water was available *ad libitum*. The experimental protocol was approved by the National Center of Toxicology (CENATOX) in accordance with institutional guidelines.

Animals were divided into five groups of five animals each. The extract was administered orally at doses of 500, 1000 and 2000 mg/kg body weight, using an intragastric cannula 16G. The positive control group received a single i.p. injection of 60 mg/kg cyclophosphamide (purchased from Asta Medica, Spain) in 0.9% saline. After 14 days of dosing the animals were euthanized

by cervical dislocation and the bone marrow was extracted. The slides were prepared essentially as described by Schmid (1976). The Genotoxic Index (GI) and Cytotoxic Index (CI), recommended by the OECD (1996) and Hayashi et al. (1994) were used for the evaluation of genotoxic activity. The Genotoxic Index was determined by the ratio of micronucleated polychromatic erythrocytes (MNPCE) and total polychromatic erythrocytes (PCE) [$GI = MNPCE / Total\ PCE$]. The Cytotoxic Index (CI) was determined recorded for 400 cells as a measure of the ratio of polychromatic erythrocyte (PCE) and normochromatic erythrocytes [$CI = (PCE / NCE)$] (CSGMT, 1995).

2.4 Saponin related toxicity tests

2.4.1 *In vitro* hemolytic assay and ocular irritation by hemoglobin denaturation

The hemolytic effect of the extract from the leaves of *Boldoa purpurascens* on rat erythrocytes was evaluated using washed erythrocytes (red blood cells, RBCs). For the preparation of rat erythrocytes, blood samples from Wistar rats were collected (each weighing 180–200 g) in citrated tubes (9:1). The blood was used within 24 h after bleeding and washed three times in phosphate buffer saline (PBS), pH 7.4. After each washing, cells were centrifuged at 150g for 5 min and erythrocytes were separated from the plasma. The supernatant was carefully removed with each wash. Washed erythrocytes were stored at 4°C and used within 6 h for the hemolysis assay. The procedure described by Pape et al. (1999) was followed to evaluate erythrocyte hemolysis. Test samples and *Quillaia saponin* A (Quill. A) (Sigma-Aldrich) as a standard were prepared at concentrations of 1 mg/mL (Sun HX et al., 2003). Two blanks were used, B1 (975 µL of PBS + 25 µL of water) and B2 (25 µL of RBCs + 975 µL of water). Test samples (1mL) with different concentrations were prepared.

Both, samples and blanks were shaken and centrifuged at 5000 rpm for 2 min. The hemolytic activity of the extract was tested *in vitro* in 96-well plates; each well contained 200 µL. The absorbance (A) of the resulting supernatant was measured at 530 nm (SUMA equipment) to determine the extent of hemolysis and at 590 nm to determine hemoglobin denaturation. The average of the readings and the percentages that they represent were determined to find the H_{50} (percent lysed erythrocytes to a given concentration). Data were processed in the statistical package MMC (Programme for Adjustment of the Least Squares method for determination of protein; Version 1.3, February 1989). The percentage of denaturation (D) was determined by the following formulas:

$$D = 100 * (R1 - Ri)$$

$$Ri = A_{\lambda\ 590} / A_{\lambda\ 530}$$

$$R2 = A_{\text{SDS } \lambda 590} / A_{\text{SDS } \lambda 530}$$

Where:

R1 = Constant value of 1.05

Ri = Absorbance of the sample for which H₅₀ was determined

R2= Absorbance of SDS 0.1%

For the calculation of the OII (Ocular Irritability Index) was used the following expression:

$$OII = H_{50} / D$$

The extract was classified taking into account the values given in Table 1.

Table 1. Response categories of eye irritation

Eye irritation	Irritability index
Non- irritant	0-10
Slightly irritant	10-20
Moderate irritant	20-30
Severe irritant	30-100

2.4.3 Evaluation of the dermal irritability

Animal models used to study skin irritating potential have scientifically and ethically been questioned, and the use of alternative models has been promoted, but for regulatory purposes in drug registration animal evaluation is still requested. For the evaluation of dermal irritability the procedure described by the OECD and Draize was used (OECD, 2006, 2010; Draize, 1965). The assay was developed in rabbits, using males from the F1 line (1.8 kg of body weight). On the day before the test each animal of a group of three rabbits was clipped free of fur from the dorsal/flank area using veterinary clippers. Only animals with a healthy intact epidermis by gross observation were selected for the study. A quantity of 0.5mL of the samples (extract and Quill. A used as standard) was applied directly to the skin at the same concentration described for the hemolysis test. The treated areas were covered by gauze and the back was wrapped with a non-occlusive bandage. After 24 h the bandage test sites were gently swabbed with cotton wool soaked in saline solution and delineated with a marker. Immediately following removal of the patches and approximately 1, 24, 48 and 72 h later, primary irritation was examined. The reactions, defined as erythema and edema, were evaluated according to the scoring system for

skin reactions (Table 2). Scores of primary irritation (SPI) at 24, 48 and 72 h were summed and divided by the number of observations for the treated sites.

SPI for each rabbit

$$= \sum \frac{\text{Erythema and Edema grade at 24, 48 and 72 h}}{\text{Number of observations}}$$

Table 2. Evaluation of skin reactions

Reaction	Score
Erythema	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation	4
Edema	
No edema	0
Very slight edema	1
Well defined edema (edges of the area well defined by defined raising)	2
Moderate edema (raising approximately 1 mm)	3
Severe edema (raising more than 1 mm and extended beyond the area of exposure)	4
Total possible score for primary irritation	8

The irritation degree was categorized as non- irritant, or slight, moderate and severe irritation according to the classification in Table 3.

Table 3. Response categories of irritation in rabbits

Primary Irritation Index	Classification of Irritancy
0 to 0.4	Non-irritant

0.5 to 1.9	Slight irritation
2 to 4.9	Moderate irritation
5 to 8	Severe irritation

3. Results

3.1 Evaluation of the oral acute toxicity

The comparison of body weight per group and sex, showed no variation after seven days (Table 4).

Table 4. Effect of the administration of the extract on the body weight

Group	Initial weight (Mean $\pm$ SD)	Weight 7 days (Mean $\pm$ SD)	Weight 14 days (Mean $\pm$ SD)
1	236.41 $\pm$ 20.07	256.1 $\pm$ 30.44	240.02 $\pm$ 26.16
2	240.17 $\pm$ 26.04	280.09 $\pm$ 34.22	249.52 $\pm$ 29.01

When evaluating the female and male animals separately, no significant differences ($p > 0.05$) were observed in the behavior of body weight, indicating that there are no gender differences (Table 5).

Table 5. Effect of the administration of the extract on the body weight for male and female animals separately.

	Males		Females	
Weight	Z	p	Z	p
1 day	- 1.57	0.1167	- 0.5227	0.6015
7 days	- 0.94	0.3772	- 0.7311	0.4647
14 days	- 1.36	0.1745	- 0.5222	0.6015

Z- Values for normal standard curve

p- Statistical significance

3.2 Genotoxicity studies

3.2.1 Mutagenic evaluation/Ames test

The results obtained in the Ames test are shown in table 6.

Table 6. Mean number of revertant colonies and Mutagenicity Ratio

	Strain TA 100				Strain TA 98			
Doses µg/mL	Number of colonies		Mutagenicity ratio		Number of colonies		Mutagenicity ratio	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Water (Control)	118.3	113.0			19.7	16.8		
50	100.4	100.9	0.85	0.89	11.7	14.8	0.59	0.88
100	112.3	102.8	0.95	0.91	20.1	20.2	1.02	1.20
150	119.1	103.2	1.01	0.91	21.8	17.1	1.11	1.02
300	137.7	109.9	1.16	0.97	29.7	17.4	1.51	1.04
500	140.2	119.4	1.19	1.06	30.4	20.0	1.54	1.19
2-Acetyl-amino-fluorene (10µg)*					48.0	2715.0	2.44	161.70
Sodium azide (5 µg)*	1235.1	3116.4	10.44	27.58				

*2-Acetyl-amino-fluorene and Sodium azide (positive controls)

As can be easily seen in the Table, the average revertant colony numbers were 100.9, 102.8, 103.2, 109.9 and 119.4 for the extract at concentrations of 50, 100, 150, 300 and 500mg/Kg with metabolic activation and 100.4, 112.3, 119.1, 137.7 and 140.2 without metabolic activation for strain TA 100. For strain TA 98 the values were 14.8, 20.2, 17.1, 17.4, 20.0 for the four tested concentrations with metabolic activation and 11.7, 20.1, 21.8, 29.7 and 30.4 without metabolic activation. Spontaneous revertants were within the normal values for the 2 strains examined.

3.2.2 Genotoxicity evaluation/ micronucleus assay of bone marrow cells

For *Boldoa purpurascens* leaves extract oral administration was used because this is the route of administration used therapeutically. For doses of 500, 1000 and 2000 mg/kg of body weight, genotoxic index (GI) values of induction of micronuclei of 0.363, 0.357 and 0.362%,

respectively, were obtained (Table 7). The values obtained for cytotoxic index (CI) were 0.680; 0.720 and 0.66% respectively for the three tested dosis (500, 1000 and 2000mg/Kg)

Table 7. *In vivo* micronucleus test in Balb/c mice of the leaves extract of *Boldoa purpurascens*

Sample	GI % (MNPCE/Total PCE)	CI (PCE/NCE)
Control	0.402 ± 0.173	0.761 ± 0.281
500mg/Kg	0.363 ± 0.243	0.680 ± 0.071
1000mg/Kg	0.357 ± 0.351	0.720 ± 0.184
2000mg/Kg	0.362 ± 0.253	0.666 ± 0.263
Ciclophosphamide (60mg/Kg)	1.211 ± 2.210	0.614 ± 0.344

PCE: polychromatic erythrocytes

MNPCE: micronucleated polychromatic erythrocytes

NCE: normochromatic erythrocytes

GI: genotoxic index

CI: cytotoxic index

Cyclophosphamide (positive control)

3.3 Saponin related toxicity tests

3.3.1 *In vitro* Hemolytic Assay / *In vitro* ocular irritation assay by hemoglobin denaturation

The results obtained for the *in vitro* hemolytic assay for the extract obtained from the leaves of *Boldoa purpurascens* and for Quill. A used as a standard are shown in Table 8. The values obtained were 51.68% for the extract and 57.77% for the standard.

Table 8. H₅₀ values, denaturation index (D) and ocular irritability index (I) for *B. purpurascens* extract and Quill. A (standard)

Samples	H ₅₀ (%)	D (%)	Ocular irritability Index / Classification
Extract, 1 mg/mL	51.68	98.59	0.53 (Irritant)
Quill A, 1 mg/mL	57.77	102.25	0.56 (Irritant)

The denaturation values (D) shown in Table 8 allow to calculate the Ocular Irritancy Index (OII), which indicates how irritating the extract is, due to denaturation of proteins in the eyes. For the extract the denaturation index was 98.59% and 102.25% for the Quill A using as standard.

3.3.2 Evaluation of dermal irritability

The observations of dermal irritability at 24, 48 and 72 h showed no erythema and edema in rabbit skin, with an irritation index equal to zero, indicating no damage to the dermis.

4. Discussion

4.1 Evaluation of the oral acute toxicity

The acute toxicity in the limit dose test in Sprague- Dawley rats was evaluated because it is an extract of a medicinal plant with no reports of toxicity in its traditional use, or in earlier studies. This test is applied to compounds of which low toxicity is expected (Hoffman, 1996; Loomis, 1992), and it would be irrelevant to conduct a study of acute toxicity at high dose levels. Thus, there must be a point at which the researcher can conclude that the product is practically non-toxic or non-lethal after acute exposure.

During the first 30 min, the first 4h and during daily observations, the single oral dose administration of the extract obtained for *Boldoa purpurascens* at the highest concentration of 2000 mg/kg body weight did not cause any mortality and revealed no clinical signs, like changes in fur and skin, eyes, mucus membrane, respiratory rate, circulatory signs, autonomic effects, and central nervous systems. At the end of the study the macroscopic pathological examination did not show abnormal histopathological changes. According to these results the median acute lethal dose (LD_{50} , expressed as weight of test substance per unit weight of test animal (mg/kg)), was assumed to be greater than 2000 mg/kg suggesting that the extract could be generally regarded as nontoxic.

Body weight is often the most sensible indicator of an adverse effect (Alemán et al., 1998) and the data referring to body weight have a high sensitivity to alterations due to chemicals with low toxicity (Mosberg et al., 1999). The rapid loss of body weight (approximately 15 to 29% loss of body weight in a period of five to seven days) is considered as the indicator that provides more information in toxicological studies. The increase in body weight for treatment group weekly were considered normal and gradual.

4.2 Genotoxicity studies

Genotoxic studies are essential in the evaluation of herbal medicines with therapeutic activity. These studies investigate the induction of genetic damage, which can cause a large number of

diseases such as tumors, alteration of genet transmission to offspring and congenital abnormalities.

4.2.1 Mutagenic evaluation/Ames test

The Ames test is commonly used with plant extracts for possible gene mutation determination. Positive results are sufficient to classify a substance as a mutagen in any bacterial strains with and without metabolic activation (Zeiger, 2001). The standard plate incorporation methods for the Ames test using *Salmonella typhimurium* standard tester strains TA98 and TA100 was performed. The test without S9 metabolic activation can only detect direct mutagens while with S9 metabolic activation allows the detection of indirect mutagens, often caused by conjugation reactions of metabolic oxidation systems. According to the results the mean of the number of revertant colonies for each dose did not exceed twice the mean number of revertant colonies of the control with and without metabolic activation, for both strains (TA 100 and TA 98) and the mutagenicity ratio (MR) was less than two.

The statistical program SALMONEL showed no dose-response relationship because there were no significant differences between strains with and without metabolic activation.

Therefore, the results meet the criteria of negativity.

4.2.2 Genotoxicity evaluation/ micronucleus assay of bone marrow cells

The micronucleus test is a widely used method to assessing genotoxicity of chemicals in the organism.

The values obtaining in the study of the clastogenic effect of *Boldoa purpurascens* are within the spontaneous range (0.10 to 0.39 %) of occurrence of micronuclei in the test line (Khan, 1998). From these results it can be concluded that, under the conditions of the experiment, the extract did not show clastogenic activity at all doses tested (Hayatsu, 1998). The range of values reported in the literature for the genotoxic index of the negative control is between 0.10 and 0.49 for rodents (Lovell, 1989) and 0.39 for Balb/c mice (Salamone, 1991). In Cuba this line is provided by CENPALAB and can have values up to 0.50 (Cancino, 1993).

The cytotoxic index (CI) provides an estimate of the exposure of the target organ to the evaluated compound (CSGMT, 1995). Therefore, a decrease in the index is an expression of disturbance in the hematopoiesis as a result of exposure to the xenobiotic (Shi et al., 1994). The observed cytotoxicity indices for animals treated with the leaves extract of *Boldoa purpurascens* demonstrated that the extract did not induce cytotoxic effects on bone marrow erythrocytes. The

maximum dose recommended for *in vivo* toxicology studies, 2000 mg/kg (OECD, 1996) was tested. As noted, there are no significant increases in the percentage of nucleated polychromatic erythrocytes at the tested doses and no dose-response relationship was observed. These results support the safety of the extract; although we cannot exclude the possibility that it was influenced by the route of administration that could limit its bioavailability.

4.3 Saponin related toxicity tests

4.3.1 *In vitro* Hemolytic Assay / *In vitro* ocular irritation assay by hemoglobin denaturation

The assessment of cytotoxicity through hemolytic activity tests has proved to be an alternative screening method for simple toxicity. Evaluation of toxicity is paramount when considering a safe treatment. Hemolysis is characterized by erythrocytes rupturing with the release of hemoglobin. The *in vitro* hemolysis test is used as a method for substance toxicity screening, estimating any likely *in vivo* damage (Aparício et al., 2005). *In vitro* hemolysis tests have also been employed by several authors for the toxicological evaluation of different plants (Gandhi and Cherian, 2000). Saponins are well known for two characteristics activities including cytotoxicity and hemolysis. However, the hemolytic activity of saponins inducing toxicity in most animals is a major drawback for their clinical development.

Taken into account the results obtained in the hemolysis test, the extract from *Boldoa purpurascens* cause less hemolysis (51.68 %) than the Quill. A standard used at the same concentration. These results could be related to the presence of different saponins in *B. purpurascens* and *Q. saponaria*.

Both, extract and Quill A. evaluated are slightly irritating, and therefore it is not recommended to apply them there and to avoid contact with the eyes.

3.3.2 Evaluation of dermal irritability

Skin irritation is one of the most common adverse effects in humans. It depends on many factors, including the concentration, duration and frequency of exposure, exposed skin site, rate of penetration and intrinsic toxic potential of the substance. For that reason, the evaluation of irritability potential to human skin of any chemicals or formulations is a necessity. This must be done to determine the risk of irritation due to the contact between these compounds and human skin (Campbell et al., 1981). The most important types of local effects that may occur are: primary irritation, corrosion, skin sensitization, phototoxicity and photoallergy. The most widely used technique is the Draize test, which is still the test of choice for the classification of substances as irritating or not-irritating (Draize, 2008).

5. Conclusions

Toxicological evaluation of the hydroalcoholic extract obtained from leaves of *Boldoa purpurascens* showed no signs or symptoms of acute toxicity, considering it as Non Classified (non toxic). Genotoxic evaluation showed that the extract was not mutagenic nor genotoxic. In the hemolysis test the extract caused lysis of 51.68% of the erythrocytes at the concentration of 1mg/mL, being hemolytic. The extract was considered non-irritant for skin but irritant for the eyes. Since the latter toxic effects are related to the presence of saponins, the toxicity is very low when given orally, as a result of their low absorption. In conclusion, the traditional use of the hydroalcoholic extract by oral administration can be considered as safe.

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