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Full Length Research Paper

Antioxidant activity and acute toxicity of *Neoglaziovia variegata* (Bromeliaceae)

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Antioxidant activities of *Neoglaziovia variegata* were evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and β-carotene-linoleic acid bleaching and was compared with ascorbic acid, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The total phenolics content of the extracts was determined by the Folin-Ciocalteu method. Total flavonoid was also determined. The most significant total phenolic content was of 543.50 ± 9.38 mg of gallic acid equivalent/g for ethyl acetate extract (AcOEt), which presented the best antioxidant activity (IC_{50} 5.08 ± 0.20 µg/ml) for DPPH scavenging. The acute toxicity of Nv-EtOH was performed 2.0 g/kg intraperitoneally and 5.0 g/kg orally in mice. No mortality and no toxicity signs were observed, indicating low toxicity of the extract. Blood was removed after 14 days for laboratory analysis of hematological and biochemical parameters. Alterations of aspartate aminotransferase (AST) and creatinine were observed. The data obtained showed that the doses induced microscopic alterations in the liver and kidney. In conclusion, the Nv-EtOH can be considered of low toxicity.

Key words: Antioxidant activity, acute toxicity, *Neoglaziovia variegata*, Bromeliaceae.

INTRODUCTION

Neoglaziovia variegata belongs to the family Bromeliaceae, subfamily Bromelioideae, and is popularly known in Brazil as "*caroá*". This species can be commonly found in the Brazilian *Caatinga* vegetation (dry woodland characteristic of semi-arid regions of Northeastern Brazil). It present economic importance centered on the leaves, which are constituted by high resistance fibers. The extraction of *caroá* reached significant levels in the 40's, before the advent of synthetic fibers, caused by the expansion of sisal plantations (Pereira and Quirino, 2008). This species, which, although endemic of *Caatinga* and of proven economic importance has not been studied regarding its chemical and pharmacological properties.

Some studies have demonstrated that species of the Bromeliaceae family have pharmacological properties such as antioedematogenic and free radical scavenging (Delaporte et al., 2004), antinociceptive and anti-inflammatory (Amendoeira et al., 2005), anti-allergic (Vieira-de-Abreu et al., 2005), antiulcer (Carvalho et al., 2010) and cytotoxic activity (Manetti et al., 2010).

Pineapple (*Ananas comosus*) contains bromelain, an enzyme that protects them from predation by insect larvae, which has great commercial value and whose use is on the rise due to its applicability in the pharmaceutical and food industries. In addition to bromelain, many other

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Abbreviations: DPPH, 2,2-Diphenyl-1-picrylhydrazyl; **BTH,** butylated hydroxytoluene; **BHA,** butylated hydroxyanisole; **AcOEt,** ethyl acetate extract.

enzymes have been isolated and identified from different bromeliads, which also have great potential to be employed in these same industries (Manetti et al., 2009).

Despite the large number of species of this family, few have been studied chemically so far. However, there is a considerable amount of identified compounds, which belong mainly to the classes of cycloartane triterpenoids, steroids (Vieira and Kaplan, 2011), anthocyanins, esters of arylpropanoid acid derivatives (Rocha et al., 2010) and flavonoids (Manetti et al., 2009).

Flavonoids are antioxidants and help to prevent diseases associated with oxidative stress, which is caused by an imbalance between reactive oxygen species (ROS) and the anti-oxidative defense systems (Daud et al., 2010). Oxidative stress is considered to be a major etiological or pathogenic agent of cardiovascular and neurodegenerative diseases, cancers, Alzheimer's, diabetes and aging. Because they inhibit or delay the oxidative process by blocking both the initiation and propagation of oxidizing chain reactions, antioxidants for the treatment of cellular degenerations are beginning to be considered (Jang et al., 2010).

Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells. Although almost all organisms possess antioxidant defense and repair systems to protect against oxidative damage, they cannot prevent the damage entirely.

Nowadays, the interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, replacing synthetic antioxidants which are often restricted due to carcinogenic effects (Wannes et al., 2010). On the other hand, it is necessary to carry out toxicological studies to evaluate safety parameters which are not observed by the popular use of the plants. Toxicological studies help to decide whether a new drug should be adopted for clinical use or not. The toxicological analysis of plant extracts is of fundamental importance, since it characterizes the deleterious effects of toxic compounds produced from its administration.

There is no previous report on the analysis of the antioxidant activity and acute toxicity of *N. variegata*. In our continuing search of the medicinal plants from Brazilian Caatinga for combine biodiversity conservation with drug discovery, the aim of this study was to evaluate the antioxidant activity and possible toxic effects of ethanolic extract of *N. variegata* in mice.

MATERIALS AND METHODS

The leaves of *N. variegata* (Arruda) Mez were collected in the city of Petrolina (Coordinates: S 08°59'16"; W 40°35'20"), State of Pernambuco, Brazil, in January of 2011. The samples were identified by André Paviotti Fontana, a botanist from Centro de Recuperação de Áreas Degradadas da Caatinga (CRAD). A voucher specimen was deposited at the Herbarium of San Francisco Valley (HVASF), of the Federal University of San Francisco Valley, with the code 6441.

Preparation of extracts

The leaves of *N. variegata* dried and pulverized (581 g) were subjected to maceration with 95% EtOH for 72 h. The solution was filtered and concentrated in a rotatory evaporator oven at 50°C, producing 30 g of crude ethanol extract (Nv-EtOH). For the evaluation of antioxidant activity, the ethanolic extract was suspended in MeOH:H_2O (3:7) and partitioned with hexane, chloroform ($CHCl_3$) and ethyl acetate (AcOEt) in crescent order of polarity to obtain the respective extracts.

Total phenolic content

Total phenolic contents were assayed using the Folin-Ciocalteu reagent, it is based on the method reported by Slinkard and Singleton (1977), only the volumes have been adjusted (Almeida et al., 2011). An aliquot (40 µl) of a suitable diluted extracts was added to 3.16 ml of distilled water and 200 µl of the Folin-Ciocalteu reagent, and mix well. The mixture was shaken and allowed to stand for 6 min, before adding 600 µl of sodium carbonate solution, and shake to mix. The solutions were left at 20°C for 2 h and the absorbance of each solution was determined at 765 nm against the blank and plot absorbance vs. concentration. Total phenolic contents of the extracts (three replicates per treatment) were expressed as mg gallic acid equivalents per gram (mg GAE/g) through the calibration curve with gallic acid. The calibration curve range was 50 to 1000 mg/l (R^2 = 0.9938). All samples were performed in triplicates.

Total flavonoid content

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen et al., 1999). An aliquot (300 µl) of Nv-EtOH, ethyl acetate extract or standard of catechin was added to flask containing 1.5 ml of distilled water. To the flask was added 90 µl $NaNO_2$ (5%). After 6 min, 180 µl $AlCl_3$ (10%) was added. After 5 min, 600 µl 1M NaOH was added and the total volume was made up with 330 µl of distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid contents were expressed as mg catechin equivalents per gram (mg CE/g) through the calibration curve with catechin. The calibration curve range was 50-1000 mg/L (R^2 = 0.9784). All samples were performed in triplicates.

Antioxidant activity

*2,2-Diphenyl-1-picrylhydrazyl (*DPPH*) free radical scavenging assay*

The free radical scavenging activity was measured using the 2,2-diphenyl-1- picrylhydrazil (DPPH) assay (Mensor et al., 2001; Falcão et al., 2006). Sample stock solutions (1.0 mg/ml) of the extracts were diluted to final concentrations of 243, 81, 27, 9, 3 and 1 µg/ml, in ethanol. One millliter (1 ml) of a 50 µg/ml DPPH ethanol solution was added to 2.5 ml of sample solutions of different concentrations, and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage of antioxidant activity (AA) using the following formula: AA% = [(absorbance of the control – absorbance of the sample)/ absorbance of the control] × 100. Ethanol (1.0 ml) plus plant extracts solutions (2.5 ml) were used as a blank. DPPH solution (1.0 ml) plus ethanol (2.5 ml) was used as a negative control. The positive controls (ascorbic acid, BHA and BHT) were

those using the standard solutions. Assays were carried out in triplicate.

β-Carotene bleaching test

The β-carotene bleaching method is based on the loss of the yellow color of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion (Wannes et al., 2010). The rate of β-carotene bleaching can be slowed down in the presence of antioxidants. β-Carotene (2 mg) was dissolved in 10 ml chloroform and to 2 ml of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40°C and 100 ml of distilled water was added, then the emulsion was vigorously shaken during two minutes. Reference compounds (ascorbic acid, BHA and BHT) and sample extracts were prepared in ethanol. The emulsion (3.0 ml) was added to a tube containing 0.12 ml of solutions 1 mg/ml of reference compounds and sample extracts. The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50°C for 120 min, when the absorbance was measured again. Ascorbic acid, BHA and BHT were used as positive control. In the negative control, the extracts were substituted with an equal volume of ethanol. The antioxidant activity (%) was evaluated in terms of the bleaching of the β-carotene using the following formula: % Antioxidant activity = $[1 - (A_0 – A_t) / (A_0^0 – A_t^0)] \times 100$; where A_0 is the initial absorbance and A_t is the final absorbance measured for the test sample, A_0^0 is the initial absorbance and A_t^0 is the final absorbance measured for the negative control (blank). The results are expressed as percentage of antioxidant activity (% AA). Tests were carried out in triplicate.

Animals

Male and female adult albino Swiss mice (30 to 40 g and aged 8 to 10 weeks), were used throughout this study. The animals were randomly housed in appropriate cages at 22 ± 2°C on a 12 h light/ dark cycle (lights on at 6:00 a.m.) with free access to food and water. When necessary, animals were deprived of food 12 h prior to the experiments. Experimental protocols and procedures were approved by the Federal University of San Francisco Valley Animal Care and Use Committee by number 21051023.

Acute toxicity

In the inquiry of the acute toxicity, animals were randomly divided in groups of five male and five female Swiss mice (n = 10). Animals were administered intraperitoneally 2.0 and 5.0 g/kg orally of the crude ethanol extract of *N. variegata*. Control group received vehicle. Subsequently, the animals were observed for 14 days to evaluate the presence of signs of toxicity. Mortality in each group within 72 h was recorded and LD_{50} was estimated by the method described by Litchfield and Wilcoxon (1949). In addition, parameters of body weight variation, consumption of food and water were assessed daily throughout the study.

Behavioral screening

The behavioral screening of the mice was performed following parameters described by Almeida et al. (1999). The animals were observed at 0.5, 1, 2, 3 and 4 h after administration of Nv-EtOH. Specific behaviors (piloerection, palpebral ptosis, abdominal contortions, locomotion, hypothermia, muscular tonus, trembling, forepaws paralysis, sedation, ambulation reduction, response to touch, analgesia and defecation) were observed and graded.

Hematological and biochemical parameters analysis of blood

For the evaluation of blood hematological and biochemical parameters, it utilized the methodology described by Vasconcelos et al. (2007) and Araújo et al. (2008) with modifications. Blood was removed after 14 days through brachial plexus for laboratory analysis of hematological parameters: Count of erythrocytes (10^6/mm^3), hemoglobin (g/dL), hematocrit (%), the mean corpuscular volume (MCV, μ^3), the mean corpuscular hemoglobin (MCH, μg), the mean corpuscular hemoglobin concentration (MCHC, %), leukocytes (10^3/mm^3), lymphocytes (%), monocytes (%) and platelets (10^3/mm^3). The biochemical parameters analyzed in serum samples were glucose (mg/dL), cholesterol (mg/dL), triglycerides (mg/dL), AST/TGO (U/L), ALT/TGP (U/L), urea (mg/dL) and creatinine (mg/dL). For the determination of hematological parameters was used hematology analyser Sysmex XT-2000, for the biochemical parameters was used an automatic analyser Wiener BT 3000 Plus.

Histopathological analysis

Sections of tissues such as kidney and liver were obtained for histopathological studies. The organs were fixed in 10% buffered formaldehyde for 18 h. After fixation, the organ was immersed in 70% alcohol and transported to the Laboratory of Cell Biology, Cytology and Histology, Campus of Agricultural Sciences of UNIVASF. It was then dehydrated in increasing series of ethanol subsequently clarified in xylene, and even embedded in paraffin. From each paraffin block containing the tissue samples, sections of 7 μm were cut in microtome and mounted on glass slides, which were stained with hematoxylin and eosin for further evaluation of tissues morphology in light microscope (400 x).

Statistical analysis

The data obtained were analyzed using the GraphPad Prism® version 4.0 and expressed as mean ± S.E.M or mean ± S.D. as appropriate. Statistically significant differences between groups were calculated by the application of Student's *t*-test. Values were considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Total phenolic, total flavonoid contents and antioxidant activity

Table 1 summarizes the results from the quantitative determination of phenolic (TP) and flavonoids (TF) as well as the effect of extracts from *N. variegata*, ascorbic acid, BHA and BHT on the DPPH free radical scavenging and β-carotene-linoleic acid bleaching test. The extracts were evaluated by comparing with ascorbic acid, BHA and BHT, which are well-known commercial antioxidants. The total phenolic contents of the extracts were determined by Folin-Ciocalteu method as gallic acid equivalents in milligrams per gram (mg GAE/g) while total flavonoid contents were calculated as catechin

Table 1. Total phenolics (TP), total flavonoids (TF) and antioxidant activity of extracts from *Neoglaziovia variegata*.

Parameter	TP (mg GAE/g)	TF (mg CE/g)	DPPH (IC_{50}, µg/ml)	β-Carotene bleaching (% AA)
EtOH	65.13 ± 1.25	7.82 ± 10.28	243.70 ± 69.99	41.87 ± 3.20
Hexane	---	---	517.30 ± 152.30	56.15 ± 2.73
$CHCl_3$	203.90 ± 10.23	32.52 ± 4.19	48.31 ± 1.65	29.84 ± 13.49
AcOEt	543.50 ± 9.38	262.30 ± 1.33	5.08 ± 0.20	40.31 ± 7.61
Ascorbic acid	---	---	4.72 ± 2.67	7.50 ± 2.12
BHA	---	---	3.50 ± 3.17	80.93 ± 3.45
BHT	---	---	17.87 ± 2.98	86.77 ± 1.14

The IC_{50} values were obtained by interpolation from linear regression analysis with 95% of confidence level. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%. Values are given as mean ± SD (n=3).

equivalents in milligrams per gram (mg CE/g). Among the four extracts, ethyl acetate extract (AcOEt) was containing the highest (543.50 ± 9.38) amount of phenolic compounds followed by $CHCl_3$ extract (203.90 ± 10.23) and crude ethanol extract (65.13 ± 1.25). Numerous publications applied the total phenols assay often found excellent linear correlations between the total phenolic profiles and the antioxidant activity (Huang et al., 2005). For the total flavonoid content, the highest value was observed in AcOEt extract (262.30 ± 1.33) while the $CHCl_3$ and crude ethanol extract (EtOH) presented 32.52 ± 4.19 and 7.82 ± 10.28 mg CE/g, respectively.

In the present study, the antioxidant ability of the *N. variegata* extracts was investigated through some *in vitro* models such as radical scavenging activity using, DPPH method and β-carotene-linoleate model system. Antioxidant activity on method of DPPH was expressed as IC_{50} which is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%. Lower IC_{50} value indicated higher antioxidant activity. In β-carotene-linoleate model system the antioxidant activity was expressed as percentage of antioxidant activity (%AA).

The DPPH reactivity is one popular method for screening of the free radical-scavenging ability of compounds that has been extensively used for screening antioxidants from plant extracts. DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolorizes the DPPH solution. The degree of color change is proportional to the concentration and potency of the antioxidants (Krishnaiah et al., 2011). The data showed that the AcOEt and $CHCl_3$ extracts exhibited excellent free radical scavenging activity. The AcOEt extract showed better antioxidant activity than BHT using by DPPH method, with a value of IC_{50} of 5.08 ± 0.20 µg/ml. BHA was the most effective antioxidant, with a value of IC_{50} of 3.50 ± 3.17 µg/ml. It appears that *N. variegata* have compounds with a strong hydrogen-donating capacity and can efficiently scavenge DPPH radicals.

The antioxidant activity of extracts was also evaluated by the β-carotene/linoleate bleaching method. This method is based on the loss of the yellow color of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. β-Carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. The rate of the β-carotene bleaching can be slowed down in the presence of antioxidants (Kulisic et al., 2004). This method is one of the antioxidant assays suitable for plant extracts. All extracts had lower antioxidant activity than BHT and BHA.

Acute toxicity

N. variegata is a plant that does not have broad popular use. However, studies are needed to prove the safety of its use, as well as the analysis of acute toxicity is fundamentally important to identify the doses that could be used, and to reveal the possible clinical signs caused by the extract under investigation.

In the acute toxicity of Nv-EtOH, behavioral and physiological alterations were not observed neither animal's death in the doses of 2.0 g/kg intraperitoneally and 5.0 g/kg orally, respectively indicating low toxicity of the extract. In this experiment, it was observed that the Nv-EtOH has LD_{50} > 5000 mg/kg. According to Kennedy et al. (1986), substances that present LD_{50} higher than 5.0 g/kg by oral route can be considered practically non-toxic.

There were significant changes ($p < 0.001$) in body weight of mice from day 1 to day 14 in group treated with the extract at dose of 5 g/kg *v. o*. At the end of the experiment, the average of control group was of 40.44 ± 0.27 g, while the treated group was of 34.59 ± 0.22 g. The variation of weight along of the experiment could be seen in Figure 1.

Generally, alterations in body weight gain of mice treated with substances reflect toxic effects, and have

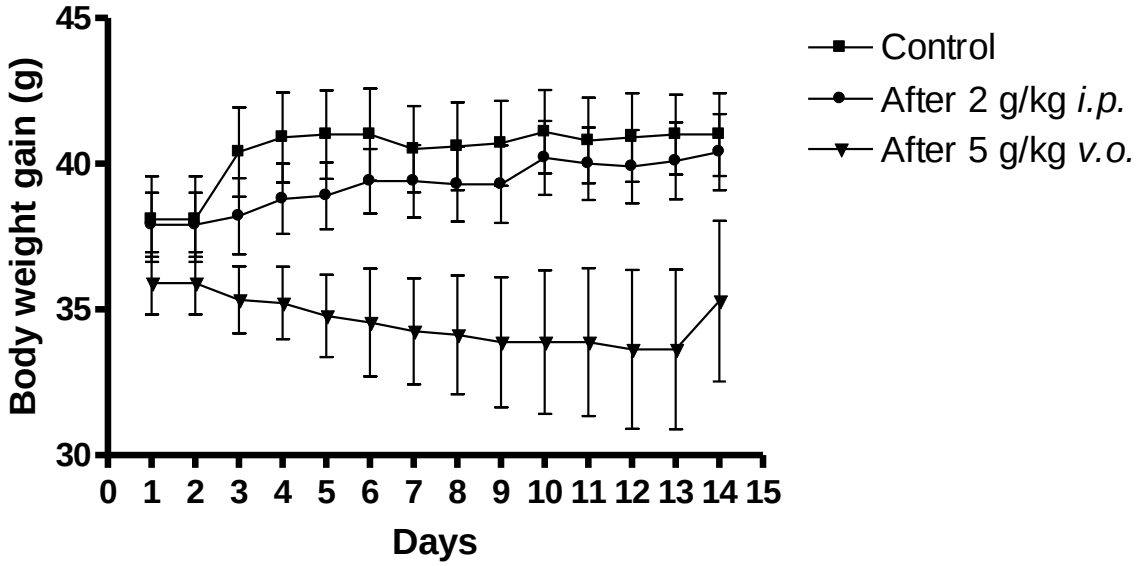

Figure 1. Body weight gain for animals treated with Nv-EtOH during 14 days (n= 10).

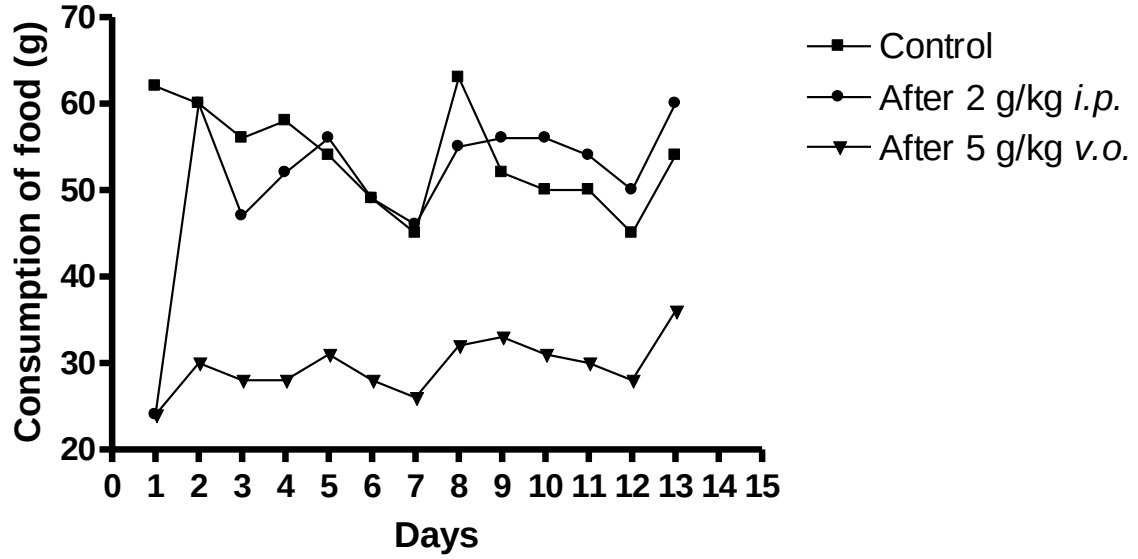

Figure 2. Consumption of food for animals treated with Nv-EtOH during 14 days (n= 10).

been used as an indicator of adverse effects of drugs and chemicals, especially if weight loss is greater than 10% of initial weight in the mice (Subramanion et al., 2011). Food and water consumption also showed significant alterations (Figures 2 and 3).

The amount of food consumed at the end of the experiment was of 53.69 ± 1.64 g for the control group, while the treated group was of 29.62 ± 0.86 g ($p < 0.001$). The amount of water consumed at the end of the experiment was of 94.38 ± 3.15 ml for the control group, while the treated group was of 65.77 ± 3.41 ml ($p < 0.001$). The analysis of food and water intake in animal experimentation is important to investigate the safety of substances studied for therapeutic purposes (Mukinda and Eagles, 2010).

Behavioral screening

The behavioral screening of animals was measured at 0.5, 1, 2, 3 and 4 h after administration of Nv-EtOH 2.0 and 5.0 g/kg (body weight) intraperitoneally and orally,

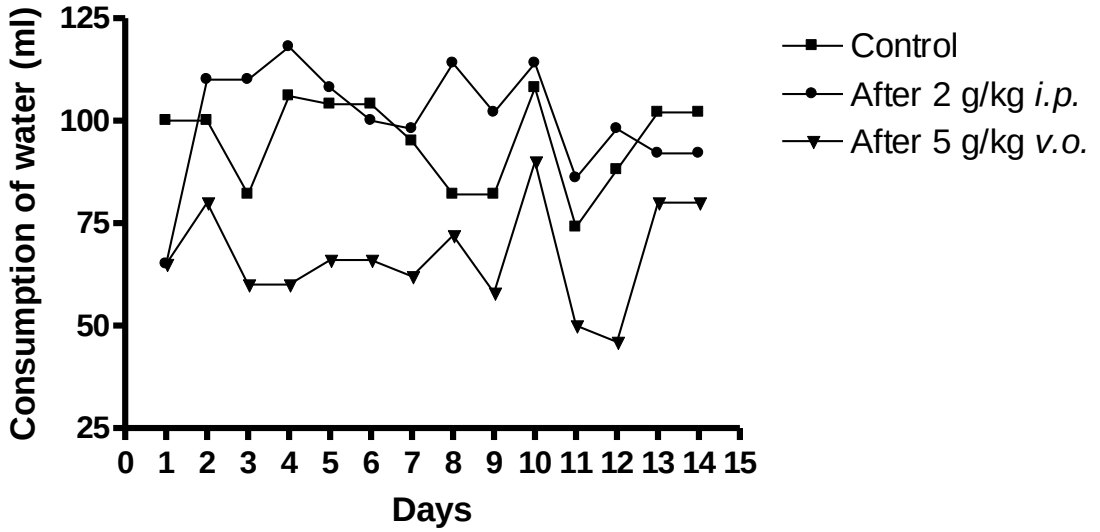

Figure 3. Consumption of liquid for animals treated with Nv-EtOH during 14 days (n= 10).

Table 2. Hematological parameters of blood of *Swiss* mice treated with 2.0 g/kg intraperitoneally and 5.0 g/kg orally of crude ethanol extract of *Neoglaziovia variegata* after 14 days (acute toxicity).

Parameter	Group		
	Control	After 2 g/kg *i.p.*	After 5 g/kg *v. o.*
Erythrocytes (10^6/mm^3)	8.47 ± 0.10	8.50 ± 0.08	8.62 ± 0.15
Hemoglobin (g/dL)	12.90 ± 0.20	13.03 ± 0.23	13.13 ± 0.12
Hematocrit (%)	34.40 ± 0.66	35.05 ± 0.32	34.70 ± 0.18*
MCV (μ^3)	41.20 ± 0.48	42.35 ± 0.46	40.40 ± 0.84
MCH (μg)	15.77 ± 0.21	15.70 ± 0.19	15.25 ± 0.16
MCHC (%)	37.40 ± 0.33	37.37 ± 0.61	37.93 ± 0.47
Leukocytes (10^3/mm^3)	2.98 ± 0.16	2.98 ± 0.21	3.11 ± 0.15
Lymphocites (%)	63.23 ± 2.00	69.73 ± 0.58*	62.01 ± 2.62
Monocytes (%)	1.45 ± 0.41	1.57 ± 0.26	2.25 ± 0.25*
Platelets (10^3/mm^3)	471.50 ± 48.66	447.00 ± 65.91	341.50 ± 59.31

Values are mean ± S.E.M, n = 8. *p < 0.05; Student's *t*-test at 5% probability.

changes in behavior. In addition, the animals did not show any sign of toxicity or change in behavioral or other physiological activities.

Hematological and biochemical parameters analysis of blood

Analyzing the results of hematological parameters (Table 2), it is possible to observe that after administration of doses of 2.0 g/kg *i. p.* and 5.0 g/kg *v. o.*, there was no significant variation in these parameters (red blood cells, hemoglobin, MCV, MCH, MCHC, leukocytes and platelets); only a few changes were found statistically significant ($p < 0.05$) in hematological parameters such as increase of percentage of hematocrit, lymphocytes and monocytes. However, the clinical significance of this increase is being investigated. This analysis is very important because the hematopoietic system is highly sensitive to toxic substances in the blood, serving thus as an important parameter to analyze the physiological and pathological status in animals (Adeneye et al., 2006).

Regarding the biochemical parameters, the results show that the glucose, cholesterol, triglycerides, aspartate amino transferase (AST/TGO), creatinine and urea have not statistical significance compared to the control group (Table 3). The levels of alanine amino transferase (ALT/TGP) after the dose of 2.0 g/kg *i. p.* were significantly lower than the control group ($p < 0.05$). The animals treated with a dose of 5 g/kg *v. o.* showed increased levels of AST and creatinine, although this was not statistically significant. AST and creatinine are good

Table 3. Biochemical parameters obtained from the serum of *Swiss* mice treated with 2.0 g/kg intraperitoneally and 5.0 g/kg orally of crude ethanol extract of *Neoglaziovia variegata* after 14 days (acute toxicity).

Parameter	Group		
	Control	After 2 g/kg *i. p.*	After 5 g/kg *v. o.*
Glucose (mg/dL)	131.00 ± 10.08	132.00 ± 8.94	141.00 ± 16.67
Cholesterol (mg/dL)	118.20 ± 8.20	107.70 ± 6.32	121.80 ± 5.74
Triglycerides (mg/dL)	142.80 ± 23.64	135.70 ± 19.08	148.80 ± 8.98
AST/GOT (U/L)	170.00 ± 11.07	197.30 ± 18.25	236.70 ± 28.26
ALT/GPT (U/L)	84.93 ± 5.82	65.72 ± 5.22*	86.40 ± 12.00
Urea (mg/dL)	73.50 ± 6.81	63.83 ± 3.16	69.75 ± 7.47
Creatinine (mg/dL)	0.73 ± 0.09	0.62 ± 0.05	0.85 ± 0.02

Values are mean ± S.E.M, n = 8. *p < 0.05; Student's *t*-test at 5 % probability.

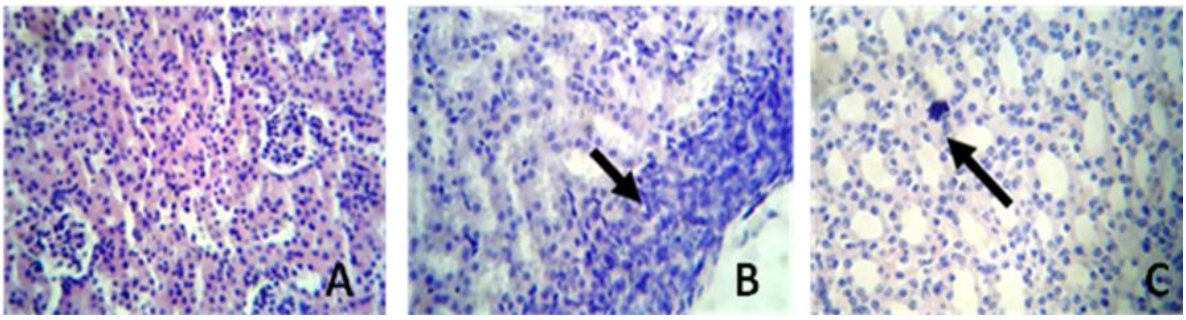

Figure 4. Photomicrographs of kidney histopathology from representative animals. (A) Control group, (B) Nv-EtOH (2 g/kg *i. p.*) and (C) Nv-EtOH (5 g/kg *v.o.*) (hematoxylin-eosin stain).

indicators of liver and kidney functions, respectively. Alterations in their levels suggest alterations in these organs. Transaminases (AST and ALT) are liver enzymes located within the hepatocytes and are used as biochemical markers to potentially toxic substances, since their biochemical values are high when an injury occurs in the liver parenchymal (Rahman et al., 2001).

Histopathological analysis

Macroscopic examination of the organs of the animals treated with extract showed no changes in color compared to the control. After the morphological analysis, the kidney tissue showed perivascular infiltrates and parenchymal cells of varying intensity, with expansive characteristics well defined, discrete lesions restricted, but multifocal lesions in large and extensive areas, and the presence of edema, areas of necrosis and the appearance of a mass of disorganized cell growth and intense (Figure 4).

In the liver, it was evident cellular infiltrates were evident, with the same characteristics, injuries ranging from mild to more extensive perivascular and parenchymal liver. In some animals the lesions are quite clear and expansive nature (Figure 5). These microscopic changes were present in both groups treated with doses of 2 g/kg *i. p*. and 5 g/kg *v. o*. The data obtained showed that the doses did not induce death of animals, but may lead to morphological alterations in the liver and kidney.

Toxic effects of substances can reach all systems and organs, however, the liver and kidneys are the organs most commonly affected because the kidneys are the organs responsible for excretion of metabolic wastes and control of homeostasis. Moreover, the liver is the main organ for drug metabolism and detoxification.

Conclusions

The present study was designed to further investigate the antioxidant activity and toxicity of ethanolic extract of *N. variegata* by using acute toxicity analysis. To our knowledge, this species is being investigated for the first time.

N. variegata could be a good source of antioxidant phenolics. It was demonstrated that the extracts contains high content of phenolic compounds and flavonoids. The antioxidant activity presented by the extracts is related to the presence of these compounds.

Based on the results presented, we conclude that the Nv-EtOH may be considered of low toxicity, since the

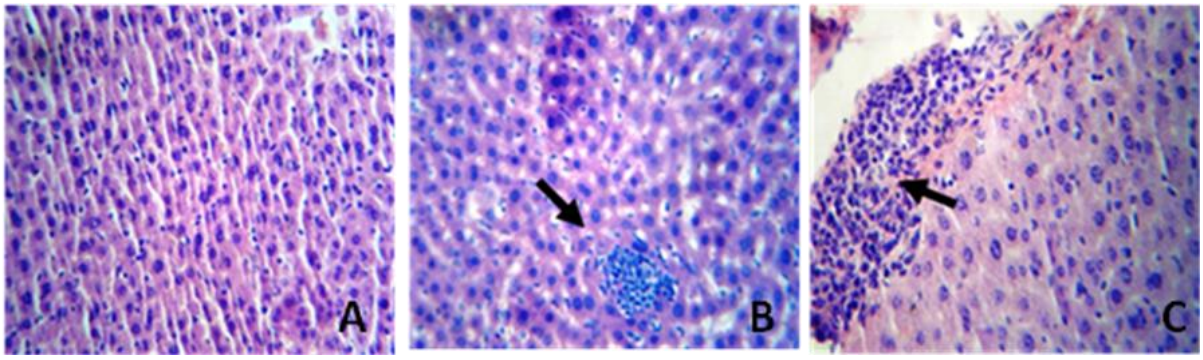

Figure 5. Photomicrographs of liver histopathology from representative animals. (A) Control group, (B) Nv-EtOH (2 g/kg *i.p.*) and (C) Nv-EtOH (5 g/kg *v. o.*) (hematoxylin-eosin stain).

acute administration intraperitoneally and orally produced no deaths or signs of toxicity in animals. The extract not induced significant alterations in almost all biochemical and hematological parameters observed. The levels of AST and creatinine were altered, indicating that there may be potential liver and kidney damage. This hypothesis was confirmed by histopathological analysis of organs. However, further long-term toxicological studies (chronic toxicity) are needed in order to establish it as medicine.

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