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<th>Affiliation</th>
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</thead>
<tbody>
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</tr>
</tbody>
</table>
ARTICLES

Anti-inflammatory and non ulcerogenic activities of acetylbergenin
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José F. de Sousa, Haroldo S. Ripardo Filho, Giselle S. Pinheiro Guilhom,
Lourivaldo S. Santos, Pergentino J. Cunha Sousa, Bruno G. Pinheiro,
Hugo A. S. Favacho and Jose C. Tavares Carvalho

Phytochemical screening and toxicity profiles of crude extracts of
Cissus quadrangularis L. and Solunum incanum L. in mice
Teka Feyera, Solomon Assefa, Endalkachew Mekonnen and
Abi Legesse
Anti-inflammatory and non ulcerogenic activities of acetylbergenin


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Endopleura uchi (Huber) Cuatrec. (Humiriaceae), the Brazilian Amazon plant, is used in folk medicine to treat arthritis and gastric ulcer. Bergenin, one of the chemical constituents of E. uchi, has anti-inflammatory properties. Its acetylation results in acetylbergenin, which is extracted to investigate its potential anti-inflammatory and anti-ulcer properties using an assay for croton oil-induced ear edema, rat paw edema induced by carrageenan and dextran, carragenin-induced peritonitis, and stress-induced gastric ulcer. In ear erythema induced by croton oil, acetylbergenin presented a significant 75.60% inhibition (p<0.001). The oral administration of 6.8 mg/kg of acetylbergenin significantly inhibited the carrageenan-induced edema formation by 35.09% (p<0.05) and the dextran-induced edema by 33% (p<0.05). The migration of neutrophils toward the peritoneal cavity was inhibited in acetylbergenin (6.8 mg/kg) treated animals by 70% (p<0.01). In the stress-induced gastric ulcer, acetylbergenin inhibited 78.55% of gastric lesions. The results suggest that, the anti-inflammatory action of acetylbergenin appears to be dependent on cyclooxygenase (COX-2) inhibition. Furthermore, although the anti-inflammatory activity of acetylbergenin is a characteristic of nonsteroidal compounds, it causes little deleterious interference in the gastric mucosa.

Key words: Endopleura uchi, bergenin, acetylbergenin, anti-inflammatory, antiulcerogenic activity.

INTRODUCTION

Endopleura uchi (Huber) Cuatrec. (Humiriaceae) is a species from the Brazilian Amazon, found in wild forests, scattered throughout the Amazon Basin, where the plant is commonly known as uchi, uxi, or uxi-pucu.

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(Cuatrecasas, 1961). *E. uchi* is widely used by the people to combat myoma and arthritis, although only few biological studies are available on the properties of its extract. bergenin (1) is a C-glucoside of 4-O-methyl gallic acid that has been isolated as the major component from the cortex of *E. uchi* and is used for treating gastrointestinal diseases such as gastritis, gastric ulcer, diarrhea, and constipation (Okada et al., 1973; Abe et al., 1980). In addition, studies report that bergenin reveals anti-inflammatory (Swarnalakshmi et al., 1984; Nunomura et al., 2009), antiarthritic (Nazir et al., 2007) and hypolipidemic effects (Jahromi et al., 1992). In our laboratory, bergenin (1) was isolated as the principal component from the aqueous extract of the *E. uchi* cortex and acetylbergenin (2) was obtained by acetylation of bergenin in order to increase its lipophilic and physiological activities. In the present study, the anti-inflammatory and ulcerogenic activities of acetylbergenin in various experimental models *in vivo* were investigated.

**MATERIALS AND METHODS**

**Extraction and isolation of bergenin (1)**

The extraction and isolation of bergenin (1) were carried out according to Borges et al. (2011). Air-dried powdered bark (1.6 kg) of *E. uchi* was extracted at room temperature with 10 L of distilled H₂O for 6 days. After filtration, the extract was lyophilized to obtain 89 g of the aqueous lyophilized extract. The lyophilized extract (5 g) was fractionated on silica gel (70-230 mesh, 125 g) column resulting in 66 fractions, after elution with n-hexane (100%), n-hexane/EtOAc (20, 25, 27.5, 30, 35, 40, 45, 50, 55, 60, 65, 70, 80 and 90%) mixtures, EtOAc (100%), EtOAc/MeOH (5, 10, 20, 40, 50, 70, and 80%) mixtures and MeOH (100%). Fraction eluted with EtOAc (100%), EtOAc/MeOH (10%) and EtOAc/MeOH (20%) were combined affording bergenin (1) (1.02 g). Bergenin was purified by recrystallization from methanol and identified by comparison of its physical and spectroscopic data (IR, 1H and 13C NMR) with those reported in the literature (Ramaiah et al., 1979).

**Acetylation of bergenin**

The method described by Borges et al. (2011) was employed in this assay. In a round-bottomed flask of 125 mL, 700 mg of bergenin (1), 17.5 mL of acetic anhydride (Ac₂O, 100% degree of purity, Synth, Brazil) and 6.5 mL of anhydrous pyridine (100% degree of purity, Synth, Brazil) were added. After agitation, the mixture was maintained for 24 h at room temperature and then transferred to a separator funnel of 125 and 25 mL of distilled water was added and the mixture was extracted with ethyl acetate (3 × 40 mL). The organic phases were collected, washed with distilled water (2 × 40 mL), 5% hydrochloric acid solution (1 × 40 mL) and then with distilled water (2 × 40 mL) until neutral pH was obtained. The organic phase was dried with anhydrous Na₂SO₄ and after filtration, the solvent was evaporated at room temperature in a chapel. The solid material obtained in the form of white crystals was recrystallized in methanol resulting in 1.13 g (yield 99%) of crystals of acetylbergenin (2) with 99% degree of purity.

**Animals**

Swiss albino mice (*Mus musculus*) male adults, weighing between 20 and 25 g and male Mac Coy rats (180 to 200 g), from the Evandro Chagas Animal Hospital of Belém, PA, Brazil, were used in this study. Male albino Wistar rats weighing between 180 and 200 g from the Multidisciplinary Center for Biological Research in the Laboratory Animal Science Area (Multidisciplinary Center for Biological Investigation in the Area of Science in Laboratory Animals) of the Faculty of Medical Sciences of Unicamp, Campinas, SP, were used in several experiments. These animals had to fast for 12 h before the experiments, and were allowed free access to water. The animals were housed in polyethylene boxes with a capacity to accommodate 5 rats or 10 mice, in an acclimatized room [22±2°C, 55±5% relative humidity (RH)], with periods of light and darkness of 12 h each, automatically controlled. The experimental procedures and use of animals was approved by the Animal Experimentation Ethics Committee of UFPA (Process MED 010/2008) in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

**Drugs, chemicals and dose used**

For the accomplishment of the experiments, the dose of acetylbergenin was based on the ED₅₀ of 6.8 mg/kg previously determined by Borges et al. (2011). Acetylbergenin was dissolved in 0.2 mL of 2% DMSO (Sigma Chemical Co., USA) and 5% Tween-80 solution (Merck, Brazil). Negative control groups received the same solution used to solubilize the substance acetylbergenin. The drugs used in the experiments were: dexamethasone (0.5 mg/kg, MSD Co., Brazil), indomethacin (5 mg/kg, MSD Co., Brazil), acetylsalicylic acid (100 mg/kg, Bayer, Brazil), and ciproheptadine hydrochloride (Chemical Co., USA) were dissolved and diluted in 0.9% physiological solution. Croton oil (2.5%, Sigma Chemical Co., USA) was solubilized in acetone (Synth, Brazil). The total volume of solution administered orally was 0.25 mL for mice and 0.5 mL for rats.

**Croton oil-induced dermatitis**

The method described by Tubaro et al. (1985) was used in this experiment. Cutaneous inflammation was induced in several groups of mice by applying 0.1 mL (1 mg/paw) of croton oil solution in acetone on the surface of the right ear. The same volume of acetone was applied to the left ear. One hour before the application, three groups of mice (n=10/group) were orally treated with acetylbergenin (6.8 mg/kg, 0.25 mL), vehicle (0.25 mL, 2% dimethyl sulfoxide (DMSO) and 2% Tween-80 in distilled water, control group, or acetylsalicylic acid (100 mg/kg, 0.25 mL). Six hours later, the mice were submitted to euthanasia and the anti-inflammatory effect was evaluated. Samples of 6 mm in diameter were extracted using a punch biopsy, and the weight difference between the samples of the control ear (left) and the croton oil-treated ear (right) was calculated. The results obtained are represented in weight (mg).

**Carrageenan-induced paw edema in rats assay**

Edema was induced by intraplantar injection of 1% carrageenan (100 µg/paw, 0.1 mL, Sigma Co., USA) into the right paw of Mac Coy rats (n = 5/group). A saline sample of equal volume was injected in the left paw (0.1 mL). The distinct experimental groups were treated with vehicle (2% DMSO and 2% Tween-80 in distilled water, negative control, 0.5 mL), acetylbergenin (6.8 mg/kg, 0.5 mL), or indomethacin (positive control, 10 mg/kg, 0.5 mL), and after 60 min they received intraplantar injections of carrageenan in the right hind paw and saline in the left hind paw. A digital pachymeter (Zaa Precision, Mitutoyo Co., Japan) was used to...
determinate the paw diameter at 1 h intervals after stimulus application over 6 h. The amount of edema was calculated by subtracting the measured volume of the paw injected with saline from the measured volume of the paw injected with carrageenan.

**Dextran-induced paw edema in rats assay**

The paw edema was induced by dextran in rats, following the method described by Carvalho et al. (1999). The Mac Coy rats were randomly divided into three groups (n = 5/group). A volume of 0.1 mL of 1% dextran (100 µg/paw, 0.1 mL, Sigma Co., USA) solution was injected on the plantar surface of the right hind paw in rats pretreated 60 min earlier with vehicle (2% DMSO and 2% Tween 80 in distilled water, 0.5 mL, control group, p.o.), acetylbergenin (6.8 mg/kg, 0.5 mL, p.o.) or the reference drug cyproheptadine (10 mg/kg, 0.5 mL, p.o.). The inflammation was quantified by measuring the volume (mL) displaced by the paw using a digital pachymeter (Zaas Precision, Mitutoyo Co., Japan) at 0, 30, 60, 90, and 120 min after dextran injection. Results were expressed as variation in volume (mL) between the right and left paws at each time.

**Carrageenan-induced peritonitis in rats**

Different groups of rats (n=8/group) were treated with acetylbergenin (6.8 mg/kg, p.o., 0.5 mL), dexamethasone (0.5 mg/kg, p.o., 0.5 mL) or vehicle (2% DMSO and 2% Tween-80 in distilled water, p.o., 0.5 mL) administered 30 min before the stimulus injection (100 µg/mL carrageenan, 4 mL intraperitoneally). All groups were given an injection of carrageenan and cell migration was evaluated 4 h later. The cell migration analysis was based on the methods described by Carvalho et al. (1999). The results obtained in the differential count were expressed as the number of neutrophils per milliliter of exudates.

**Stress-induced acute gastric ulcer**

Ulcers were induced according to the method described by Basile et al. (1990). Wistar rats were fasted with free access to water for 24 h and were further treated with vehicle (2% DMSO and 2% Tween-80 in distilled water 0.5 mL, p.o), acetylbergenin (6.8 mg/kg, 0.5 mL, p.o), and indomethacin (10 mg/kg, 0.5 mL, p.o). Groups of five animals each were treated and 30 min later, each animal was kept for 17 h in a contender tube, which was immersed vertically until the water reaching the neck region of the animal in a tank with current water at 25°C. Furthermore, the rats were submitted to euthanasia by CO2 inhalation. Their stomachs were immediately excised, opened by cutting along the greater curvature, and the inner wall was examined for lesions using a binocular stereomicroscope with a magnification of 10x (Nikon SMZ-10). The number and the severity of the acute lesions were enumerated and graded as follows: light (1+) = presence of hyperemia and single mucosal punctiform hemorrhages (petechiae); moderate (2+) = presence of submucosal hemorrhagic lesions with small erosions; severe (3+) = presence of hemorrhagic edges with severe erosions and some invasive lesions. A lesion index was determined following the formula reported by Basile et al. (1990).

**Statistical analysis**

Results were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using Student’s t-test and analysis of variance (ANOVA), followed by Student-Newman-Keuls. A value of p<0.05 was considered as statistically significant. The analysis was performed using a GraphPad Prism 5.0 program.

**RESULTS**

**Bergenin and acetylbergenin**

Compounds 1 and 2 (Figure 1) were identified as bergenin and acetylbergenin, respectively, by the aid of 1H-1H, 1H-13C COSY, DEPT, and HMBC spectra, and by comparison of its NMR spectral data with those related to the literature (Ramaiah et al., 1979; Borges et al., 2011).
**Croton oil-induced dermatitis**

Pretreatment with acetylbergenin (6.8 mg/kg, p.o.) inhibited 75.42% of the ear edema formation induced by croton oil injection (p<0.001) when compared with the control group. This inhibition of the edematogenic process was similar to that observed with the group treated with acetylsalicylic acid 100 mg/kg (positive control) decreasing the inflammatory process by 78.53% (Figure 2).

**Carrageenan-induced paw edema in rat**

Carrageenan injection in the animal paws produced a visible and measurable edema, with maximum inflammation observed 4 h after the injection of the inflammatory agent. The group treated with acetylbergenin at a dose of 6.8 mg/kg inhibited the edema formation over the 6 h of the experiment (Figure 3). The maximum edema inhibition was 35.09% (p<0.05, Student’s test, and ANOVA).

**Dextran-induced paw edema in rats assay**

Dextran 1%–induced intense paw edema in rats, an effect that reached a maximum level at 1 h after administration and decreased over the subsequent hours. The oral administration with 6.8 mg/kg of acetylbergenin inhibited the dextran-induced edema by 33% (p<0.05). The reference drug cyproheptadine (10 mg/kg, p.o.) significantly (p<0.05) inhibited the dextran-induced paw edema at 30, 60, 90, and 120 min after administration when compared with the control (Figure 4).

**Carrageenan-induced peritonitis in rats**

In this model of carrageenan-induced leukocyte migration, it was possible to observe an acute inflammatory response in the peritoneal cavity of rats by neutrophil concentration of $3893.25 \times 10^6$ cells/mL after 4 h. The acetylbergenin (6.8 mg/kg, p.o.) was able to significantly reduce the carrageenan-induced neutrophil count (70%), when compared with the control group treated with distilled water (Figure 5). Treatment of the animals with dexamethasone (0.5 mg/kg, p.o.) 1 h before the experiment, used as a positive control, significantly reduced (94.23%) the cell migration.

**Stress-induced acute gastric ulcer**

In the stress ulcer experiment, the animals treated with indomethacin (10 mg/kg, p. o) produced more lesions when compared with those treated with acetylbergenin at a dose of 6.8 mg/kg (p.o) (Table 1). Acetylbergenin at a dose of 6.8 mg/kg has revealed significant effect with a ulcer index of 10.08 and protection of 78.55% when
Figure 3. Effect of the p.o. administration of acetylbergenin (6.8 mg/kg), indomethacin (5 mg/kg) and only vehicle (2% DMSO and 2% Tween-80 in distilled water) on rat paw edema, induced by the intraplantar injection of carrageenan (1000 µg/paw). The data is expressed as mean±SEM of five animals, *p<0.05, compared to the control group, Student's "t" test.

Figure 4. Effect of the p.o. administration of acetylbergenin (6.8 mg/kg), cyproheptadine (10 mg/kg) and control (2% DMSO and 2% Tween-80 in distilled water) on rat paw edema induced by the intraplantar injection of dextran (1000 µg/paw). The data is expressed as mean±SEM of five animals; *p<0.05, compared to the control group, Student's "t" test.
**DISCUSSION**

This study aimed to investigate the anti-inflammatory and antiulcerogenic activities of acetylbergenin using various experimental models in vivo. Acetylbergenin was extracted by acetylation of bergenin, according to Borges et al. (2011). It is well known that several plants in nature comprise a huge reservoir of bioactive molecules that can be developed as new chemical entities, analogs, derivatives, and synthetic compounds to form a natural product (Rastogi and Rawat, 2008).

Experimental models using carrageenan as an inflammatory agent are widely used to investigate the pathophysiology of the inflammatory response, as well as to characterize the novel anti-inflammatory drugs (Tobacman, 2001). Carrageenan induces a measurable local inflammatory response. This model of paw edema is most frequently used to evaluate the effects of anti-inflammatory drugs. This model presents two inflammatory phases and a third, uncharacteristic one. In the first hour after the carrageenan injection, an increase in vascular permeability mediated by histamine and serotonin is observed. In the second hour, the permeability increase is caused by kinines. In the third hour, the increase of vascular permeability occurs due to prostaglandin action (Perazzo et al., 2005).

The data presented in this study revealed that pretreatment of the animals with acetylbergenin at a dose of 6.8 mg/kg, 1 h prior to the intraplantar injection of carrageenan, inhibited the development of paw edema at all evaluation times; however, this inhibition was higher during the second and third peaks (2 and 3 h), revealing the participation of prostaglandins in the third hour. Indomethacin (10 mg/kg, p.o.), considered as a nonspecific cyclooxygenase inhibitor, also significantly compared with indomethacin group (p<0.001).

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<th>Treatment</th>
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<th>Ulcer index</th>
<th>Curative ratio (%)</th>
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<td>Control</td>
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<td>0.6±1.34</td>
<td>0.4±0.89</td>
<td>0.6±0.40</td>
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<td>Acetylbergenin</td>
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<td>6.8±1.39</td>
<td>9.2±2.15</td>
<td>8.4±2.29</td>
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<td>Indomethacin</td>
<td>5</td>
<td>10</td>
<td>16±3.50</td>
<td>13.6±5.73</td>
<td>32.6±5.73</td>
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***P<0.001 when compared with indomethacin, Student’s t-test and ANOVA.
reduced the volume of carrageenan-induced paw edema at all evaluation times, compared to the respective negative control used. In order to demonstrate the participation of prostaglandins in carrageenan-induced edema, some authors have confirmed that pretreatment with aspirin, a nonsteroidal anti-inflammatory drug, promotes endogenous prostaglandin and edema reduction, whereas administration of high doses of prostaglandin E2 (PGE2) or prostacyclin elicited an increase in paw edema (Lewis et al., 1975; Vane and Botting, 1987).

Another phlogistic agent used in the present study was dextran. This substance is a high molecular weight polysaccharide that induces an anaphylactic reaction, characterized by extravasation and formation of edema with little protein and few neutrophils, besides the degranulation of mast cells and subsequent release of histamine and serotonin (Vinegar et al., 1969). Thus, it was possible to observe that in the dextran-induced edema, oral administration of 6.8 mg/kg of acetylbergenin inhibited the edema at the initial stage (1 and 2 h), where the degranulation of inflammatory cells occurred with a release of biogenic amines, serotonin, and histamine.

Swarnalakshmi et al. (1984) reported the anti-edematogenic activity of bergenin, with a dose dependent inhibition in the third hour, with 44.1, 53.6, and 65.5% inhibition at oral doses of 60, 120, and 240 mg/kg, respectively; however, considering this data, it is possible to verify that acetylbergenin was more effective at an oral dose of 6.8 mg/kg, since at this dose it was able to cause 47% edema inhibition induced by carrageenan in the third hour besides being significant in other instances.

Acetylbergenin activity on leukocyte migration was studied in the carrageenan-induced peritonitis model in rats. Oral administration of acetylbergenin (6.8 mg/kg) inhibited neutrophil cell migration by 70%, following the profile similar to that of dexamethasone which caused 94.23% inhibition. This model of acute inflammation allows the quantification of leukocytes that migrate to the peritoneal cavity under the action of chemotactic agents, mainly leukotrienes LTB₄ (Bastos et al., 2001).

Therefore, in general, the initial process of the acute inflammatory response is characterized by the increase of neutrophils in the circulating blood, being the first line of physiological defense, followed by lymphocytes and monocytes (Male, 2003). The mode of action of carrageenan in inducing the leukocyte migration may be a result of synergism between PGE2, LTB₄, and other potent chemotactic agents such as complement (C5a) and interleukins (IL-8), promoting vasodilation, plasma exudation, and leukocyte accumulation in the lesion sites (Thomazzi et al., 2009). Hence, it is possible that nonsteroidal anti-inflammatory drugs (NSAIDs), by inhibiting the synthesis of vasodilatory PGE2, promote blood flow reduction by compromising the leukocyte migration to the area of inflammatory reaction (Almeida et al., 1980). The results suggest that the anti-inflammatory activity of acetylbergenin is related to the biosynthesis of prostaglandins and lipoxygenase products.

While testing dermatitis induced by the topical application of croton oil, the activation of phospholipase A₂, releasing arachidonic acid with consequent biosynthesis of leukotrienes and prostaglandins, by cyclooxygenase and lipoxygenase pathways, respectively, was observed. This dermatitis is sensitive to the action of topical anti-inflammatories, also responding to the systemic administration of steroidal anti-inflammatory drugs (Tubaro, 1986). Acetylbergenin at a dose of 6.8 mg/kg significantly inhibited (p<0.001) the dermatitis and presented a similar result to the nonsteroidal anti-inflammatory acetylsalicylic acid (100 mg/kg).

On the evaluation of the ulcerogenic effect of 6.8 mg/kg acetylbergenin, the substance produced 78.55% less ulcerative damages. In addition, 1+ and 3+ (hemorrhagic) gastric lesions in animals submitted to stress were significantly reduced by treatment with 6.8 mg/kg of acetylbergenin when compared with indomethacin. These results suggest that, despite having performed significantly on carrageenan edema, however, did not show an ulcerogenic effect such as indomethacin, which is a nonspecific cyclooxygenase inhibitor. This effect was like that found for bergenin, at a dose of 30 mg/kg, i.p., when it exerted a non-ulcerogenic effect in rats submitted to the stress-induced gastric ulcer test (Abe et al., 1979).

Gastric cytoprotection is conferred by certain substances, such as prostaglandins. The clinical use of traditional NSAIDs for treating inflammation and pain is often accompanied by adverse gastrointestinal effects. The pharmacological effects of NSAIDs are due to the inhibition of a membrane enzyme called cyclooxygenase (COX), which is involved in the prostaglandin biosynthesis. There are two isoforms, COX-1 and COX-2, which share the same substrates, produce the same products, and catalyze the same reaction using identical catalytic mechanisms, but differ in inhibitor selectivity. The isoform, COX-1, chiefly plays a physiological role in the kidneys and the stomach, whereas COX-2, induces inflammatory conditions and is involved in the production of prostaglandins that mediate pain. Inhibition of COX-1 is responsible for the adverse gastrointestinal and renal effects of NSAIDs, while the inhibition of COX-2 accounts for NSAIDs therapeutic effects. All classical NSAIDs, such as aspirin and indomethacin are nonselective inhibitors of both COX-1 and COX-2, but bind more tightly to COX-1 (Alanazi et al., 2015).

Chung et al. (2001) reported that acetylbergenin, presented greater activity in vivo when compared with bergenin, against the hepatotoxicity in rats. Notably, acetylbergenin is more readily absorbed due to its ability to cross the bilayer of intestinal cell membranes, resulting in an increase in the protective activity after being hydrolyzed into a hydrophilic polyphenol such as norbergenin and bergenin.
Other studies have demonstrated the importance of adding an acetyl radical to a molecule for anti-inflammatory activity (Carvalho et al., 1999). This may be partially explained by the high specificity of acetylated anti-inflammatory compounds (Souza et al., 2004), such as aspirin, which inhibit prostaglandin synthesis by inactivating COX. Aspirin selectively acetylates the hydroxyl group of a serine residue (Ser 53), among the terminal 70 amino acids of the PGEs enzyme chains. Acetylation leads non-selectively to the irreversible inhibition of isoenzymes (COX-1 and COX-2) (Cerella et al., 2010).

Conclusion

The anti-inflammatory action of acetylbergenin appears to be dependent on COX-2 inhibition. Furthermore, although the anti-inflammatory activity of acetylbergenin is a characteristic of nonsteroidal compounds, it causes little deleterious interference in the gastric mucosa. Based on these results, it was concluded that acetylbergenin has a potential anti-inflammatory activity. The addition of five acetyl groups, from the natural prototype, increased the anti-inflammatory activity of bergenin compared to the original isocoumarin bergenin, as changes in the molecule were associated with changes in the anti-inflammatory properties. Nevertheless, detailed investigations are still necessary in order to study the relationship between the structure and pharmacological activity of acetylbergenin, since its results were quite promising.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

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Full Length Research Paper

Phytochemical screening and toxicity profiles of crude extracts of *Cissus quadrangularis* L. and *Solunum incanum* L. in mice

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³Department of Basic Sciences, College of Medicine and Health Sciences, Jigjiga University, Jigjiga, Ethiopia. 
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Plant derived bioactive molecules are always in demand and are the main focus of research. Despite the growing market demand for herbal medicines, safety of medicinal plants is still a concern. The present work aimed at generating preliminary phytochemical and biosafety information of crude methanolic and chloroform extracts of *Cissus quadrangularis* L. (aerial parts) and *Solunum incanum* L. (fruit). Both plants are extensively used by traditional healers among the agro-pastoralist communities of Fafan Zone in Eastern Ethiopia. The investigation employed standard phytochemical screening procedures and acute (2000mg/kg single dose) and sub-acute (200 and 400mg/kg repeated doses) oral toxicity studies in Swiss albino mice. Changes in body weight, packed cell volume (PCV), Hb level and mortality were recorded to evaluate the toxicity profile of the crude extracts. The phytochemical study revealed the presence of several secondary metabolites in both plants. The acute toxicity study did not show extracts related mortality and body weight reduction at 2000 mg/kg of methanol and chloroform extracts of both plants. However, the sub-acute toxicity study exhibited that crude extract of fruit of *S. incanum*(400 mg/kg) showed relatively higher toxic effects of causing a more pronounced (p<0.05) mortality, body weight loss, and reduction in PCV and Hb levels, compared to negative control. Extracts of *C. quadrangularis* revealed low mortality and a marginal increase of the hematological parameters. A detailed experimental analysis of these herbs extensively used by the agro-pastoralists of the area is essential to establish their therapeutic value and safety in use.

**Key words:** Cissus quadrangularis, Solunum incanum, phytochemical, toxicity.

INTRODUCTION

Therapeutic use of plants dates back to human civilization and continuous efforts are being made

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towards its improvement. About 200,000 different types of natural products are known, which are plant origins. Moreover, many more are being identified from higher plants and microorganisms (Mosihuzzaman, 2012; Kinghorn et al., 2011). Some plant-based drugs have been used for centuries and for some such as cardiac glycosides, there is no alternative conventional medicine. Therefore, medicinal plants and their bioactive molecules are always in demand and are the main focus of research. This led to the recent (WHO, 2011) surge in the demand for herbal medicine.

To date, herbs have remained useful not only as remedy for different diseases that affect humans and animals, but also as good starting points for the discovery of bioactive molecules for drug development. The medicinal importance of a plant is due to the presence of some endogenous substances like alkaloids, glycosides, resins, volatile oils, gums, tannins, and others in one or more parts of the plant (Himesh et al., 2011). Despite the growing market demand for herbal remedies, there are still concerns associated with not only their use, but also their safety. The herbal products which are standardized to identify their bioactive metabolites is less than 10% of herbal products available in the world market. Moreover, strict quality control measures are not always meticulously followed (Cragg et al., 2005).

In Fafan zone of Ethiopian Somali Regional State, two well-known medicinal plants called Cissus quadrangularis and Solanum incanum (solanaceae) are extensively utilized by traditional healers of the locality (Feyera et al., 2017). C. quadrangularis, which belongs to Vitaceae family, is a succulent perennial plant commonly found in tropical and subtropical xeric wood (Kavitha and Manimekalai, 2015). The plant is known by a local name in Somali called ‘Gaad’ and traditionally indicated for the treatment of different human and livestock ailments in the area. In humans, it is traditionally claimed to be effective in the management of helminthiasis, anorexia, skin diseases, hemorrhage, swellings, anemia, burns and wounds whereas in livestock, it was reported to have been used traditionally to treat tick infestation, lice infestation, wounds, leach infestation, bites of poisonous insects, skin sores/saddle sores (donkeys and camels), retained placenta and helminthiasis (Njoroge and Bussman, 2006; Rao and Merugu, 2013; Bharti et al., 2014).

S. incanum locally called ‘kiriir’, similarly serves several medicinal values in the studied area against both livestock and human health problems. It has been reported to be used for the treatment of such health problems in livestock as tick, mange and lice infestations, ringworm infection, and hyena or jackal bites, non-specific wounds, to manage infertility, swollen joints, retained placenta and other reproductive purposes. Different plant parts are also widely used in human for the treatment of skin problems, including skin infections, gonorrhea, ringworm, burns, sores, rashes, wounds, bleeding and painful conditions (Tolossa et al., 2013; Lulekal et al., 2008; Regassa, 2000; Sori et al., 2004).

However, despite the widespread usage of these herbs, there is gap of information regarding their phytochemical composition and safety profile. The present study was, thus, initiated in view of generating preliminary phytochemical and biosafety information of C. quadrangularis (aerial part) and S. incanum (fruit) extensively used by traditional healers among the agro-pastoralist communities of Fafan zone in Eastern Ethiopia.

METHODOLOGY

Plant extracts preparation

The fresh aerial part of C. quadrangularis and fruits of S. incanum were collected from their natural habitat, at the beginning of a rainy season in this specific livelihood zone (The zone geographically lies between 8° 44’ N to 11° 00’ N latitude and 40° 22’ E to 44° 00’ E longitude). Both plants were taxonomically identified at the National Herbarium, Department of Biology, College of Natural Sciences, Addis Ababa University, Herbarium specimen (collection number SA 001 for C. quadrangularis and SA 002 for S. incanum) was deposited. The plant materials were separately washed with distilled water, air dried, mechanically ground and coarsely pulverized using mortar and pestle. The powdered plant material were then subjected to cold maceration extraction using two solvents; methanol and chloroform separately, to obtain the crude methanolic and chloroform extracts, respectively. The selection of these solvents is based on their widespread use as a solvent for substances intended for human/animal contact and consumption, polarity, and to mimic the same or similar methods used by local people to obtain or prepare botanical products.

For the cold maceration technique, a similar step used by Tadesse et al. (2015) with slight modification was applied. A total of 250 g of the pulverized materials was soaked in each extraction solvents (1:10 ratio) followed by frequent agitation for three days and then filtered. The residue left after maceration was successively extracted twice. This is to make the solvents extract substantial quantities of the chemical constituents from the pounded plant materials. The resulting liquid was filtered using Whatman No. 3 filter paper (Whatman Ltd., England). Finally, the filtered extracts were dried in hot air oven at a temperature of 45 to 50°C. Labeled vials were used to keep the crude extract and stored in a refrigerator at 4°C until required for experimentation.

Preliminary phytochemical screening

Chloroform and methanol extracts of the two plants were screened for the presence of active principles, such as alkaloids, anthraquinones, flavonoids, cardiac glycosides, polyphenols, saponins, phytosteroids, tannins, and terpenoids. A combination of several methods was used to identify the phytochemicals of the medicinal plants. Standard screening tests using conventional protocol, procedure and reagents, were conducted on both the methanolic and chloroform extracts using standard procedures to identify the constituents as stated by WHO (1978), Trease and Evans (1989) and Sofowara (1993).

Toxicity studies

Experimental animals

For this biosafety test of the crude extracts, female Swiss albino
mice (OECD, 2001), 8 to 12 weeks old and weighing 25 to 35 g, were obtained from the breeding colony of Akililu Lemma Institute of Pathobiology, Addis Ababa University. They were kept in clean polypropylene cages in a 12 h light/dark cycle with litter changed every week. The animals were provided with commercial pelleted ration (mice cubes) and clean water ad libitum and left under controlled conditions for one week to acclimatize before conducting any experimental procedure. The acute toxicity study was conducted in accordance with the guidelines on care and wellbeing of research animals (ILAR, 1996). The experimental procedure was approved by research and ethics committee of the Jigjiga University.

**Acute oral toxicity study**

Acute toxicity study protocols were done using the limit test dose of 2000 mg/kg (OECD, 2001). Six randomly selected female Swiss albino mice were used for each crude extract. For each extract, two groups of mice were required, one treatment and one control group. Before the administration of a single dose of the extract, the mice were fasted for 2 h (water allowed) (CDER, 1996; OECD, 2001). The treatment and the control groups respectively received a single dose of crude extract (2000 mg/kg) and normal saline (10 ml/kg). The mice were observed continuously for 1 h after administration of the extracts intermittently for 4 h, over a period of 24 h and for 14 days. During this period, the mice were observed for behavioral, neurological, autonomic or physical changes such as alertness, motor activity, restlessness, convulsions, hair erection, coma, diarrhea and lacrimation and other signs of toxicity manifestation (OECD, 2001). Body weight was monitored on days 0, 7 and 14.

**Sub-acute oral toxicity test**

The sub-acute toxicity study on plant extracts was performed as per the OECD guidelines 407 (OECD, 2008). The animals were divided into three groups. Groups 1 (n=6) and 2 (n=6) received extract doses of 200 and 400 mg/kg, respectively. The doses were selected based on the acute toxicity study finding. Group 3 (n=6) received 10 ml/kg body weight of normal saline and served as control. After extract treatments, mortality, food and water consumption as well as observation for any abnormal clinical signs of the animals were evaluated daily for 28 days, whereas body weight was recorded once in a week throughout the study period. At the end of 14 days observation period, the animals were anaesthetized and their blood samples were collected through cardiac puncture.

**Hematological parameters**

All experimental animals were humanely sacrificed at the end of the experiment by diethyl ether in desiccators. Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes. The blood samples were analyzed for haemoglobin (Hb) content using standard techniques (Dacie and Lewis, 1984). Packed cell volume (PCV) was determined using micro-haematocrit centrifuge and microhaematocrit tube reader according to Ekaidem et al. (2006).

**Data analysis**

For the toxicity studies, differences in haematological parameters and body weights for all treated and control mice were determined using a One-Way Analysis of Variance (ANOVA). A P values less than 0.05 were considered significant. All data were expressed as mean ± standard error of the mean.

**RESULTS AND DISCUSSION**

**Phytochemical screening**

The methanolic and chloroform extract yields of the plant materials were 12.4 and 5.8% for *C. quadrangularis* and 11.6 and 4.2% for *S. incanum*, respectively. In both plants, better yields were obtained from methanolic extraction. The preliminary phytochemical screening of the plant materials showed the presences of different secondary metabolites which are of medicinal importance (Table 1). Alkaloids, flavonoids, tannins, polyphenols, and cardiac glycosides are present in the methanolic extracts of both plants. Chloroform extract of the plant materials exhibited positive result for terpenoids and phytosteroids.

The active principles usually remain concentrated in the storage organs of the plants (Himesh et al., 2011).

<table>
<thead>
<tr>
<th>Constituents</th>
<th><em>C. quadrangularis</em></th>
<th><em>S. incanum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanolic extract</td>
<td>Chloroform extract</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phytosteroids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+, Present; -, absent.
Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in traditional practice (Das et al., 2010).

The preliminary phytochemical screening tests may be useful to identify bioactive principles present in the plant and encourages novel drug discovery and development from plant origin. These tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds as well (Usha and Karpagam, 2017). The phytochemical screening in the present study has revealed the presence of alkaloids, flavonoids, tannins, polyphenols, cardiac glycosides terpenoids, and phytosteroids in both herbs. Furthermore, fruit of S. incanum presented anthraquinones in its chloroform extract. The presence of these and other different phytoconstituents in the crude extracts of these medicinal plants may be responsible for their claimed therapeutic properties in human and veterinary diseases.

Plant phenolics (Flavonoids and tannins) are a major group of compounds that act as primary antioxidants. Flavonoids enhance the effects of vitamin C and function as a free radical scavenger. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Korkina and Afanas'ev, 1997). Since these compounds were found to be present in the extracts, it might be responsible for the antioxidant capacity of both plants. Saponins also cause hemolysis of red blood cells (Winter et al., 1993).

Toxicity studies

Only a few of the traditional herbal medicines have been verified by clinical trials. Moreover, their efficacy and safety are still questioned by users (Cheng et al., 2009). Exposure to plant based chemicals can be hazardous and results to adverse effects on human being and animals. Hence, evaluation of toxic properties of natural products is crucial when considering human and animal protection. In practice, the evaluation typically includes acute, sub-acute, sub-chronic, chronic, genotoxic and teratogenic effects (Asante-Duah, 2002). The present investigation assessed the safety profile of crude extracts of C. quadrangularis aerial part and S. incanum fruits using acute and sub-acute mice models.

Acute oral toxicity study

The acute toxicity test indicated a similar safety profile in all the crude extracts tested. For C. quadrangularis, both the methanolic and chloroform crude extracts caused no visible signs of acute toxicity at the maximum dose administered (2000 mg/kg). The test animals did not display any visible signs of acute toxicity such as lacrimation, restlessness, loss of appetite, tremors, hair erection, salivation, diarrhoea, convulsions and coma when compared with the control during the 14 days observation period. The mice were physically active and death was not observed.

For S. incanum, amongst the Swiss albino mice treated with methanolic fruit extract, there was no mortality or any signs of toxicity or side effects recorded. However, some of the animals which received its chloroform extract showed mild signs of toxicity. Toxicity signs observed in all the cases were initial excitement, restlessness, and difficulty in breathing, loss of appetite, general weakness and depression in the first 4 h. All these signs were reversed on the second day and the animals remained normal thereafter. With continues monitoring, none of the experimental animals were dead in the first 24 h and throughout the period of experiment.

Generally, test substance related mortality was not recorded at 2000 mg/kg. Therefore, the approximate medium acute toxicity lethal value (LD50) of experimental plants was determined to be higher than 2000 mg/kg and as such could be generally regarded as safe (GRAS). This finding is in concordance with those of Clarke and Clarke (1967), who reported that any compound or drug with oral LD50 estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe. Thus, it can be said that the crude extracts of both plants are not acutely toxic because there was no mortality recorded even at 2000 mg/kg bodyweight, thus indicating the safety of the extracts. This is in line with OECD guideline for testing of chemicals using Swiss albino mice (OECD, 2001).

Similarly, a study done by Ilavarasan et al. (2005) using methanolic bark extract of Cassia fistula showed that the plant did not cause any mortality up to 2000 mg/kg and was thus considered as safe. Another study done by Sangetha (2008) also exhibited similar results for a single dose (2000 mg/kg) administration of Cassia spectabilis leaf extracts that was revealed to be non-lethal to the tested mice. Acute toxicity test gives clues on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index of drugs and xenobiotics (Rang et al., 2001).

In vivo acute toxicity studies in mice could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that a toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose. However, these studies are not able to detect effects on vital functions like the cardiovascular, central nervous and respiratory systems which are not usually assessed during the study. Such effects of natural products should be evaluated prior to their therapeutic use (Syahmi et al., 2010). In principle, the limit test serves as a suggestion for classifying crude extracts based on the expected outcome at which dose
level the animals are able to survive (Jothy, 2011).

The effects of crude extracts on the percentage change in body weight of the control and treated mice are shown in Table 2. Normal body weight increment was observed in all the experimental animals without any strong difference between control and extract treated groups.

One of the indicators for drugs' toxic effect is change in body weight. The adverse effect will be significant if the body weight loss occurred in animals is more than 10% of their initial weight (Raza et al., 2002). During the 14 days of acute toxicity evaluation, all animals which were orally treated with crude extracts (methanol and chloroform) of both plants at single dose of 2000 mg/kg exhibited body weight increment and did not show significant changes in behavior. Apart from that, the physically observed features such as skin, fur and eyes were found to be normal. This indicates that the administration of single dose (2000 mg/kg) of the crude extracts had insignificant level of toxicity on the growth of the animals. Besides, evaluation of mice feeding and water consumption is important in the acute toxicity study of a product with therapeutic purpose (Iversen, 2003). In this study, the food intake and water consumption also was not affected by the administration of all extracts of the plant materials and none of the extracts induced appetite suppression and caused no deleterious effects. Thus, it can be speculated that there was no disturbance in carbohydrate, protein or fat metabolism (Klaassen, 2001).

Sub-acute oral toxicity test

Administration of different sub-acute doses of crude extracts of both plants caused a variable loss in body weight of the treated mice with a more pronounced loss which was recorded at higher dose (400 mg/kg), while the control group gained weight after 28 days of experimental observation. Particularly, chloroform extract of S. incanum (400 mg/kg) and methanol extract of C. quadrangularis (400 mg/kg) caused a statistically significant (p<0.05) body weight loss as compared to the control group. Similarly, in mice administered with high doses (400 mg/kg) of S. incanum chloroform extract and C. quadrangularis methanolic extract, a statistically significant (p<0.05) steady drop in the body weight was observed as against the control and pre-treatment value. The effects of the crude extracts on the change in mean body weight and mortality of the control and treated mice are shown in Table 3.

Phytochemicals may have a useful or harmful effect on animals. Tannins and anthraquinones are reported to have both pro-oxidant as well as antioxidant effects which causes tissue damage and protection on the body, respectively. The observed weight changes in animals treated with higher doses of the extracts indicate the presence of tannins and other phenolics which are responsible for altered absorption of nutrients and food intake (Kumar and Singh, 1984). According to this study, even though the animals were fed with adequate diet, the repeated chloroform extract at higher doses probably caused interference with absorption of nutrient such as proteins, resulting in weight loss.

In the present study, the significant decrease in body weight was observed in the groups repeatedly treated with chloroform extract of S. incanum (400 mg), methanolic extract of C. quadrangularis (Meth400) and other dose could be attributed to the suppression of the animals’ appetite by the extracts leading to reduced food intake (Ogbonnia et al., 2010).

The assessment on the hematological parameters is important as it can point directly to the systemic effects caused by the administered extract (Pariyani et al., 2015). The effect of sub-acute administration of the crude extracts on hematological parameters in the animals fed with the extracts for 28 days is presented in Table 4.

This study recorded significant increase in hemoglobin and PCV in all the groups treated with both doses of methanolic extracts of C. quadrangularis when compared with the control group and those treated with methanolic extracts of S. incanum (p<0.05). However, the observation of these parameters in groups treated with both doses of methanolic extracts of S. incanum showed slightly

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>D₀</th>
<th>D₇</th>
<th>D₁₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. quadrangularis</td>
<td>ME</td>
<td>26.00±0.82</td>
<td>26.75±0.66</td>
<td>27.00±0.82</td>
</tr>
<tr>
<td></td>
<td>CE</td>
<td>26.91±1.01</td>
<td>26.75±0.87</td>
<td>28.00±0.82</td>
</tr>
<tr>
<td>S. incanum</td>
<td>ME</td>
<td>26.50±0.99</td>
<td>26.75±0.98</td>
<td>27.75±0.12</td>
</tr>
<tr>
<td></td>
<td>CE</td>
<td>26.58±0.82</td>
<td>26.91±0.74</td>
<td>27.83±0.87</td>
</tr>
<tr>
<td>Control</td>
<td>Normal saline</td>
<td>26.75±0.85</td>
<td>27.33±0.96</td>
<td>28.50±0.92</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n: 6; D: Day; D₀: day treatment commenced; D₇, 7th day after treatment; D₁₄, 14th day after treatment; ME, methanol extract; CE: chloroform extract.

Table 2. Effect of administering 2000 mg/kg of C. quadrangularis and S. incanum methanolic and chloroform crude extracts on body weight of mice over a period of four weeks.
decreased values. This decrement in hematological parameters are not significantly (p>0.05) different from the control. Consumption of the different doses of the chloroform extracts of both plants did not cause significant changes in the PCV and Hb when compared with the control. Only higher dose (400 mg/kg) of chloroform extract of C. quadrangularis seemed to show a marginal increase of both parameters. These increases were not significantly different between the two extracts and with the control at p < 0.05.

It was clearly noted that crude extracts of C. quadrangularis positively affected the level of Hb and PCV more than S. incanum treated mice. This was particularly significant for the methanolic extract of C. quadrangularis. The observed significant increment in hemoglobin concentration and PCV suggests that this crude methanolic extract may have properties that stimulate erythropoiesis in the bone marrow when orally administered and may be very useful in the treatment of anemia. The phyto-constituents such as phenols (Ofokansi et al., 2005) and flavonoid (anti-oxidant and free radical scavenger) (Salahdeen and Yemitan, 2006) are highly implicated in such phenomenon.

Some of the biological functions of flavonoids, for example, include protection against allergies, free radicals, platelet aggregation microorganisms, ulcers, hepatotoxins and tumors (Okwu, 2004). Tannins and saponins were not present in C. quadrangularis. It is noteworthy that the presence of tannins and saponins in the methanolic extract of S. incanum may be a contributing factor to the slight marginal decrease in the haematological parameters. Saponins, for instance, have the properties of precipitation of proteins, cholesterol-binding and haemolysis. Other phyto-components such

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**Table 3.** Potential lethal toxic effects (number of deaths) and body weight changes caused by crude extracts of C. quadrangularis and S. incanum in mice over four weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of deaths</th>
<th>Number survived</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D&lt;sub&gt;0&lt;/sub&gt;</td>
<td>D&lt;sub&gt;28&lt;/sub&gt;</td>
<td>D&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td>CQ-Meth200</td>
<td>0/6</td>
<td>6/6</td>
<td>28.00±0.73</td>
</tr>
<tr>
<td>CQ-Meth400</td>
<td>1/6</td>
<td>5/6</td>
<td>27.17±0.70</td>
</tr>
<tr>
<td>CQ-Chlor200</td>
<td>0/6</td>
<td>6/6</td>
<td>27.00±0.97</td>
</tr>
<tr>
<td>CQ-Chlor400</td>
<td>0/6</td>
<td>6/6</td>
<td>26.50±0.99</td>
</tr>
<tr>
<td>SI-Meth200</td>
<td>0/6</td>
<td>6/6</td>
<td>27.00±0.73</td>
</tr>
<tr>
<td>SI-Meth400</td>
<td>1/6</td>
<td>4/6</td>
<td>27.33±0.7</td>
</tr>
<tr>
<td>SI-Chlor200</td>
<td>0/6</td>
<td>6/6</td>
<td>26.67±1.02</td>
</tr>
<tr>
<td>SI-Chlor400</td>
<td>3/6</td>
<td>3/6</td>
<td>27.17±0.75</td>
</tr>
<tr>
<td>Control</td>
<td>0/6</td>
<td>6/6</td>
<td>26.57±0.64</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 6; D<sub>0</sub>, day treatment commenced; SEM, standard error of mean; SI, S. incanum; CQ, C. quadrangularis; Chlor, chloroform; Meth, methanol; all superscripts indicate significance at p < 0.05 (*compared to negative control; *compared to SI-Meth200 and CQ-Chlor200)

**Table 4.** Packed cell volume (PCV) and hemoglobin [Hb] changes of experimental mice following sub-acute administration of crude extracts of C. quadrangularis and S. incanum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Packed cell volume (%)</th>
<th>Hb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D&lt;sub&gt;0&lt;/sub&gt;</td>
<td>D&lt;sub&gt;28&lt;/sub&gt;</td>
</tr>
<tr>
<td>CQ-Meth200</td>
<td>45.00±1.00</td>
<td>50.83±0.70&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>CQ-Meth400</td>
<td>46.00±0.58</td>
<td>51.00±0.56&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>CQ-Chlor200</td>
<td>46.00±0.26</td>
<td>47.50±0.62</td>
</tr>
<tr>
<td>CQ-Chlor400</td>
<td>46.33±0.61</td>
<td>48.17±0.47</td>
</tr>
<tr>
<td>SI-Meth200</td>
<td>46.67±0.71</td>
<td>44.83±0.65</td>
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<tr>
<td>SI-Meth400</td>
<td>46.67±0.99</td>
<td>44.12±0.70</td>
</tr>
<tr>
<td>SI-Chlor200</td>
<td>48.33±0.91</td>
<td>48.00±0.68</td>
</tr>
<tr>
<td>SI-Chlor400</td>
<td>47.67±1.14</td>
<td>47.00±1.12</td>
</tr>
<tr>
<td>Control</td>
<td>46.67±0.88</td>
<td>47.16±1.04</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 6; D<sub>0</sub>, Day; D<sub>28</sub>, day treatment commenced; SEM, standard error of mean; SI, S. incanum; CQ, C. quadrangularis; Chlor, chloroform; Meth, methanol; all superscripts indicate significance at p < 0.05 (*compared to negative control; *compared to SI-Meth200; *compared to SI-Meth400; *compared to all extracts of S. incanum).
as alkaloids and glycosides found in these plants also do not have properties relating to increased haematopoiesis or hemolysis (Vadivel and Janardhanan, 2000). Thus, *C. quadrangularis* appeared to be more effective than *S. incanum* in modulating haematopoiesis and protecting hemolysis.

**Conclusion**

The phytochemical screening indicated the presence of different secondary metabolites in both plants, which are responsible for bioactivity of the plants. The results show that methanol and chloroform extracts of *C. quadrangularis* and *S. incanum* did not cause any clear acute toxicity in an animal model. The sub-acute toxicity on the other hand revealed that crude extract of fruit of *S. incanum* showed toxic effects and mortality at high doses and thus prolonged use should be discouraged and low doses are recommended. However, an advanced experimental analysis of chronic toxicity of both plants is essential to establish therapeutic value and the safety in use.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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**REFERENCES**


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