In vivo analgesic, muscle relaxant, sedative and toxicological studies of *Senna bicapsularis* (L.) Roxb

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**ABSTRACT**

*Senna bicapsularis* is an important medicinal plant that has been traditionally used as a purgative and muscle relaxant as well as for the treatment of pain. The current work focused on phytochemicals to explore the analgesic, muscle relaxant, sedative and toxicological effects of the aerial part of *Senna bicapsularis* in an animal model. An acetic-acid-induced writhing screening showed that pretreatment of methanolic, ethyl acetate and aqueous extracts exhibited excellent analgesic activity at 500 mg/kg i.p. In chimney and traction, the crude butanolic and aqueous extracts showed a promising muscle relaxation effect at 500 mg/kg i.p., while the sedative effect of the ethyl acetate and methanolic extracts exhibited good activity at 500 mg/kg i.p. Crude extracts of *S. bicapsularis* were assessed for anti-inflammatory potency during a 5-h experiment, and the ethanolic and methanolic extract exhibited excellent inhibition in carrageenan-induced paw oedema among entire extracts. These provided natural products chemists for the treatment of pain and relaxation of muscles.

1. Introduction

*Senna bicapsularis* (L.) Roxb is commonly known as yellow candlewoods and belongs to the family Fabaceae [1,2]. It is native to West Indies, South America, and has been found from Panama south to Venezuela as well as in Colombia and China. It is a shrub that ranges from 1.5 to 3.5 m tall. *S. bicapsularis* leaves are 2.5–9 cm long, pinnate, and contain 6–8 leaflets; the length of the leaflets range from 1.6 to 4.5 cm, while the broad leaves range from 1.1 to 2.3 cm. A few flowers are produced together on a short raceme, the size of yellow flowers range from 12 to 16 mm in length [1]. The leaves of this plant are used in traditional treatments of skin ailments [3]. The ethanoic and aqueous extracts of *S. bicapsularis* have been documented for the presence of some phytochemicals, including phenolic compounds, anthocyanins, flavonoids and tannins. Due to these active phytochemicals, this plant has shown excellent antioxidant properties and anti-microbial activity [3]. Various secondary metabolites such as 1,8-dihydroxi-3-methyl anthraquinone (crysophanol), 1,8-dihydroxi-3-methoxy-6-methyl anthraquinones (physcion) and stigmasterol-dehidrostigmasterol mixtures were isolated from *S. bicapsularis* [4]. Also, previously, some major constituents such as rhein [1] and emodin [2] have been isolated from *S. bicapsularis* (Figure 1) [5]. These major compounds 1 and 2 might be responsible for in vivo biological activities. Traditionally, this plant has multiple medicinal uses. Based on traditional usage, the current study was aimed to evaluate the crude extracts and their fractions of *S. bicapsularis* for their analgesic, muscle relaxant, sedative, and toxicological potency.

2. Material and methods

2.1. Plant material

*Senna bicapsularis* plant samples were kindly provided by the Department of Chemistry, University of Swabi, KPK, Pakistan. The samples were collected from various regions of Swabi, KPK, Pakistan. The plant specimen was recognized by Dr Mohammad Ilyas Head Department of Botany, University of Swabi, KPK, Pakistan. The voucher specimen No UOS (Bot404) was placed in the herbarium of said Department.

2.2. Preparation of extraction

*Senna bicapsularis* plant material was cleaned and shade dried for approximately 20 days. The shade-dried plant material was ground by a local grinder machine, and then the powder was ground and the plant material was soaked in different organic solvents such as methanol, butanol, hexane, chloroform and ethyl acetate for 15 days until extraction of secondary metabolites was completed. The extracts were concentrated to obtain...
crude extract using a rotary evaporator using previously reported methods [6,7]. The plant was also kept in distilled water to obtain aqueous extract. The obtained crude extracts, including methanol (2.11 g), butanol (1.88 g), hexane (2.10 g), chloroform (2.73 g), ethyl acetate (2.60 g) and aqueous (1.64 g) extracts, were stored in a freezer and then used for biological activities.

2.3. Phytochemical analysis

The chemical screening test of the extracts was performed to recognize secondary metabolites using previously reported methods [8–11]. For detection of steroids, 0.6 g of each solvent extracts was added to 2 mL of acetic anhydride (C₄H₆O₃) and 2 mL of sulfuric acid (H₂SO₄) in a test tube. The colours of the reaction mixture changed from violet to green indicate the presence of steroids moiety. For alkaloids identification, the crude extracts (0.5 g of each solvent extracts) were warmed with 2% of sulfuric acid (H₂SO₄) for 2 min. For removal of insoluble impurities, the solution was firstly filtered, followed by adding few drops of Dragendorff reagents to each extracts of the plant. The development of the orange-red colour precipitate in the solution exhibited the occurrence of alkaloids groups in the extract. While for the tannins determination, 0.6 m of each extract was firstly dissolved in water and then put on a water bath for removal of insoluble impurity. The solution was filtered and then heated using a water bath. When the filtration was completed, 3–5 drops of ferric chloride (FeCl₃) was mixed with each extract. The development of a dark green colour exhibited the occurrence of tannins. For determination of phlobatanins, 0.7 g of each extract was mixed with distilled water, and then the solution was filtered to remove impurities. Then, the obtain filtrate was boiled with 2% of hydrochloric acid solution. The development of a red colour displayed the occurrence of phlobatanins. The fatty acids were detected in each extracts by adding 0.3 g of each extract of the plant with 5 mL of ether. The extracts were allowed for 4 h to evaporate on filter paper, and then the filter paper was dried. The presence of transparence on the filter paper indicated the presence of fatty acids. The presence of flavonoids was identified by adding 0.4 g of crude extracts with diluted sodium hydroxide (NaOH), and then a few drops of hydrochloric acid (HCl) were mixed. The formation of a yellow solution that turn colourless indicated the flavonoids moiety. The reducing sugars in each extract were identified by adding each extract with distilled water, and then the solution was filtered. This was followed by adding Fehling’s solution (Fehling’s A and Fehling’s B reagents) in a few drops to the filtrate, and then the mixed solution was boiled in a water bath. The presence of an orange-red colour in the mixed solution indicated the presence of reducing sugars. For terpenoids identification in each extract, 0.4 mg of extracts was combined with 2 mL of chloroform, and this was followed by carefully adding 3 mL of concentrated sulfuric acid (H₂SO₄) to the solution, which then formed a layer. The development of a reddish colour at the interface indicated the presence of terpenoids moiety. The glycoside was detected in each extract when each extract of the plant was hydrolyzed with hydrochloric acid (HCl) and neutralized with sodium hydroxide (NaOH) solution. This was followed by adding few drops of Fehling’s solution (Fehling’s A and Fehling’s B reagents) and mixed. Then the formation of a red colour in the solution indicated the presence of glycoside group in each extract. The cardiac glycoside was detected in each 2 mg extract by adding 1 mL of glacial acetic acid (CH₃COOH) and 5% ferric chloride (FeCl₃) in test tube and mixed. A few drops (2-4 drops) of concentrated sulphuric acids were added. The appearance of a greenish blue colour indicated the presence of cardiac glycoside in each extract.

2.4. Animals

BALB/c mice (weight: 20–26 g) of both sexes were used in this in vivo screening of each extract. The animals used in this study were bought from the animal house facility of the King Saud University (KSU), Saudi Arabia. The animals were reserved in standard laboratory conditions at room temperature. All animals were served with standard food and water ad libitum. The experiments were conducted following the guidelines
of the University of London Animal Welfare Society, Wheathamstead, England. The experiments were conducted in accordance to the ethical procedures and policies of the local bioethics committee of Qassim University, Saudi Arabia.

2.5. Analgesic activity

The crude methanolic, ethanolic, hexane, chloroform and ethyl acetate extracts were assessed for their analgesic activity using a standard method [12]. All mice were separated into various groups and each group comprised five mice \( (n = 5) \), weighing 20–26 g. One group of mice was treated with diclofenac as a standard drug (0.5 mg/kg, i.p.), and the second group was administered with normal saline (10 mL/kg i.p.). The remaining groups of mice received various extracts including methanolic, ethanolic, hexane, chloroform and ethyl acetate at the following doses: 50, 100, 250 and 500 mg/kg (i.p.). After the completion of 40 min treatment, the pain was induced in the mice by intraperitoneal injection (i.p) of acetic acid (0.9%) \( (v/v, 0.1 \text{ mL/10 g body weight}) \). The muscle contraction of every mouse was noted above a period of 20 min after injection of acetic acid. All groups and each animal were compared with controls for the number of writhes [12].

2.6. Muscle relaxant activity (inclined plane screening)

The crude extracts including methanolic, ethanolic, hexane, chloroform and ethyl acetate of title plant were assessed in an inclined plane model following previously reported methods [12,13]. This plane model comprised two plywood boards attached to each other, where one plywood board formed the base, while the another plywood board was fixed to the base at approximately 65 degrees. Animals of various groups in this study were administrated with diazepam (1 mg/kg) as a standard drug, normal saline (10 mL/kg), and the tested extracts. After the administration of each sample (extracts and standard) with various intervals (30, 60 and 90 min), the mice were monitored to fall or not for approximately 30 s after keeping on the upper portion of the inclined plane [12,13].

2.7. Traction screening

The crude extracts were also subjected to a traction screening test. A metal wire coated with rubber was used in this test where the ends of this metal wire were tightly stretched and reinforced with stands, approximately 60–70 cm above a laboratory bench. To perform this test, mice were randomly divided into 14 groups, and each group contained five mice. Distilled water and diazepam were used to treat two groups of mice as negative and positive control groups, respectively. The other groups of mice were treated with various extracts in the following doses: 50, 100, 250 and 500 mg/kg i.p. of the bodyweight of animals. Bioassays were conducted for all treated mice after the administration of treatment with different time intervals of 30, 60 and 90 min. Each mouse in this test was suspended on the metal wire from their hind legs, and thus the floppy time was noted for approximately 5 s. Then, the failure of mice to hang in the metal wire in less than 5 s reveals the occurrence of muscle relaxation potency of each extract and vice versa [12,13].

2.8. Sedative activity

The sedative properties of each extract were investigated by using an open-field screening technique. The apparatus design for the current study comprised an arena of white wood with a diameter of 150 cm and surrounded by stainless steel wall in which it is divided into 19 squares by drawing black lines. All experiments using this open-field occurred in a room with light- and sound-attenuated. Before starting of the experiment, all mice groups were adapted under red light (40-W red bulb) with food and water accessible ad libitum. Diazepam was used as a reference drug, and normal saline was used as a negative control. Other mice groups were treated with the extracts of the title plant at doses of 50, 100, 250 and 500 mg/kg i.p. Each mouse in this study was kept in the centre of the white wood arena, then the number of lines crossed by each group of mice was counted for every mouse following a previously reported procedure [14].

2.9. Anti-inflammatory

The crude extracts such as hexane, butanol, methanol, ethyl acetate and chloroform were tested for anti-inflammatory activity in this study by using a carrageenan-induced paw oedema model following a standard procedure. The animals were randomly divided into various groups, and each group contained six mice \( (n = 6) \). One group was considered as a negative control (normal saline) for analysis. Another group was treated with standard drug (diclofenac; 10 mg/kg), while the other groups of animals were treated in this study with different extracts at various doses: 50, 100 and 250 (i.p). After passing 30 min, the treated animals were also injected with 0.05 mL carrageenan solution sub planter to the region of paw SC. The observed volume of paw edema produced was noted at different intervals of 1–5 h of carrageenan administration. Also, the paw size was also quantified at the beginning of the experiment. The % activity was measure by using below formula:

\[
\% \text{ inhibition} = \frac{A - B}{A} \times 100
\]
3.2. Toxicological profile

To determine the toxicity of each extract, mice were divided in this study into 14 groups in which each group comprised five mice (n = 5). The combined crude extracts (methanolic, ethanolic, hexane, chloroform, ethyl acetate) of *S. bicapsularis* samples at 50, 100, 250 and 500 mg/kg (body weight of mouse) were used as test doses. Mice were kept in this study under observation for any behavioural effect and mortality for approximately 24 h after intraperitoneally (i.p) administration of the trial doses. The numbers of dead and live mice were noted by calculating the % of mortality [15,16].

3.11. Statistical analysis

All obtained results in this study are represented as mean ± standard error of the mean (SEM). One-way ANOVA was used among all groups for assessment screening, followed by Dunnett’s multiple calculation post-tests. For each results calculation, the significance level was defined as p < .05 or .01 and measured for each screening test in the current study. All results of biological activities were also shown in this study as mean ± SEM, and their statistical investigation was performed using the Graphpad software package (version 6.0, Graphpad Software Inc., San Diego, CA, USA).

3. Results

3.1. Phytochemical composition

Phytochemical composition is an important step before bulk isolation can be performed with new and novel compounds. The crude methanolic, aqueous, butanolic, hexane, chloroform and ethyl acetate extracts were subjected to phytochemical screening, as shown in Table 1. The methanolic and butanolic extracts indicate the presence of many phytochemicals, including steroids, alkaloids, tannins, phlobatanins, fatty acids, reducing sugars, terpenoids, flavonoids and glycosides. The aqueous extract exhibited the presence of the following phytochemicals: alkaloids, tannins, phlobatanins, steroids, reducing sugars, terpenoids and glycosides. The chloroform and ethyl acetate exhibited the presence of steroids, fatty acids, reducing sugars, terpenoids, flavonoids and glycosides, while the no polar n-hexane indicated the presence of fatty acids and steroids.

The analgesic effect of the various solvents extracted such as methanolic, aqueous, butanolic, hexane, chloroform and ethyl acetate were screened in mice with doses of 50, 100, 250 and 500 mg/kg body weight (Table 2). Diclofenac sodium was used in this study as a standard drug at 0.5 mg/kg i.p. The dose-dependent analgesic activity was observed, and pretreatment of methanolic, ethyl acetate and aqueous extracts exhibited excellent analgesic activity at 500 mg/kg. i.p. in acetic-acid-induced writhing screening.

### Table 1. Phytochemical screening of different solvent extract of *Senna bicapsularis*.

| Phytochemicals | Methanolic ext | Aqueous ext | Butanolic ext | Hexane frt | Chloroform frt | Ethyl acetate frt |
|----------------|----------------|-------------|---------------|------------|---------------|-----------------|
| Steroids       | +              | +           | +             | +          | +             | +               |
| Alkaloids      | +              | +           | +             | −          | −             | +               |
| Tannins        | +              | +           | +             | −          | −             | −               |
| Phlobatanins   | +              | +           | +             | −          | −             | −               |
| Fatty acids    | +              | −           | +             | +          | +             | +               |
| Flavonoids     | +              | −           | +             | −          | −             | −               |
| Reducing sugars| +              | +           | +             | −          | −             | −               |
| Terpenoids     | +              | +           | +             | +          | +             | +               |
| Glycosides     | +              | +           | +             | +          | +             | +               |

Note: ext; Extract; frt: Fractions.

### Table 2. Analgesic activity of crude extract and fractions of *Senna bicapsularis*.

| Sample                  | Dose   | No. of writhes |
|-------------------------|--------|---------------|
| Normal Saline           | 10 mL/kg | 62.0 ± 2.01  |
| Diclofenac sodium       | 0.5 mg/kg | 15.1 ± 1.93*** |
| Methanol extract        | 50     | 29.1 ± 2.19** |
|                         | 100    | 25.5 ± 2.00** |
|                         | 250    | 21.2 ± 1.99** |
|                         | 500    | 17.2 ± 1.96** |
| Aqueous extract         | 50     | 37.3 ± 2.78*  |
|                         | 100    | 31.9 ± 1.82*  |
|                         | 250    | 25.8 ± 1.70*  |
|                         | 500    | 20.2 ± 1.68*  |
| Butanol extract         | 50     | 40.3 ± 2.80*  |
|                         | 100    | 34.0 ± 1.88*  |
|                         | 250    | 30.6 ± 1.84*  |
|                         | 500    | 25.71 ± 1.79* |
| Hexane fraction         | 50     | 44.1 ± 2.80*  |
|                         | 100    | 39.6 ± 2.60*  |
|                         | 250    | 35.3 ± 2.00*  |
|                         | 500    | 33.9 ± 1.80*  |
| Chloroform fraction     | 50     | 40.9 ± 2.90*  |
|                         | 100    | 37.7 ± 2.71*  |
|                         | 250    | 31.5 ± 2.01*  |
|                         | 500    | 29.8 ± 1.88*  |
| Ethyl acetate fraction  | 50     | 39.9 ± 2.89*  |
|                         | 100    | 30.8 ± 2.81*  |
|                         | 250    | 24.4 ± 1.87*  |
|                         | 500    | 19.8 ± 1.86** |

All values are given as mean ± SEM of five animals in comparison with control.

where A is the oedema volume of negative control, and B is the oedema volume of the tested groups.
Table 3. Muscle relax activity of crude extract and fractions of Senna bicapsularis (Chimney & Traction).

| Group          | Dose mg/kg | Chimney test (%) | Traction test (%) |
|----------------|------------|------------------|-------------------|
|                | 30 min     | 60 min | 90 min | 30 min | 60 min | 90 min |
| Distilled water| 10 mL/kg   | 0.± 0.00 | 0.± 0.00 | 0.± 0.00 | 0.± 0.00 | 0.± 0.00 |
| Diazepam       | 1 mg/kg    | 100± 0.00 | 100± 0.00 | 100± 0.00 | 100± 0.00 | 100± 0.00 |
| Methanol extract| 50         | 30.29  | 39.22 | 48.22 | 28.49 | 37.98 | 46.21 |
|                | 100        | 36.22  | 44.21 | 52.17 | 37.32 | 45.29 | 53.98 |
|                | 250        | 39.21  | 47.99 | 55.88 | 38.76 | 46.21 | 57.99 |
|                | 500        | 42.98  | 51.22 | 66.21 | 43.97 | 52.21 | 68.22 |
| Aqueous extract| 50         | 34.29  | 43.22 | 52.22 | 34.42 | 42.11 | 51.58 |
|                | 100        | 39.33  | 47.44 | 57.77 | 40.11 | 45.21 | 58.00 |
|                | 250        | 44.87  | 54.98 | 69.11 | 45.71 | 54.29 | 70.22 |
|                | 500        | 51.99  | 60.33 | 77.22 | 50.65 | 61.86 | 78.00 |
| Butanol extract| 50         | 36.21  | 45.75 | 55.21 | 36.56 | 46.98 | 57.78 |
|                | 100        | 41.66  | 50.22 | 60.71 | 43.98 | 48.26 | 64.01 |
|                | 250        | 47.87  | 54.98 | 73.19 | 48.22 | 58.11 | 76.21 |
|                | 500        | 56.11  | 60.33 | 77.22 | 50.65 | 61.86 | 78.00 |
| Hexane fraction| 50         | 10.22  | 19.12 | 26.65 | 10.87 | 17.12 | 25.66 |
|                | 100        | 15.87  | 22.87 | 29.67 | 14.81 | 21.88 | 29.01 |
|                | 250        | 19.34  | 23.88 | 30.00 | 18.31 | 24.87 | 30.01 |
|                | 500        | 21.67  | 28.55 | 37.78 | 21.99 | 27.51 | 38.71 |
| Chloroform fraction| 50       | 20.21  | 30.10 | 38.61 | 19.83 | 26.11 | 37.61 |
|                | 100        | 25.83  | 32.82 | 40.66 | 24.56 | 32.99 | 42.60 |
|                | 250        | 29.35  | 36.77 | 44.01 | 28.36 | 34.81 | 39.56 |
|                | 500        | 36.67  | 40.51 | 49.72 | 35.91 | 38.55 | 47.22 |
| Ethyl acetate fraction| 50     | 22.20  | 32.98 | 41.66 | 21.80 | 27.66 | 39.38 |
|                | 100        | 27.55  | 34.81 | 43.88 | 28.11 | 35.90 | 44.61 |
|                | 250        | 30.39  | 39.71 | 49.03 | 29.88 | 37.84 | 48.51 |
|                | 500        | 39.61  | 45.53 | 57.71 | 38.90 | 44.54 | 56.29 |

All values are given as mean ± SEM of five animals in comparison with control.

3.3. Muscle relax effect

The muscle relaxation effect of polar (methanolic, aqueous, butanolic and ethyl acetate) and non-polar (hexane and chloroform) extracts are displayed in Table 3. Pretreatment with different solvent extracts at a dose of 50, 100, 250 and 500 mg/kg and standard drug (Diazepam) of 1 mg/kg decreased fall off as well as sliding time and increased climbing time. In the chimney screening test, the tree fractions (butanolic, aqueous, and methanolic) at 50, 100, 250, and 500 mg/kg exhibited maxima of 56.11, 67.19 and 81.21; 51.99, 60.33 and 77.22; and 42.98, 51.22 and 66.21% effect after 30, 60 and 90 min of treatment at 50, 100, 250 and 500 mg/kg i.p., respectively. While in traction screening, butanolic and aqueous extracts exhibited a maximum of 55.98, 66.81 and 80.09; 50.65%, 61.86% and 78.00% muscle relaxant effect. This was followed by methanol extract (43.97%, 52.21% and 68.22% activity) after 30, 60 and 90 min of treatment at 50, 100, 250 and 500 mg/kg i.p., respectively. The maximum activity exhibited by the butanolic extract might be due to the presence of the identified secondary metabolites in the extract.

3.4. Sedative effect

The sedative properties of the different extracted including methanolic, aqueous, butanolic, hexane, chloroform and ethyl acetate in mice with doses of 50, 100, 250 and 500 mg/kg bodyweight are listed in Table 4. Bromazepam was used as a standard drug at 0.5 mg/kg i.p. Dose-dependent sedative effects were observed among the tested extracts, and the ethyl acetate extract exhibited excellent locomotive activity at 500 mg/kg i.p. as compared to the standard drug.

3.5. Anti-inflammatory

The anti-inflammatory effects of crude extracts such as methanolic, aqueous, butanolic, hexane, chloroform and ethyl acetate in mice with doses of 50, 100, 250...
Figure 2. Anti-inflammatory activity of crude extract and fractions of *Senna bicapsularis*.

Table 5. Toxicological profile of crude extract and fractions of *Senna bicapsularis* (Chimney & Traction).

| Treatment               | Doses (mg/kg) | Number of died animals/5% mortality | Gross behaviours changes |
|-------------------------|---------------|------------------------------------|--------------------------|
| Normal saline           | 10mL/kg       | 0/5                                | Nil                      |
| Methanol extract        | 50            | 0/5                                | Nil                      |
|                         | 100           | 0/5                                | Nil                      |
|                         | 250           | 0/5                                | Nil                      |
|                         | 500           | 0/5                                | Nil                      |
| Aqueous extract         | 50            | 0/5                                | Nil                      |
|                         | 100           | 0/5                                | Nil                      |
|                         | 250           | 0/5                                | Nil                      |
|                         | 500           | 0/5                                | Nil                      |
| Butanol extract         | 50            | 0/5                                | Nil                      |
|                         | 100           | 0/5                                | Nil                      |
|                         | 250           | 0/5                                | Nil                      |
|                         | 500           | 0/5                                | Nil                      |
| Hexane fraction         | 50            | 0/5                                | Nil                      |
|                         | 100           | 0/5                                | Nil                      |
|                         | 250           | 0/5                                | Nil                      |
|                         | 500           | 0/5                                | Nil                      |
| Chloroform fraction     | 50            | 0/5                                | Nil                      |
|                         | 100           | 0/5                                | Nil                      |
|                         | 250           | 0/5                                | Nil                      |
|                         | 500           | 0/5                                | Nil                      |
| Ethyl acetate fraction  | 50            | 0/5                                | Nil                      |
|                         | 100           | 0/5                                | Nil                      |
|                         | 250           | 0/5                                | Nil                      |
|                         | 500           | 0/5                                | Nil                      |

All values are given as mean ± SEM of five animals in comparison with control.

and 500 mg/kg body weight are listed in (Figure 2). Diclofenac sodium was used as a positive control at 0.5 mg/kg i.p. The activities of all extracts were compared with standard diclofenac sodium, which was the most active, followed by butanolic extract.

### 3.6. Toxicological profile

The results of various extracts such as methanolic, aqueous, butanolic, hexane, chloroform and ethyl acetate of *Senna bicapsularis* are given in Table 5. All tested animals were found safe up to the maximum tested dose (500 mg/kg) for the various solvent extracts. Also, no gross behavioural changes were observed during the 24-h assessment.

4. Discussion

This study deals with promising analgesic, muscle relaxant and sedative effects of the aerial part of *S. bicapsularis* in an animal model experiment. The acetic-acid-induced writhing test is most often used to examine preliminary analgesic properties of plant extracts and pure compounds [12]. The intraperitoneal injection of acetic acid to animals causes the liberation of various pain-stimulating mediators that induce constrictions of the abdominal muscles [12,15]. The results of our study suggest a promising analgesic effect of various extracts including methanolic, aqueous, butanolic, hexane, chloroform and ethyl acetate. Therefore, the pharmacological active compounds of these extracts may play a role in inhibiting the release of pain-stimulating mediators. *In vivo* traction and chimney screening tests are the maximum common tools for the assessment of skeleton muscle relaxation potency [12]. The current finding on the different solvent extracts such as methanolic, aqueous, butanolic, hexane, chloroform and ethyl acetate of *S. bicapsularis* displayed the strong potential of the plant for skeleton muscle relaxant phytochemicals. The sedative activity of the crude extracts such as crude methanolic, aqueous, butanolic, hexane, chloroform and ethyl acetate of *S. bicapsularis* is given in Table 5. All tested animals were found safe up to the maximum tested dose (500 mg/kg) for the various solvent extracts. Also, no gross behavioural changes were observed during the 24-h assessment.

Diclofenac sodium was used as a positive control at 0.5 mg/kg i.p. The activities of all extracts were compared with standard diclofenac sodium, which was the most active, followed by butanolic extract.

| Treatment       | Doses (mg/kg) | % Inhibition |
|-----------------|---------------|--------------|
| Normal saline   | 10mL/kg       | 100          |
| Methanol extract| 50            | 80           |
|                 | 100           | 70           |
|                 | 250           | 60           |
|                 | 500           | 50           |
| Aqueous extract | 50            | 90           |
|                 | 100           | 80           |
|                 | 250           | 70           |
|                 | 500           | 60           |
| Butanol extract | 50            | 90           |
|                 | 100           | 80           |
|                 | 250           | 70           |
|                 | 500           | 60           |
| Hexane fraction | 50            | 90           |
|                 | 100           | 80           |
|                 | 250           | 70           |
|                 | 500           | 60           |
| Chloroform fraction | 50  | 90    |
|                 | 100           | 80           |
|                 | 250           | 70           |
|                 | 500           | 60           |
| Ethyl acetate fraction | 50  | 90    |
|                 | 100           | 80           |
|                 | 250           | 70           |
|                 | 500           | 60           |

All values are given as mean ± SEM of five animals in comparison with control.
specific secondary metabolites responsible for the sedative effect of *Senna bicapsularis*.

The crude extracts of *S. bicapsularis* were assessed for their anti-inflammatory potency during a 5-h experiment. The activity of all extracts was compared with standard diclofenac sodium, which was used as a positive control and found to be the most active. This was followed by butanolic extract, which was found to be the most active of all tested extracts. It exhibited 33% inhibition in carrageenan-induced paw oedema, which was potent (84%) after 3 h and was found significant after 5 h of experimental time. The methanolic extract exhibited 30% attenuation in carrageenan-induced paw oedema, and the effect became promising (80%) after 3 h, while it remained for the 5 h of the experimental duration. Aqueous, chloroform and ethyl acetate extracts exhibited mild activity, while the n-hexane was found least active among all extracts.

Interestingly, the crude extracts (methanolic, aqueous, butanolic, hexane and chloroform extracts) of *S. bicapsularis* neither impacted any change in animal behaviour in the acute toxicity screening test, did not cause any mortality and was found completely safe during the 24-h assessment. The potent activity of *S. bicapsularis* might be due to the reported compounds during the 24-h assessment. The potent activity of *S. bicapsularis* might be due to the reported compounds including rhein (1) and emodin (2) (Figure 1).

It is concluded that *S. bicapsularis* is a rich source of active secondary metabolites. In addition, the crude extracts showed excellent analgesic, muscle relaxant, sedative activities and thus provided natural products for the treatment of pain and as a muscle relaxant. This finding directed the researchers to work on the various extracts of *S. bicapsularis*, which may lead to the isolation of new, rare and novel compounds. Further toxicological studies are needed to examine the effects of *S. bicapsularis* on organ function and also on foetal development for pregnant mothers.

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