Anti-ulcerative colitis activity of *Calotropis procera* Linn.

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ABSTRACT

The aim of the present study was to evaluate the anti-ulcerative colitis activity of *Calotropis procera*. Different extracts of the investigated plant were evaluated; total alcohol extract, polar extract and non-polar extract. All the investigated extracts at doses 200 & 400 mg/kg possessed a dose-dependent anti-ulcerative colitis potential when administrated for 5 consecutive days after colitis induction by acetic acid in rats. They reduced different parameters of UC. Only polar extract at both doses (200, 400 mg/kg) was more effective than the standard drug Prednisolone (50 mg/kg), it produced percent protection of control colitis by 63.8% and 78.4% respectively, while the standard drug Prednisolone produced 54.9% protection. The anti-ulcerative colitis activity may be attributed to the active principles i.e. flavonoids. Preliminary phytochemical screening showed that the plant contains flavonoids, unsaturated sterols and/or triterpenoids, cardiac glycosides, carbohydrates or glycosides, proteins and/or amino acids, tannins and coumarins. The total alcohol extract was safe up to 4000 mg/kg and there were no side effects reported on liver and kidney functions.

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1. Introduction

*Calotropis procera* Linn. Family Asclepiadaceae is an important wild growing medicinal plant, it is an erect, tall, large, highly branched and perennial shrub or small tree that grows to a height of 5.4 m, with milky latex throughout. It is known by various names like Swallow wart in English, madar in Hindi and Alarka in Sanskrit. It is found in most parts of the world with a warm climate in dry, sandy and alkaline soils. Different parts of the plant are used in the treatment of various diseases in folk medicine. The inner bark of Calotropis is used to make strong fibers called behenate and glyceryl-1, 2-dicapriate-3-phosphate. The root bark contains calotropaceous, procerursenyl acetate and proceranol, N-dotriacont-6-ene, glyceryl mono-oleolyl-2-phosphate, methyl myrisate, methyl behenate and glyceryl-1, 2-dicapiate-3-phosphate. The root bark has also been found to possess 1-amyrin, 2-amyrin, lupeol, 1-sitosterol and flavanols like quercetin-3-rutinoside. In the leaves, mudarine is the principal active constituent as well as a bitter yellow acid, resin and 3 toxic glycosides calotropin, uscharin and calactoxin. (Perwez and Mohammad, 2009).

Chemical investigation of *C. procera* has shown the presence of triterpenoids, calotropursenyl acetate and calopfriedelenyl, a nortiterpenyl ester, calotropternyl ester oleane triterpines like calotropoleanyl ester, procerleanol A and B and cardiac glycosides calotropogenin, calotropin, uscharin, calotoxin and calactoxin. In addition to cardenolides and anthocyanins (Quazi et al., 2013). Phytochemical investigation of the roots showed phytoco- stituents, procursenyl acetate and proceranol, N-dotriacont-6-ene, glyceryl mono-oleolyl-2-phosphate, methyl myrisate, methyl behenate and glyceryl-1, 2-dicapiate-3-phosphate. The root bark has also been found to possess 1-amyrin, 2-amyrin, lupeol, 1-sitosterol and flavanols like quercetin-3-rutinoside. In the leaves, mudarine is the principal active constituent as well as a bitter yellow acid, resin and 3 toxic glycosides calotropin, uscharin and calactoxin. (Perwez and Mohammad, 2009).

There are many biological activities reported for *C. procera*. The previous pharmacological studies include reports of anticancer, anti-fungal and insecticidal activity (Ahmed et al., 2006). The flowers of the plant exhibit hepatoprotective activity (Setty et al., 2007), anti-inflammatory, antipyretic, analgesic, antioxidant and anti-ulcerative colitis activity (Awaad et al., 2017). The inner bark of Calotropis is used to make strong fibers called madar which are used in the manufacture of weave carpets, ropes, sewing thread and fishing nets (Rajesh et al., 2014).

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antibacterial activities (Yesmin et al., 2008). In addition, the plant is reported to possess anthelmintic, anticancer, antidiabetic, gastroprotective, cardiovascular and hypolipidemic, wound healing and anticonvulsant effects (Al-Snafi, 2015). Furthermore, there are also studies of contraceptive activities reported in rats (Silva et al., 2010).

Ulcerative colitis (UC) is an inflammatory bowel disease that primarily affects the colonic mucosa and sub-mucosa. The most common symptoms of UC are ulcers and inflammation of the inner lining of the colon that lead to symptoms of bloody diarrhea, passage of pus, mucus, and abdominal cramping during bowel movements. Currently, there is no effective therapy to cure the disease but the mainstream treatment depends on the reduction of the symptoms. The treatment depends on the severity of the disease; therefore, treatment is adjusted for each individual (El-Meligy et al., 2015).

2. Material and methods

2.1. Phytochemical part

2.1.1. Plant materials

The aerial part of C. procera were collected during flowering stage in 2016 from Al-Yamamah territory (Al-Khurj, South of Riyadh, KSA). The Sample was identified by Dr. Jacob T. Pandalayil (Assistant Professor of Plant Taxonomy, Botany and Microbiology). Voucher specimen (KSU. NO. 6015) was kept in the herbarium. Plant material was air-dried in shade, reduced to fine powder, refillable in tightly closed containers and storage for photochemical and pharmacological studies.

2.1.2. Extraction

The air-dried powder (250 g) of C. procera was extracted by percolation in 95% V/V aqueous ethanol, with occasional shaking for 72 h. The ethanol extract of the powder was filtered and the residues were re-percolated for three times. The combined filtrates were concentrated under reduced pressure at low temperature to yield 85 g (total alcohol extract). The residues obtained was suspended in water (300 mL), filtered over a piece of cotton. The materials obtained on the top of the cotton piece were non-polar components (non-polar extract) while the aqueous layer obtained represent the polar components (polar extract).

2.1.3. Preliminary phytochemical screening

For determination of the active constituents, the air-dried powders of the investigated plant were subjected to preliminary phytochemical screening according to the published methods (Tiwari et al., 2011).

2.2. Pharmacological part

2.2.1. Animals

Swiss albino mice of both sex (26–30 g) and male Wistar rats (180–200 g) were purchased from the animal house of King Saud University, KSA. Animals were housed in standard polypropylene cages with wire mesh top and maintained under standard conditions (temperature 23 ± 1 °C, humidity 55 ± 10%, 12 h light/12 h dark cycle). They fed with a standard pellet diet with water ad libitum and were allowed to adapt to the laboratory environment for one week before experimentation.

2.2.2. Preparation of the extracts for biological studies

The extracts of C. procera were freshly suspended in distilled water just before administration by the aid of Tween 80.

2.2.3. Acute toxicity (LD50) test

The oral median lethal dose (LD50) of the total alcohol extract of C. procera was determined as described by El-Meligy et al. (2015). Swiss albino mice in groups of six, received one of 500, 1000, 2000, or 4000 mg/kg doses of the tested extract. Control animals were received the vehicle and kept under the same conditions. Signs of acute toxicity and number of deaths per dose within 24 h were recorded.

2.2.4. Sub-chronic toxicity

Wistar rats were randomly divided into two groups each of 10 animals. Rats of the 1st group received the vehicle in a dose of 5 mL/kg and left as normal control. Rats of the 2nd group were administered the total alcohol extract of C. procera (400 mg/kg).

All medications were administered orally daily for 35 consecutive days. Animals were maintained under identical conditions with food and water ad libitum for the entire period with close observation. At the end of the experimental period, blood samples (2 mL) were drawn by puncturing retro-orbital venous sinus of each rat (under ether anesthesia) and centrifuged at 10,000 rpm for 5 min. Sera were separated to be used for the biochemical estimations.

2.2.4.1. Measurement of liver and kidney function markers. Liver functions were evaluated by measuring the serum activity of ALT, AST, in addition to levels of total bilirubin, albumin and total proteins. Levels of blood urea, and Serum creatinine were determined as measures of kidney functions for the total alcohol extract.

2.2.5. Effect on ulcerative colitis

Male Wistar rats were divided into 9 experimental groups, each of 6 animals. Rats of groups 1 and 2 received the vehicle (5 mL/kg) and served as normal control and control colitis groups. Group 3 administered dexamethasone (0.1 mg/kg) and served as Reference Drug group. The alcohol extract, polar extract and non-polar extract of C. procera were administrated at dose 200 mg/kg to rats of groups 4, 5 and 6 and at dose of 400 mg/kg to rats of groups 7, 8 and 9 respectively. All medications were administered orally, once daily for 5 consecutive days, the first doses were administrated in all groups one hour after colitis induction with 2 mL (4%, v/v) acetic acid in saline. At the end of the experiment, animals were sacrificed using ether anesthesia, colonic segments (8 cm in length and 3 cm proximal to the anus) were excised, opened along its mesenteric border, washed with saline, and were used for macroscopic scoring (El-Meligy et al., 2015).

The colon specimens were weighted and wet weight/length ratio was calculated for all the rats. The specimens were examined under a dissecting microscope and the lesion scores were quantified by scoring system (0 – 5).

Ulcer area was measured using plane glass square. Each cell on the glass square was 1 mm² in area and the number of cells was counted and the ulcer area was determined for each colon.

Ulcer index (UI) was measured by summing the lesion score and the ulcer area for each colon specimen. The curative ratio was determined according to the formula:

Curative ratio = Control UI – Test UI/Control UI × 100

2.2.6. Statistical analysis

All values were expressed as mean ± S.D. Comparisons between means were carried out using a one-way ANOVA test followed by the Tukey HSD test using SPSS, version 14 (SPSS, Chicago, IL). Differences at p<0.05 were considered statistically significant.
3. Results and discussion

3.1. Preliminary phytochemical screening

The plant contains; unsaturated sterols and/or triterpenoides, Flavonoids, cardiac glycosides, Carbohydrates or glycosides, Proteins and/or amino acids, Tannins and Coumarins.

3.2. Pharmacological activity

3.2.1. Acute toxicity (LD50) test

The tested extract is characterized by a low degree of toxicity. The obtained results indicated that different doses of the alcohol extract *Calotropis procera* (500, 1000, 2000 and 4000 mg/kg) did not produce any symptom of acute toxicity and none of the mice died during 24 h of observation. It was suggested that oral LD50 of the tested extracts were higher than 4000 mg/kg and the tested extract is considered safe.

3.2.2. Sub-chronic toxicity

The non-toxic nature of the alcohol extract of *C. procera* in acute toxicity study is well supported by the results of sub-chronic toxicity study. Oral dosing of *C. procera* alcohol extract (400 mg/kg) for 35 days did not show any significant effect on the levels of ALT, AST, total bilirubin, total proteins, albumin, urea and creatinine in their sera as compared to control non-treated rats (Table 1).

3.2.3. Effect on ulcerative colitis

The model of acetic acid induced colitis shares many of the histologic features of ulcerative colitis in human beings including mucosal edema and submucosal ulceration (Awaad et al., 2016).

In rats of normal control group, no abnormal changes were observed suggesting that handling procedure had no interference with the experimental outputs. Macroscopic damage parameters of the colon of control colitis rats, two days after rectal infusion of acetic acid revealed dark brown lesions, mucosal hyperemia, edema, erosion, and ulceration. Control colitis rats showed lesion score, ulcer area and ulcer index values of 4.7 ± 0.29, 5.5 ± 0.34 cm² and 10.2 ± 0.63, respectively (Table 2).

The inflammatory changes of the intestinal tract were associated with a significant increase of wet weight/length of the colon

### Table 1
Effect of alcohol extract of *Calotropis procera* on liver and kidney functions.

| Parameter                  | Normal Control | Total alcohol extract |
|----------------------------|----------------|-----------------------|
| ALT (U/L)                  | 67.50 ± 1.48   | 69.3 ± 2.73           |
| AST (U/L)                  | 146.30 ± 5.20  | 160.4 ± 6.54          |
| Total bilirubin (mg/dl)    | 1.70 ± 0.11    | 1.73 ± 0.10           |
| Total protein (g/dl)       | 8.65 ± 0.22    | 8.35 ± 0.34           |
| Albumin (g/dl)             | 3.72 ± 0.01    | 3.20 ± 0.25           |
| Urea (mg/dl)               | 37.00 ± 2.35   | 38.05 ± 2.55          |
| Creatinine (mg/dl)         | 0.43 ± 0.22    | 0.40 ± 0.25           |

### Table 2
Effects of *Calotropis procera* extracts on the macroscopic parameters of ulcerative colitis induced by acetic acid in rats.

| Groups                           | Lesion score (0–5) | Ulcer area (cm²) | Ulcer index | Wet W/L (g/cm) |
|----------------------------------|--------------------|------------------|-------------|----------------|
| Normal control                   | 0.0 ± 0.0          | 0.0 ± 0.0        | 0.0 ± 0.0   | 0.34 ± 0.03    |
| Control colitis                  | 4.71 ± 0.29        | 5.55 ± 0.34      | 10.20 ± 0.63| 0.98 ± 0.07    |
| Prednisolone (50 mg/kg)          | 2.22 ± 0.12        | 2.53 ± 0.15      | 4.75 ± 0.27 | 0.46 ± 0.02    |
| *C. procera* total extract (200 mg/kg) | 2.50 ± 0.23        | 3.80 ± 0.30      | 6.30 ± 0.43 | 0.56 ± 0.05    |
| *C. procera* total extract (400 mg/kg) | 1.91 ± 0.15        | 2.50 ± 0.27      | 4.41 ± 0.34 | 0.48 ± 0.05    |
| *C. procera* polar extract (200 mg/kg) | 1.50 ± 0.19        | 2.00 ± 0.28      | 3.50 ± 0.31 | 0.53 ± 0.06    |
| *C. procera* polar extract (400 mg/kg) | 1.35 ± 0.20        | 1.65 ± 0.25      | 3.00 ± 0.27 | 0.42 ± 0.04    |
| *C. procera* non-polar extract (200 mg/kg) | 3.35 ± 0.27        | 3.65 ± 0.27      | 7.00 ± 0.39 | 0.55 ± 0.04    |
| *C. procera* non-polar extract (400 mg/kg) | 3.00 ± 0.24        | 2.40 ± 0.23      | 5.40 ± 0.27 | 0.62 ± 0.05    |

Prednisolone (50 mg/kg), the tested extracts (200, 400 mg/kg) (n = 6). *Significantly different from the control colitis at, p < 0.05.

Fig. 1. Effect of investigated extracts on acetic acid-induced colitis in rats. The figure shows % protection of control colitis for 7 groups of animals (n = 6), treated with alcohol, polar & non-polar extracts of *Calotropis procera* (200 and 400 mg/kg) and Prednisolone (50 mg/kg) for 5 successive days after ulcerative colitis induction by acetic acid.
specimens as an indicator of inflammation. These inflammatory indices were significantly improved by oral dosing of Prednisolone, alcohol, polar and non-polar extracts of *C. procera* and for 5 days after colitis induction (Fig. 1).

All the investigated extracts possessed a dose-dependent anti-ulcerative colitis potential. They reduced different parameters of UC (Table 2). Polar extract at 400 mg/kg was more effective than Prednisolone (50 mg/kg), it produced percent protection of control colitis by 78.4%, while the standard drug Prednisolone produced 54.9% protection. In addition, the other evaluated dose of the polar extract (200 mg/kg) was also more effective than Prednisolone (50 mg/kg), it produced percent protection of control colitis by 63.8%.

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