Effect of some plants' extracts used in Sudanese folkloric medicines on carrageenan-induced inflammation

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Abstract: Investigations for anti-inflammatory potential and categorization of Sudanese medicinal plants according to their potency. Anti-inflammatory effect of plants' extracts of 17 genera were studied using the carrageenan induced inflammation in rats' paws. The plant extracts were obtained using methanol and dichloromethane as solvent and administered intra peritoneally at the concentration of 2g/kg body weight. The results obtained in this experiment strongly support and validate the traditional uses of these Sudanese medicinal plants to treat various inflammatory diseases. 63.9% of plants extracts showed marked inhibition of inflammation induced by carrageenan (78.3% out of this percentage represented by methanolic extract), 27.8% showed no activity and 8.3% enhanced the carrageenan induced inflammation. The anti-inflammatory effect of many of these plants has not been reported previously, yet they have been extensively used in Sudanese folkloric medicine. The result of this study justify the traditional medicinal use of the evaluated plants species in treating inflammatory disorders and helped in categorizing the investigated plants into most useful, moderately useful and least useful category for inflammatory diseases. Out of the 17 investigated plant species 05 belongs to most useful and 06 belongs to moderately useful category. However, toxicity studies are required to prove the safety of these plant materials.

Keywords: Sudanese medicinal plants, anti-inflammatory activity, carrageenan.

INTRODUCTION

Inflammation is a complicated pathophysiological process in which a number of signaling molecules are involved and these molecules are produced by macrophages, mast cells and leukocytes as well as by the activation of complement factors. The formation of these signaling molecules causes extra vasation of fluid and proteins and accumulation of leukocytes leading to inflammation of the site. Treatment of inflammation requires removal of key inflammatory cells and down regulation of pro-inflammatory mediators in the inflamed sites. This two-way control process is actively regulated by biochemical mediators possessing anti-inflammatory effect (Lee and Surh, 2012). Some chronic inflammatory diseases remain one of the world's major health problems. Recent epidemiological studies state that in development of ulcerative colitis consumption of a type of unsaturated fatty acids play a key role (Marion-Letellier *et al*., 2013). The popularity of most of the anti-inflammatory agents is decreasing day by day because of some serious adverse reactions like sodium, potassium and water retention, gastric intolerance, increase in blood urea and creatinine, bleeding and bone marrow suppression resulting from prolonged use (Louis and George, 2012). Therefore, development of new, economic, potent and safe anti-inflammatory drug from natural source is the need of hour especially for developing countries. This study was aimed to evaluate the scientific basis for the traditional uses of some Sudanese medicinal plants for inflammatory disorders.

MATERIALS AND METHODS

Plant material

The 17 plant genera belong to different families (listed in table 1) were collected from different region in Sudan: Kordofan and Nuba Mountain (western Sudan), Khartoum (central Sudan) and Ingassana area (Blue Nile). Most of these plants are indigenous while others are grown and cultivated in Sudan. The botanical identity of the plants material was confirmed by taxonomist and voucher specimens were deposited in the Medicinal and Aromatic Plant Research Institute Herbarium (Sudan).

Preparation of the extracts

Collected plants were dried under shade and then powdered. The powdered plants materials (200 mg) were sequentially extracted two times with sufficient quantities of dichloromethane and 80% methanol at room

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temperature for 48 hour. The extracts were filtered and the filtrates were concentrated under reduced pressure and stored at room temperature.

Drug administration

All the extracts were suspended in 0.25% Sodium carboxy methylcellulose solution followed by homogenization. The suspended agent alone served as control.

Animals

Male Wistar rats weighing 200-220g were used in this study. The studies were carried out using three rats as experimental models of inflammation for each plant extract.

Carrageenan induced rat paw edema

The anti-inflammatory effect was evaluated by Carrageenan induced rat paw edema according to the method given by Sudhir *et al*., 1986. Each animal was marked on its right ankle in a circular manner using a non-erasable blue ink. The volume of each paw up to the ankle mark was then measured using Ugo-Basil plythesmometer using 0.45% Sodium chloride solution as a displacement fluid. The volume of the immersed paw was then read in the digital display. Groups of three animals were injected, each with a dose of a different extract in a dose of 2g/kg intra peritoneal. After 60 minutes each rat was then injected intra plantarly with 0.2% aqueous Carrageenan using a fine 1-ml hypodermic syringe. The control group was injected with the suspending agent solution intra peritoneally in a volume equivalent to the test volumes injected. After 60 minutes, Carrageenan was injected intra plantarly as described above.

Following Carrageenan injections, paw volumes to the marked sites were read at 1, 2 and 3hours intervals. The volume of formed oedema was then calculated using the following formula:

$$\text{Percentage inhibition} = \frac{(\text{Ct} - Co)\ \text{control} - (\text{Ct} - \text{Co})\ \text{treated}}{(\text{Ct} - Co)\ \text{control}} \times 100$$

Where Co is volume of edema in control group and Ct is volume of edema in test group. Net edema volumes formed two hours following injection of Carrageenan were used to calculate the effect of the extracts on the induced edema. Data were expressed as the mean± SEM. Significant difference between the control and the treated groups was performed using Student's t-test and P values. The difference in results was considered significant when $P<0.001$.

RESULTS

The widely used basic model for evaluation of inflammation as well as anti-inflammatory drugs is carrageenan induced rat hind paw edema. The inflammation caused by carrageenan involves three phases: initiation, promotion and progression, caused by the release of histamine and serotonin; Bradykinin and prostaglandins respectively (Thais *et al*., 2012). The vascular permeability is increased by both histamine and serotonin. The maximum vascular responses during the third phase of inflammation are mediate by prostaglandins (Lundequist *et al*., 2010). The present investigation gives a wide range of potency of the extracts showing anti-edematous effect mentioned in table 2. These results comply with the findings of other scientists, e.g. anti inflammatory *activity of Cyperus rotundus*, *Ocimum basilicum*, *Tamarindus indica* and *tribulus terrestris* is through their ability to inhibit nitric oxide and super oxide production (Tuncer *et al*., 2009). *Tamarindus indica* have high inhibitory activity against human neutrophil elastase (Fook *et al*., 2005). *Acacia nilotica*, *Capparis deciduas, Senna alata and Trigonella foenum-graecum* have a known NSAID like mechanisms to suppress the inflammatory processes through inhibition of COX enzyme (Eldeen and Staden, 2008; Vivian *et al*., 2008). *Senna alata* and *Solenostemma argel* containing kaempferol which modulate the COX pathway via inhibition of nitric oxide production and that can contribute to its anti-inflammatory activity (Eldeen *et al*., 2010). Anti-inflammatory activity of *Achyranthes aspera* could be attributed to presence of alkaloids (Innocenti *et al*., 2005; Barua *et al*., 2010), saponins (Das *et al*., 2012) and oleanolic acid, which also has anti-viral properties (Mukherjee *et al*., 2012).

DISCUSSION

In this study methanolic extracts of most of the plants (*Cyperus rotundus, Tribulus terrestris ,Trgonella foenum-graecum, Capparis deciduas, Mimosa pigra, Senna alata -stem*) showed good antiedematous effect (% of inhibition more than 53%), indicating their good ability to combat with the inflammatory mediators, while methanolic extract of other plants (*Ocimum basilicum, Tamarindus indica, Achyranthes aspera, ambrosia martima, Solenostemma argel and Acacia nilotica*) showed moderate to weak activity as mentioned in table 3. *Tribulus terrestris* and *Trgonella foenum-graecum* being evaluated as one of the most potent anti-inflammatory drugs indicate the role of saponin glycosides in inflammatory disorders.

Some metanolic extracts of the studied plants (*Triathema portulacastrum and Echinocloa colona*) showed no anti-inflammatory activity as mentioned in table 4, while *Zaleya pentandra, Glinus lotoides and Heliotropium bacciferum* were showed no great differences in activity between methanol and dichloromethane extracts.

Table 1: Sudanese medicinal plants selected for anti-inflammatory screening

Species	Family	Local name	Traditional uses
Acacia nilotica (L.) Del.	Mimosaceae	Garad, Sideer	Pods are used for cough and as gargle for tonsillitis (Gamal *et al*., 1987)
Achyranthes aspera L.	Amaranthaceae	Abu Rukab, Abu el-leseing	Maceration of the roots are externally to enhance wounds healing (Gamal *et al*., 2003)
Ambrosia martima	Amaranthaceae	El-Damsessa	Infusion of whole plant is used for renal nephritis (Gamal *et al*., 1998)
Senna alata L.	Mimosaceae	El-Nawama	Leaves together with roots are used for fever (Gamal *et al*., 1998)
Capparis decidua (Forssk.) Edgew.	Capparidaceae	Tundub, Humbuk	Fumigant of stems are used as anti-rheumatic (Gamal *et al*., 1997)
Cyperus rotundus L.	Cyperaceae	Seida, Najil	Corms powder are used as demulcent (Gamal *et al*., 1994)
Echinocloa colona (L,) Link.	Poaceae	El-Difra	Diuretic (Khare, 2007)
Glinus lotoides L.	Aizoaceae	Raba'a El Bahr	The dried plant is a helpful purgative in abdominal disorder (Dangol, 2012) and the poultice made up of leaves is applied over wounds and inflammation (Qureshi *et al*., 2010)
Heliotropium bacciferum Forssk.	Boraginaceae	Danab El Agrab	Maceration is used to treat fever (Schmelzer *et al*., 2008)
Mimosa pigra L.	Mimosaceae	Al Sit El-Mustahia	the roots are sniffed for head colds, a decoction of the leafy stem is used as a mouthwash for toothaches, and that the fruits are used in eye medicines (Anon, 1980)
Ocimum basilicum L.	Lamiaceae	Al-Rehan	Seed powders are used as demulcents (Gamal *et al*., 1997).
Slenostemma argel (Delile) H ayne.	Asclepiadaceae	El Hargel	Infusion of he leaves are used for renal nephritis (Gamal *et al*., 1998)
Tamarindus indica L.	Caesalpiniaceae	El-Ara'deb	Infusion of the seeds is used for treatment of jaundice (Gamal *et al*., 1998).
Triathema portulacastrum L.	Aizoaceae	Raba'a	A decoction of the whole plant is used in treatment of rheumatism (Kirtikar and Basu, 1975), the plant is analgesic, stomachic, and serves as alternative cures for bronchitis and inflammation (Banu *et al*., 2009).
Tribulus terrestris L.	Zygophyllaceae	Dreisa	Infusion of the aerial parts is used as demulcent and renal nephritis (Gamal *et al*., 1997).
Trigonella foenum-graecum L.	Mimosaceae	Helba	Seeds powder is used as poultice for swellings and rheumatism (Gamal *et al*., 1998).
Zaleya pentandra (L.) Jeffrey	Aizoaceae	El Rabaa'	Macerations of the whole plants are used against scorpion bites (Gamal *et al*., 1994)

Table 2: Net edema 2 hours after injection of Carrageenan and % inhibition by various extracts

Number	Treatment	Part extracted	Solvent	Net oedema volume (ml)	%inhibition
1	Control *Cyperus rotundus*	Roots	Methanol	1.6±0.1 0.42±0.06	- 73.5±1.5
2	*Tribulus terrestris*	Aerial parts	Methanol	0.45±0.09	72.0±3.9
3	*Trigonella foenum-graecum*	Seeds	Methanol	0.57±0.1	64.1±4.9
4	*Capparis deciduas*	Leafy stems	Methanol	0.6±0.11	62.3±5.3
5	*Mimosa pigra*	Stems	Methanol	0.64±0.07	60.0±3.7
6	*Cyperus rotundus*	Leaves	Methanol	0.75±0.08	53.2±5.1
7	*Senna alata*	Stems	Methanol	0.75±0.08	53.0±3.6
8	*Ocimum basilicum*	Aerial parts	Methanol	0.8±0.09	50.0±3.9
9	*Tamarindus indica*	Fruits	Methanol	0.83±0.04	48.0±2.1
10	*Senna alata*	Leaves	Methanol	0.95±0.07	40.8±6.3
11	*Senna alata*	Stems	Dichloromethane	0.98±0.04	39.0±3.9
12	*Zaleya pentandra*	Whole plants	Methanol	0.98±0.03	39.0±2.3
13	*Zaleya pentandra*	Whole parts	Dichloromethane	0.98±0.03	38.7±2.3
14	*Achyranthes aspera*	Whole parts	Methanol	1.13±0.04	28.9±1.7
15	*Heliotropium bacciferum*	Aerial parts	Dichloromethane	1.15±0.09	28.2±3.1
16	*Mimosa pigra*	Leaves	Methanol	1.17±0.08	27.1±3.6
17	*Ambrosia martima*	Whole parts	Methanol	1.18±0.09	26.4±1.3
18	*Glinus lotoides*	Whole parts	Methanol	1.24±0.06	22.3±0.9
19	*Senna alata*	Leaves	Dichloromethane	1.28±0.05	20.1±1.3
20	*Heliotropium bacciferum*	Aerial parts	Methanol	1.29±0.07	19.5±3.1
21	*Solenostemma argel*	Aerial parts	Methanol	1.38±0.05	13.7±1.3
22	*Glius lotoides*	Whole plants	Dichloromethane	1.42±0.02	11.5±0.3
23	*Acacia nilotica*	Leaves	Methanol	1.42±0.08	11.3±2.7

Table 3: Plants extracts tested at doses of 2g/kg and showed anti-inflammatory activity

Number	Species	Part extracted	Solvent	% inhibition
1	*Cyperus rotundus*	Roots	Methanol	73.5±1.5
2	*Tribulus terrestris*	Aerial parts	Methanol	72.0±3.9
3	*Trigonella foenum-graecum*	Seeds	Methanol	64.1±4.9
4	*Capparis deciduas*	Leafy stems	Methanol	62.3±5.3
5	*Mimosa pigra*	Stems	Methanol	60.0±3.7
6	*Cyperus rotundus*	Leaves	Methanol	53.2±5.1
7	*Senna alata*	Stems	Methanol	53.0±3.6
8	*Ocimum basilicum*	Aerial parts	Methanol	50.0±3.9
9	*Tamarindus indica*	Fruits	Methanol	48.0±2.1
10	*Senna alata*	Leaves	Methanol	40.8±6.3
11	*Senna alata*	Stems	Dichloromethane	39.0±3.9
12	*Zaleya pentandra*	Whole plants	Methanol	39.0±2.3
13	*Zaleya pentandra*	Whole parts	Dichloromethane	38.7±2.3
14	*Achyranthes aspera*	Whole parts	Methanol	28.9±1.7
15	*Heliotropium bacciferum*	Aerial parts	Dichloromethane	28.2±3.1
16	*Mimosa pigra*	Leaves	Methanol	27.1±3.6
17	*Ambrosia martima*	Whole parts	Methanol	26.4±1.3
18	*Glinus lotoides*	Whole parts	Methanol	22.3±0.9
19	*Senna alata*	Leaves	Dichloromethane	20.1±1.3
20	*Heliotropium bacciferum*	Aerial parts	Methanol	19.5±3.1
21	*Solenostemma argel*	Aerial parts	Methanol	13.7±1.3
22	*Glius lotoides*	Whole plants	Dichloromethane	11.5±0.3
23	*Acacia nilotica*	Leaves	Methanol	11.3±2.7

*The volume of oedema formed in the control rats two hours after Carrageenan was 1.6±0.1 ml.
*Values represent the mean ±S.E.M. of three rats.

Table 4: Plants extracts tested at doses of 2g/kg and showed no anti-inflammatory activity

Number	Species	Part extracted	Solvent
1	*Achyrathus aspera*	Whole plants	Dichloromethane
2	*Acacia nilotica*	Leaves	Dichloromethane
3	*Ambrosia martima*	Whole plants	Dichloromethane
4	*Trigonella foenum-graecum*	Seeds	Dichloromethane
5	*Triathema portulacastrum*	Whole plants	Dichloromethane
6	*Cyperus rotundus*	Leaves	Dichloromethane
7	*Mimosa pigra*	Leaves	Dichloromethane
8	*Capparis deciduas*	Leavey stems	Dichloromethane
9	*Echinocloa colona*	Whole plants	Methanol
10	*Triathema portulacastrum*	Whole plants	Methanol

*The volume of oedema formed in the control rats two hours after Carrageenan was 1.6±0.1 ml.
*Values represent the mean ±S.E.M. of three rats.

Table 5: Plant extracts tested at doses of 2g/kg and enhanced the Carrageenan induced inflammation

Number	Species	Part extracted	Solvent	% enhancement
1	*Ocimum basilicum*	Aerial parts	Dichloromethane	17.4±1.9
2	*Echinocloa colona*	Aerial parts	Dichloromethane	8.1±1.3
3	*Solenostema argel*	Aerial parts	Dichloromethane	6.5±3.2

*The volume of oedema formed in the control rats two hours after Carrageenan was 1.6±0.1 ml.
*Values represent the mean ±S.E.M. of three rats.

Table 6: Category assigned on the basis of anti-inflammatory potential of plants

Most Potent Plants	
Cyperus rotundus L.	Cyperaceae
Mimosa pigra L.	Mimosaceae
Tribulus terrestris L.	Zygophyllaceae
Trigonella foenum-graecum L	Mimosaceae
Capparis decidua (Forssk.) Edgew.	Capparidaceae
Moderately Potent Plants	
Senna alata L.	Mimosaceae
Ocimum basilicum L.	Lamiaceae
Tamarindus indica L.	Caesalpiniaceae
Zaleya pentandra (L.) Jeffrey	Aizoaceae
Achyranthes aspera L.	Amaranthaceae
Heliotropium bacciferum Forssk.	Boraginaceae
Least Potent Plants	
Triathema portulacastrum L.	Aizoaceae
Echinocloa colona (L,) Link.	Poaceae
Slenostemma argel (Delile) Hayne.	Asclepiadaceae
Glinus lotoides L.	Aizoaceae
Acacia nilotica (L.) Del.	Mimosaceae
Ambrosia martima	Amaranthaceae

In contrast to methanol extracts, dichloromethane extracts of the same studied plant material showed range of activities varied from moderate, weak, no activity and even some species capable to induce inflammation (table 5). So the selection of methanol as extracting solvent is the best decision since it can extract most of active anti-inflammatory constituents contained in traditional preparations of Sudanese medicine.

CONCLUSION

The anti-inflammatory effect of many of these plants has not been reported previously, yet they have been extensively used in Sudanese folkloric medicine. The result of this study support the rationale behind the traditional use of these plants in inflammatory conditions and helped in categorizing the investigated plants into

most useful, moderately useful and least useful plants for anti-inflammatory disorders (table 6). Out of the 17 investigated plant species 05 belongs to most useful and 06 belongs to moderately useful category. Now, depending on our requirement a poly-herbal formulation can be designed for inflammatory disorders by using different proportions of the investigated plant extracts. However, toxicity studies are required to prove the safety of these plant materials.

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