Anti-Inflammatory Activity of Selected Plants from Saudi Arabia

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Thirteen selected Saudi Arabian plants, belonging to seven different families, were tested for possible anti-inflammatory activity using the carrageenin-induced paw edema model in rats. The methanolic extracts of *Vernonia schimperi*, *Trichodesma trichodesmoides* var. *tomentosum*, and *Anabasis articulata* exhibited the highest anti-inflammatory activity. The active extracts were further subjected to fractionation with chloroform, ethyl acetate, and *n*-butanol and tested together with their mother liquor for their anti-inflammatory activity in the same rat model. The most potent fractions were the *n*-butanol fractions of *Anabasis articulata* and *Vernonia shimperi* and the aqueous mother liquor of *Trichodesma trichodesmoides*. Nevertheless, the three potent methanolic extracts showed higher anti-inflammatory activities than their individual fractions. The antioxidant properties were assessed by their *in vitro* 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activities. It was concluded that the anti-inflammatory activity is dependent, at least in part, on the reduction of prostaglandin (PGE_2) and tumour necrosis factor-α (TNF-α) levels and cyclooxygenase-2 (COX-2) activity.

Key words: Anti-Inflammatory, Saudi Arabian Plants, COX

Introduction

Inflammation is a protective response to tissue injury caused by physical trauma, noxious chemicals, microbiological agents or even autoimmune disease. This protective response may lead to potentially damaging consequences. Proinflammatory molecules like tumour necrosis factor-α (TNF-α) and prostaglandins (PGs), and even pathogenic concentrations of nitric oxide (NO) are responsible for eliciting such a response (van der Vliet *et al.*, 2000).

Unfortunately, drugs currently available to treat pain and inflammation are associated with several side effects and low efficacy, especially in chronic diseases. As alternative to chemical therapeutics, natural products have shown good efficacy and few side effects. The study of plants used in traditional medicine as anti-inflammatory or pain-killer agents is considered a successful strategy in the search for analgesic and anti-inflammatory drugs (Calixto *et al.*, 2000). Thus, many pharmacognostic and pharmacological investigations are carried out to find new lead structures for the treatment of human diseases in which inflammation plays an important role, such as rheumatoid arthritis and atherosclerosis (Gurib-Fakim, 2006).

The Saudi Arabian flora comprises about 2250 plants distributed throughout the Kingdom (Collenette, 1999). Many plants have been used by local communities for the treatment of a large number of ailments including inflammation. Therefore, the current study was conducted to investigate the possible anti-inflammatory activity of extracts from thirteen Saudi Arabian plants. Promising extracts were subjected to further fractionation with different organic solvents in a bioguided manner to identify the most active fraction(s). The mechanisms underlying the observed anti-inflammatory activity of the bioactive fractions were explored by determination of PGE_2 and TNF-α levels in rat

paw exudates, as well as of the activities of cyclooxygenases 1 and 2 (COX-1 and -2) in human peripheral blood mononuclear cells (PBMC).

Material and Methods

Plant material

The flowering aerial parts of the selected plant samples (Table I) were collected from different localities in the Kingdom of Saudi Arabia. The collected plants were kindly identified by the staff of the Department of Taxonomy, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. A herbarium specimen of each collected plant was prepared and kept at the herbarium of the Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University. The plant samples were air-dried, ground, and kept for extraction.

Extraction, fractionation, and phytochemical screening

Each plant sample (500 g) was extracted twice with methanol (2 x 1000 ml) using an IKA Ultra-Turrax T 25 digital instrument (IKA Labortechnik, Staufen, Germany). The solvent was distilled off under reduced pressure, and the dried methanolic extracts were kept at 4 °C till biological tests. The crude methanolic extracts showing highest anti-inflammatory activity in the primary *in vivo* screening against the carrageenin-induced rat paw edema model (see below) were suspended in a suitable volume of water and fractionated against chloroform, ethyl acetate, and *n*-butanol (saturated with water). The previous fractions as well as their mother liquor were further tested *in vitro* for their anti-inflammatory activity. Biologically active extracts were screened for their chemical constituents (Ayoola *et al*., 2008)

Determination of total phenolic content

Total phenolic content was determined in triplicate employing the Folin-Ciocalteu method described by Ainsworth and Gillespie (2007). Briefly, 7 ml distilled H_2O, 0.5 ml Folin-Ciocalteu reagent (2 N), and 0.5 ml of each extract (0.40–1.0 mg/ml) were mixed. After 3 min, 2 ml of 20% Na_2CO_3 were added and the mixture heated at 100 °C for 1 min in a water bath. Absorbance was measured at 685 nm after cooling in the dark, and the results were expressed in mg of gallic acid equivalents (GAE)/g dry weight.

Determination of total flavonoid content

Total flavonoid content was measured by the aluminum chloride colorimetric assay described by Lamaison *et al*. (1990). An aliquot (1 ml) of each extract (3.0–7.0 mg/ml) or standard solution of rutin (0.03–0.3 mg/ml) was added to 1 ml of 2% methanolic $AlCl_3$ solution. The absorbance was measured 10 min later at 430 nm. The total flavonoid content was determined from the calibration curve and expressed as mg rutin equivalents (RE)/g dried extract. All determinations

Table I. Plant species selected for anti-inflammatory study, their family, place of collection, herbarium specimen number, and yield.

Plant	Family	Place of collection	Specimen no.	Yield (%)
Hypoestes forskaolii R. Br.	Acanthaceae	El-Shefaa, Al-Taif	HF1005	17
Achyranthes aspera var. *aspera* L.	Amaranthaceae	Al-Hadda Road	AA1010	18
Caralluma russelliana (Courbon ex Brongn.) Cufod	Asclepiadaceae	Akabet Al-Bnah, Al-Baha	CR1050	14
Caralluma tuberculata N. E. Br.		El-Shefaa, Al-Taif	CT1027	19
Achillea biebersteinii Afanasiev	Asteraceae	Al-Baha-Al-Taif Road	AB1064	12
Conyza stricta Wall.		Wadi Kama	CS1058	10.1
Echinops galalensis Schweinf.		El-Shefaa, Al-Taif	EG1001	15
Vernonia schimperi DC.		Al-Hadda Road	VS1054	8
Trichodesma trichodesmoides var. *tomentosum* R. Mill.	Boraginaceae	Al-Hadda Road	TT1024	9
Anabasis articulata Forssk.	Chenopodiaceae	El-Shefaa, Al-Taif	AA1011	28
Chenopodium schraderianum Schult.		El-Shefaa, Al-Taif	CS1057	7
Astragalus abyssinicus Steud. ex A. Rich	Leguminosae	Al-Makhwah, Al-Baha	AA1012	8
Crotalaria microphylla Vahl		Al-Hadda Road	CM1013	11.6

were carried out in triplicate, and the mean values were calculated.

Determination of antioxidant activity

In order to determine the antioxidant activity of the investigated extracts, the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay as described by Sharma and Bhat (2009) was used. The dried methanolic extracts were re-dissolved in methanol at different concentrations (10–70 μg/ml). Aliquots were taken and the volume adjusted to 3.0 ml with methanol. The reaction was started by addition of 1.0 ml 50 μM DPPH in methanol. The reaction mixture was kept at 30 °C for 30 min, and the absorbance was measured at 517 nm. Radical scavenging activity was calculated using the equation: % inhibition = $(A_{blank} - A_{test}) \cdot 100/A_{blank}$, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract), and A_{test} is the absorbance of the test extract. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. Tests were carried out in triplicate. Ascorbic acid (1 mM) was used as standard.

Chemicals

Indomethacin and carboxymethylcellulose (CMC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Inflammatory-grade carrageenin was purchased from FMC Corporation (Rockland, ME, USA). PGE_2 kits were purchased from Schwan's Research and Development Inc. (Marshall, MN, USA), TNF-α kits were purchased from ID Labs (London, ON, Canada), and COX assay kits were obtained from Cayman Chemical Co. (Ann Arbor, MI, USA). Solvents and all other chemicals were of the highest available commercial grade.

Animals

Adult male Sprague-Dawley rats, weighing 150–175 g (8 weeks old), were obtained from the animal facility of the National Research Center (Dokki, Giza, Egypt). Animals were housed at a temperature of (23 ± 2) °C with free access to water and standard food pellets (unless indicated otherwise). Rats were left to acclimatize in the animal facility for 1 week prior to experimentation. Procedures involving animals and their care were conducted in conformity with the institutional guidelines of the bioethical committee for animal use and care.

Measurement of paw volume in carrageenin-induced rat edema model

Ninety rats were randomly equally divided into fifteen groups, assigned to numbers 1–15. Animals were fasted, with free access to water, 16 h before the experiment. Groups 1 and 2 were given CMC [10 ml/kg body weight (BW) of 0.5% CMC in distilled water] vehicle, while groups 3–15 were treated with plant extracts at a dose of 250 mg/kg BW. Animals in group 2 received indomethacin as standard anti-inflammatory drug [12.5 mg/kg BW, orally (p.o.)]. All solutions were administered to the rats using an intragastric tube, and the volume of the dose was kept constant (10 ml/kg BW) and completed with saline when required. One h after oral treatment, group 1 received 0.05 ml saline, while groups 2–15 were subcutaneously (s.c.) injected with 0.05 ml of freshly prepared carrageenin (1% solution in distilled water) on the plantar surface of the left hind paw. The left hind paw volume baseline value was measured immediately before carrageenin injection by water displacement using a UGO-BASILE 7140 plethysmometer (Comerio, Italy) (Winter *et al.*, 1962). The paw volume was re-measured 1, 2, and 3 h after carrageenin injection. Edema inhibition (EI) was calculated as percentage change related to the corresponding value of the untreated control at 3 h.

The bioactive extracts with highest potency were suspended in a minimal amount of water and fractionated against different solvents (chloroform, ethyl acetate, *n*-butanol) and again tested, along with the remaining mother liquor, for anti-inflammatory activity by the same procedure as described above. After decapitation, the right hind paw was dissected. A volume of 0.1 ml saline containing 10 μM indomethacin was injected to aid removal of the eicosanoid-containing fluid and to stop further production of PGE_2. Paws were incised with a scalpel, and the inflammatory exudates were collected. For recovery of the inflammatory exudates, paws were centrifuged at 1800 x *g* for 15 min (Mnich *et al.*, 1995).

Determination of PGE_2 level

PGE_2 level was quantified in the collected exudates using a quantitative PGE_2 enzyme immunoassay kit. The kit uses a monoclonal antibody to bind, in a competitive manner, the PGE_2 in the sample as well as alkaline phosphatase-labelled PGE_2 provided in the kit. The enzyme bound through the PGE_2 molecules to the monoclonal antibodies processes the specific substrate to a coloured product that was measured spectrophotometrically (Virella, 1998).

Determination of TNF-α level

TNF-α level was assayed using a rat TNF-α enzyme immunometric assay kit. Rat TNF-α was immobilized on polyclonal antibodies bound to a microtitre plate. Excess sample was washed off. A monoclonal antibody specific to rat TNF-α, coupled to horseradish peroxidase, was added. The monoclonal antibody binds specifically to the immobilized rat TNF-α. Excess monoclonal antibody was washed off, and the substrate, tetramethyl benzidine, was added. After an incubation period, the developed colour was determined spectrophotometrically at 450 nm (Virella, 1998).

Isolation of human PBMC

Human PBMC were isolated from whole blood obtained from healthy donors of whom informed consent had been obtained that their donated blood might be used for scientific purposes. Separation of blood cells was performed using density centrifugation (lymphocyte separation medium; Lonza, Basel, Switzerland) according to the manufacturer's instructions. After isolation, PBMC were washed three times in phosphate-buffered saline containing 1 μM EDTA. Cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (Lonza), 2 mM glutamine (Serva, Heidelberg, Germany), and 0.1% gentamicin (BioWhittaker Inc., Walkersville, MD, USA) in a humidified atmosphere containing 5% CO_2 for 48 h.

Stimulation of PBMC

Isolated PBMC were plated at a density of $1.5 \cdot 10^6$ cells/ml in a supplemented RPMI 1640 medium and stimulated with 10 μg/ml of nitrogen phytohaemaglutinin (PHA) for 48 h (Neurauter *et al.*, 2003; Jenny *et al.*, 2011).

Assays of COX-1 and COX-2 activities

Stimulated PBMC were collected 48 h after exposure to PHA using trypsin/EDTA. Cells were lysed by sonication in a cold buffer [0.1 M Tris-HCl, pH 7.8, containing 1 mM EDTA, while keeping the sample as concentrated as possible (to a cell pellet of 100 μl no more than 400 μl buffer were added)]. After centrifugation at 10000 x g for 15 min at 4 °C, the supernatant was kept on ice for the enzyme assays. The inhibitory activity of the test compounds was assessed using Cayman's COX assay kit that measures the peroxidase activity of COX (Kulmacz and Lands, 1983) according to the manufacturer's instructions. Briefly, 40 μl of the cell lysate were added to the designated wells of a 96-well plate in the presence of 10 μl of diluted heme (an aliquot of 88 μl of heme was diluted in 1912 μl dilution buffer) and the test agent at 800 μg/ml to measure total COX activity. Specific wells were dedicated to incubation of cell lysate with the test agent in the presence of either the COX-1 inhibitor SC-560 or the COX-2 inhibitor DuP-697. The plate was cautiously shaken for a few seconds and then incubated for 20 min at 25 °C. Then 20 μl of the colourigenic substrate were added to each well. The reaction was initiated by adding 20 μl of 2.2 mM arachidonic acid to each well. The plate was carefully shaken again for a few seconds and then incubated for 20 min at 25 °C. The absorbance was measured at 590 nm using a ChroMate 4300 microplate reader (Awareness Technology, Inc., Palm City, FL, USA). Percent inhibition of COX-1 and COX-2 activities was calculated according to the following equation: % COX-1 inhibition = (total COX activity – total COX activity in COX-1(2) inhibitor-treated sample)/total COX activity.

Statistical analysis

Data were expressed as mean ± standard error (SE) of the mean. Unless otherwise indicated, statistical analyses were performed using one-way analysis of variance (ANOVA). If the overall *F*-value was found statistically significant ($p < 0.05$), further comparisons among groups were made according to the *post hoc* Tuckey's test. All statistical analyses were performed using GraphPad InStat version 3 (GraphPad Software, Inc., La Jolla, CA, USA) software.

Results and Discussion

Anti-inflammatory effect

Thirteen selected Saudi Arabian plants (Table I) were screened for their potential anti-inflammatory activity using the carrageenin-induced rat paw edema test. Intraplantar injection of carrageenin to rats resulted in severe inflammation with a significant increase in the mean volume of the challenged paws compared to that of the untreated paws (Table II). This initial phase of inflammation has been reported to be mediated mainly by histamine, serotonin, and bradykinin, and an increased synthesis of prostaglandins (PGs) around the damaged tissue was observed (Di Rosa, 1972). After the first hour, the inflammation continued to increase gradually during the following 3 h. This second, late, phase is thought to be sustained by the release of PGs (Di Rosa, 1972) due to the induction of inducible COX-2 (Seibert *et al*., 1994).

According to the inhibition of edema formation (EI) 3 h after carrageenin injection, the methanolic extracts of all tested plants possessed significant ($p < 0.05$) anti-inflammatory activity except that of *Chenopodium schraderianum* (Table II). The three most active extracts were those from *Vernonia shimperi*, *Anabasis articulata*, and *Trichodesma trichodesmoides*, with EI values of 81, 78, and 77%, respectively.

These three extracts were subjected to fractionation and the fractions tested for their anti-inflammatory activity as above (Fig. 1). The *n*-butanol fractions of *A. articulata* and *V. shimperi* were highly effective with EI values of 68 and 65%, respectively. On the other hand, the aqueous fraction was the most potent fraction of *T. trichodesmoides* that inhibited edema formation by 70%. However, no significant differences between the individual fractions and the total methanolic extract were observed and hence the compounds in the fractions do not seem to act synergistically.

To explore the mechanism of action for the anti-inflammatory activity of the tested methanolic extracts of *V. shimperi*, *T. trichodesmoides*, and *A. articulata*, PGE_2 and TNF-α levels were determined in paw exudates.

Prostaglandins are important cell growth factors and are recognized as major mediators of inflammation. Especially PGE_1 and PGE_2 serve as chemotactic and activating factors for inflammatory cells and have been used in the evaluation of the effects of non-steroidal anti-inflammatory agents for decades (Vane, 1971). They are produced from arachidonic acid by the COX-1 and COX-2 isozymes. The anti-inflammatory ac-

Table II. Effect of different plant extracts on carrageenin-induced paw edema in rats.

Plant	Change in paw volume (%)			EI[a] (%)
	Time after carrageenin injection			
	1 h	2 h	3 h	
Untreated control	35.9 ± 8.2	60.9 ± 8.8	95.3 ± 8.2	–
Indomethacin	27.5 ± 12.5	48.0 ± 17.9	27.8* ± 11.1	70.8
Achillea biebersteinii	23.6 ± 6.9	48.2 ± 10.6	32.4* ± 7.6	66.0
Achyranthes aspera var. *aspera*	9.7 ± 5.186	27.6 ± 9.7	31.7* ± 6.1	66.7
Anabasis articulata	44.8 ± 3.8	30.8 ± 4.9	21.1* ± 3.8	77.9
Astragalus abyssinicus	21.5 ± 11.6	51.5 ± 22.8	36.6* ± 14.2	61.6
Caralluma russelliana	31.3 ± 8.0	32.4 ± 9.2	28.9* ± 8.6	69.7
Caralluma tuberculata	28.6 ± 2.9	69.7 ± 6.9	37.5* ± 6.3	60.7
Chenopodium schraderianum	51.3 ± 15.9	79.4 ± 20.8	53.9 ± 12.4	43.4
Conyza stricta	18.5 ± 5.9	33.7 ± 6.3	30.3* ± 10.9	68.2
Crotalaria microphylla	60.1 ± 8.9	52.3 ± 7.9	47.9* ± 6.7	49.7
Echinops galalensis	53.7 ± 7.9	56.1 ± 13.3	45.0* ± 9.4	52.8
Hypoestes forskaolii	43.9 ± 6.3	37.2 ± 6.6	33.2* ± 6.2	65.2
Trichodesma trichodesmoides var. *tomentosum*	16.9 ± 3.7	33.1 ± 5.7	22.1* ± 8.5	76.8
Vernonia schimperi	28.3 ± 8.8	21.9 ± 7.1	17.8* ± 6.0	81.3

* Significantly different from control group at $p < 0.05$.
[a] EI, edema inhibition after 3 h in %.

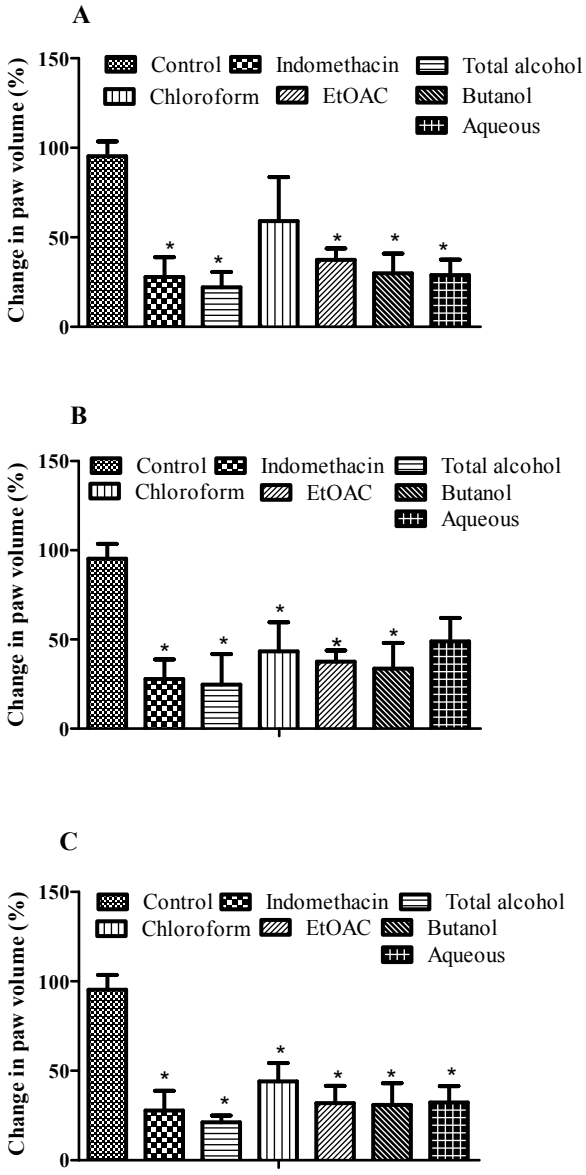

Fig. 1. Percentage change in paw volume after 3 h of administration of different fractions of (A) *Trichodesma trichodesmoides*, (B) *Vernonia schimperi*, and (C) *Anabasis articulata*. *Significantly different from control at $p < 0.05$.

tivity of the methanolic extracts of *V. shimperi*, *T. trichodesmoides*, and *A. articulata* was accompanied by a significant reduction of the PGE_2 level (Fig. 2A).

TNF-α is a pleiotropic cytokine which plays a critical role in both acute and chronic inflammation (Holtmann *et al.*, 2002). Several inflammogens have the ability to induce TNF-α synthesis. The formation of a number of small molecular mediators of inflammation is linked with TNF-α and thus contributes to the range of mediators that critically control inflammation (Harada *et al.*, 1994). TNF-α facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cells (Gamble *et al.*, 1985). The anti-inflammatory activity of extracts from *T. trichodesmoides*, *V. schimperi*, and, to a lesser extent, *A. articulata* was accompanied by a decreased TNF-α level (Fig. 2B).

PGs are arachidonic acid metabolites synthesized by cyclooxygenase (COX). COX-2 is the inducible isoform of COX, since its levels increase in response to various stimuli, including inflammation, in different types of tissues (Teather *et al.*, 2002). In contrast, COX-1, the constitutive form of COX, is involved in housekeeping of cellular function (Herschman, 1996). The three most potent plant extracts were tested at 800 μg/ml for inhibition of COX-1 and COX-2 using indomethacin as a reference inhibitor (800 μg/ml). Plant extracts giving a minimum inhibition of 50% were considered to have good activity (Eldeen and Van Staden, 2008). The three tested plant extracts showed good activity against both COX-1 and COX-2 (Figs. 2C and D), but inhibition of COX-2 was more pronounced, while the reverse was true for indomethacin. This exceptional effect on COX isozymes can be advantageous since COX-2 inhibition has been linked to the therapeutic effects in the management of inflammation, pain, cancer, and neuropathologic conditions, while COX-1 inhibition results in many undesirable side effects (Loren, 2002; Jachak, 2006; Blobaum and Marnett, 2007).

Content of flavonoid and phenolic compounds

Among the three highly active extracts, the flavonoid content, expressed as rutin equivalents (RE), was highest in the *V. schimperi* methanolic extract (2.87 mg RE/g dried extract) followed by *T. trichodesmoides* (1.99 mg RE/g dried extract) and *A. articulata* (1.64 mg RE/g dried extract). The highest phenolic content, determined as gallic acid equivalents (GAE), was found in the extracts of *A. articulata* (13.50 mg GAE/g dried extract) followed by *T. trichodesmoides* (7.66 mg GAE/g dry weight) and *V. schimperi* (7.00 mg GAE/g dry weight).

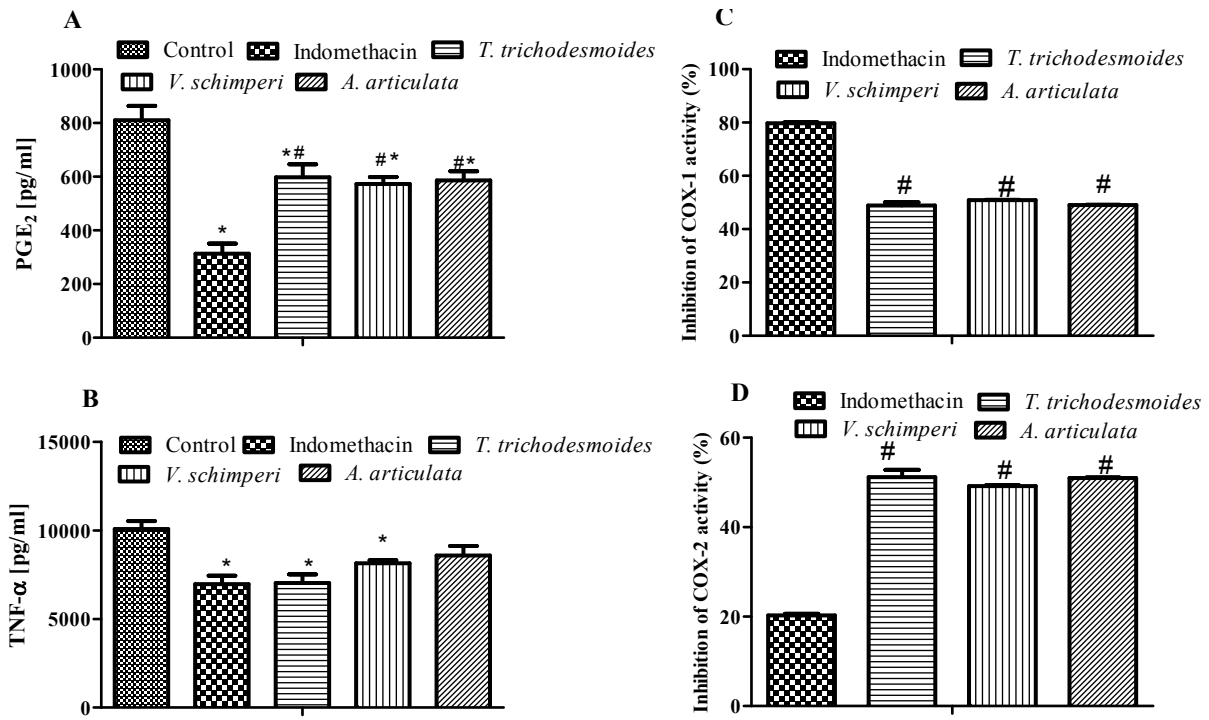

Fig. 2. Effect of total methanolic extracts of *Vernonia schimperi*, *Trichodesma trichodesmoides*, and *Anabasis articulata* on the level of (A) PGE_2 in exudates of rat paw edema, (B) TNF-α of rat paw edema, (C) COX-1, and (D) COX-2 activities. *Significantly different from control at $p < 0.05$; # significantly different from indomethacin at $p < 0.05$.

Determination of antioxidant activity

Production of reactive oxygen species (ROS) has been linked to an acute inflammatory response (Dyugovskaya *et al.*, 2002). These ROS mediate cell damage in many pathophysiological conditions associated with inflammation or oxidative stress through a number of independent mechanisms including the initiation of lipid peroxidation, inactivation of a variety of antioxidant enzymes, and glutathione depletion (Kojda and Harrison, 1999; Kobayashi *et al.*, 2003). All tested extracts were able to reduce the stable radical DPPH to the yellow coloured diphenylpicrylhydrazine. *V. schimperi* possessed the most potent DPPH scavenging activity (IC_{50} 20 μg/ml), followed by *T. trichodesmoides* (IC_{50} 32 μg/ml) and lastly *A. articulata* (IC_{50} 61 μg/ml). The IC_{50} value for the positive reference, ascorbic acid, was 10.8 μM.

The anti-inflammatory activity of the potent extracts can be explained on the basis of their high contents of phenolics (*V. schimperi*, *T. trichodesmoides*) and saponins (*A. articulata* and *V. schimperi*). The anti-inflammatory activity of many phenolic compounds has been attributed to cyclooxygenase and lipoxygenase inhibition, as well as to their antioxidant activity (Kim *et al.*, 2004; Oomah *et al.*, 2010). Flavonoids have been reported to inhibit the expression of isoforms of inducible nitric oxide synthase, cyclooxygenase, and lipoxygenase, with subsequent elevation of nitric oxide, prostanoids, and leukotrienes, as well as other mediators of the inflammatory process such as cytokines, chemokines or adhesion molecules (Tuñón *et al.*, 2009). The saponins in *Anabasis* and *Vernonia* species are another factor that may be responsible for the anti-inflammatory effect of the studied plants (Safayhi and Sailer, 1997; Abdou *et al.*, 2013).

Vernonia is a genus of about 1000 species belonging to the family Asteraceae. Chemical investigation of some *Vernonia* species revealed the presence of saponins (Cioffi *et al.*, 2004). *V. cinerea* and *V. condensata* showed anti-inflammatory effects due to their phenolic and saponin

constituents, respectively (Abeysekera *et al*., 1999; Valverde *et al*., 2001). The phytochemical investigation (Ayoola *et al*., 2008) of the *n*-butanol fraction of *V. schimperi*, which was the most effective fraction, revealed the presence of saponins as major constituents in addition to flavonoid glycosides. This fact is confirmed by our finding of the high antioxidant activity (IC_{50} 20 μg/ml) and high flavonoid (2.87 mg RE/g dried extract) content of the methanolic extract.

A. articulata, the second most effective plant, belongs to the family Chenopodiaceae, which is well known for its phenolic acid and saponin contents (Hussein, 1985). Phytochemical screening of *A. articulata* revealed the presence of saponins as major constituents of the *n*-butanol fraction. The anti-inflammatory activity of *A. articulata* may be attributed to its saponin content as proven for *A. setifera*, a related species containing cyclooxygenase inhibitory saponins (Abdou *et al*., 2013)

Finally, the least effective plant of the three, *T. trichodesmoides*, belongs to the family Boraginaceae. *Trichodesma* species have been examined for pyrrolizidine alkaloids (Wassel *et al*., 1987) and triterpenes (Singh *et al*., 2006). Aerial parts of *T. amplexicaule* (Singh *et al*., 2006) as well as the chloroform fraction of *T. indicum* root (Perianayagam *et al*., 2006) showed anti-inflammatory activity against carrageenin-induced paw edema. The anti-inflammatory activity of *T. trichodesmoides* may be based on the phenolics detected in its aqueous fraction. The high phenolic content is in agreement with its potent antioxidant activity (IC_{50} 32 μg/ml) and phenolic (7.66 mg GAE/g dry weight) as well as flavonoid (1.99 mg RE/g dried extract) contents.

Conclusion

Of a total of thirteen investigated plant species, the methanolic extracts of *V. shimperi*, *A. articulata*, and *T. trichodesmoides* possessed significant anti-inflammatory activity, presumably due to their phenolic and/or saponin contents. This activity is likely to be mediated, at least in part, through reduction of PGE_2 and TNF-α levels and COX-2 activity (Abdou *et al*., 2013). All three plant extracts inhibited both COX enzymes, but with more selectivity towards the COX-2 isozyme, contrary to indomethacin. The extracts exhibited antioxidant activity that might participate in the anti-inflammatory activity. In future work, the active constituents responsible for the anti-inflammatory activity will be isolated.

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