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Full Length Research Paper

Evaluation of anti-inflammatory potential of *Atropa acuminata* in carrageenan induced inflammation in rats

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The present study was envisaged to assess the rationality for the use of traditionally in vogue herb *Atropa acuminata* as an anti-inflammatory agent. The drug, that is, *A. acuminata* when tested against carrageenan induced paw oedema showed significant anti-inflammatory activity at the dose of 1 g/kg body weight. The effects were analyzed by following the evaluation of various biochemical parameters including succinate dehydrogenase (SDH), gamma-glutamyl transpeptidase (GGT), lipid peroxidation (LPO), nitric oxide (NO), superoxide dismutase (SOD), glutathione, xanthine oxidase (XO), catalase and reduced glutathione peroxidase (GPx) were measured and the results suggested that the extracts possess significant anti-inflammatory activity, and with regard to certain parameters, the extracts performed fairly well than the allopathic drug, Diclofenac.

Key words: *Atropa acuminate*, anti-inflammatory lipid peroxidation activity, carrageenan.

INTRODUCTION

Reactive oxygen species (ROS) are the oxygen derived free radicals that have well defined roles in the inflammatory process (Winyard et al., 1999). Their actions include the killing of microorganism as well as participation in cell to cell communication among phagocytes via the activation of a superoxide dependent chemo-attractant (Davies et al., 2001). The role of ROS and reactive nitrogen species (RNS) in inflammation is well documented (Bazzichi et al., 2002; Karatas et al., 2003; Winter et al., 1962). These highly reactive intermediates interact with several extracellular and intracellular molecules and with each other, thus, generating a complex network of responses culminating in outcome that may be detrimental or beneficial for the host. Studies indicate that the increased oxidative and/or defective antioxidant status has a big potential to contribute to the pathology of inflammation (Winter et al., 1962). Other control mechanisms which may be perturbed in inflammation include: the oxidative modification of low density lipoprotein, the oxidative inactivation of α-1-protease inhibitor, DNA damage, lipid peroxidation (LPO) and heat shock protein associated with the activation of neutrophil, nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) and endothelial cell xanthine dehydrogenase, which have been found to contribute significantly to the inflammatory processes.

A number of modern therapies and drugs are employed for the treatment of inflammation. Most of these are directed at non-specific suppression of the inflammation but these therapies are mostly loaded with severe side effects.

In the present work, the anti-inflammatory potential of traditionally in vogue herb *Atropa acuminata* was studied, through *in vitro* and *in vivo* pharmacological and biochemical experimentation that may also aid in the understanding of its mode of action.

Atropa acuminate (mait brand)

The genus *Atropa* comprises four species of medicinal plants, distributed in the Mediterranean region, Southern

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Europe and Asia. Of these, *Atropa belladona* has long been a reputed drug in Europe but its properties do not seem to have attracted the attention of Indian systems of medicine. *A. belladona*, the common European species is cultivated in some places on the Himalayas. The species found wild in India is *A. acuminata* Royle.

Atropa acuminata Royle.

It is a tall straight plant of about 3 to 5" high. The leaves are stalked, elliptic-lanceolate acuminata, 3 to 6" long and 2 to 4" broad. The aerial shoots die every autumn and new ones arise in the following season and form a large taproot with many rootlets. They are woody, pale brown in colour, 6" or more in length and 3/8 to 3/4" in diameter. They have short transverse scars due to the folding of outer bark. The bell shaped flowers are solitary, short stalked, about an inch long and are generally yellow in colour. The fruit is a purple black berry of the size of a cherry (Wealth of India).

The plant is a wild one in Apharwat area of Gulmarg, Ferozpur and Thajwass glaciers of Sonamarg in the valley of Kashmir. It is abundantly used as a traditional medicine for treating arthritis and related inflammatory disorders by Gujars and nomads inhabiting the higher reaches of the valley of Jammu and Kashmir. In literature, it has been also found to be present in abundance in Muzaffarabad, Kanawar (PAK, 8,500'), and many other places in Baluchistan. During the collection of leaves, a portion of stem is also removed. The rest of the plant is then uprooted and dried.

Indian *bellodona* is the most studied species of *Atropa*. It was exploited during worldwar I and large quantities of roots were exported from Himalayas and other outlying parts of the Himalayas. Indian *belladonna* is now used in India also for the manufacture of tinctures, plasters etc. The roots may serve as a source of atropine. *A. belladonna* is a perennial herb widely distributed throughout Central and Southern Europe. It is cultivated for medicinal purposes in England, Central Europe and on an increasingly large scale in the USA. The plant contains several alkaloids, chiefly l-hyoscyamine, $C_{17}H_{23}O_3N$ and small amounts of atropine.

MATERIALS AND METHODS

The chemicals, bovine serum albumin (BSA), H_2O_2, tertiary butyl hydroperoxide, tertiary butyl alcohol (TBA), Folins ciocalteus phenol reagent (CDH India), ethanol (Bengal chemicals), ascorbic acid, ferric nitrate, trichloroacetic acid (TCA), sodium dihydrogen monophosphate, sodium hydrogen diphosphate (Sigma Aldrich), ethylene diamine tetraacetic acid (EDTA) (Hi media). 2,2-diphenyl-1-Picrylhydrazyl (DPPH), NaOH, sodium potassium tartarate (Sigma Aldrich), ammonium thiocyanate (Qualigens), ferrous chloride (BDH-Analar), linoleic acid (Sigma Aldrich), xanthine, reduced glutathione (GSH) and 5,5-dithiobis-2 nitro benzoic acid (DTNB) were obtained from Thomas baker India. Diclofenac sodium (vovran) was available with the local chemist.

Preparation of plant extract

The rhizome of *A. acuminata* was authentically identified and collected from higher reaches of kongdoori, Afarwath areas of Gulmarg and Thajwas glacier of Sonamarg in the month of May to June, 2007. The plants were identified by the courtesy of library and expert facilities of the Kashmir University herbarium (KASH) in centre of plant taxonomy (COPT) University of Kashmir. The voucher specimen was deposited under herbarium no. Kash-bot/KU/Atr-Rb-001 at the centre.

The authentically identified plant material (roots of *A. acuminata*) was shade dried. It was powdered and then subjected to different extraction procedures. The absolute ethanolic extract was prepared using Soxhlet extractor. The extract was dried under reduced pressure using a rotary flash evaporator. The percentage yield of absolute ethanolic extract was 18 g.

The aqueous extract was prepared by decoction method. The powdered material was suspended in just boiled distilled water (10 g/50 ml) and kept overnight (18 to 20 h). The extract was separated from the residue using a muslin cloth, and kept in autoclaved screw capped glass bottle in refrigerator. The residue was re-extracted in further 50 ml distilled water and the decoction was prepared as previously described. The supernatant was mixed with the earlier one and stored. The total volume of water used to prepare the decoction was thus, 100 ml. The percentage yield for aqueous extract was 8 to 10 g. All the extracts were re-dissolved in autoclaved deionized distilled water (DDW) and stored at 4°C for experimental purpose.

Animals

Pathogen free adult Wistar strain of rats (150 to 180 g body weight) were used throughout the study. Rats were procured, kept and used in an environmentally controlled room with a 12 h light-dark cycle at constant room temperature (24 ± 20°C and relative humidity (60 ± 15%). Animals were acclimatized for 1 week before starting the experiment. At a maximum, six rats were kept in each polypropylene cage. Animals had free access to pellet diet and water *ad libitum*.

Determination of the dose of extract

Toxicity of the extracts was determined by dividing the animals in different groups; each group containing eight animals. Lethal dosage (LD_{50}) was determined by testing a dose up to a level of 3 g/kg body weight orally for 7 days at 24 h interval. No mortality or other apparent effects were noticed. The dose of test extract that was used in this study was 1 g/kg body weight.

Assessment of anti-inflammatory activity

Carrageenan induced inflammation

Anti-inflammatory activity of the extracts was determined by carrageenan described by Wright et al. (1981). The rats were divided into five groups, each containing six animals. Two groups were given test extracts orally, declofenac treated group was considered as positive control, normal saline was initially given to the remaining two groups. Immediately after dosing, paw volume were measured by Plethesmiometer. After 1 h of dosing, carrageenen was injected locally in the hind paw under the plantar

region except in normal group. A single injection of 0.1 ml of 1% carrageenen solution prepared by suspending 0.1 g carrageenan in 10 ml normal saline solution produces acute inflammatory oedema leading to marked increase in volume of the limb. After 1 h of carrageenan, injection paw volume was again measured and final reading was taken after 3 h of injection with maximum inflammation. Percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{(Vc - Vt)}{Vc} \times 100$$

Where VC and VT are average oedema volume of control and treated group, respectively.

Preparation of tissue lysate for biochemical analysis

Immediately after pharmacological studies, animals in each group were sacrificed by cervical dislocation under light anesthesia in an ethically proper way. The animals were immediately dissected and the liver of each animal was removed and washed in ice-cold saline (0.9% NaCl). The extraneous material was removed from the tissue, and the tissue as blotted gently between the folds of a filter paper. A portion of the liver was cut and weighed to prepare a 10% homogenate. The liver homogenate (or lysate) was prepared in ice-cold buffer (phosphate buffer) (0.1 M, pH 7.4) using a Heidolph Diax 900 homogenizer and subsequently subjected to sub-cellular fractionation for biochemical analysis on various fractions (phenazine methosulphate (PMS) obtained by centrifugation at 10,500 × g for 20 min).

Biochemical analysis

Lipid peroxidation (LPO)

The assay of LPO was done by the method of Claiborne (1985). Reaction mixture in a total volume of 2.0 ml contained 1.8 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml of the liver homogenate (10% w/v). The reaction mixture was incubated at 37°C in a water bath shaker for 1 h. The reaction was stopped by the addition of 0.67% TBA (w/v prepared in distilled water). Following the addition of 1 ml of 0.67% TBA (w/v prepared in warm distilled water), all the tubes placed in boiling water bath for 20 min. In the end, solutions were cooled by keeping the tubes in ice and then centrifuged at 2500 × g for 10 min. The supernatant containing the thiobarbituric acid reactive substance (TBARS) formed in each sample was measured at 535 nm using spectrophotometer against reagent blank. The results were expressed as n mole malonaldehyde (MDA) formed / g of tissue using a molar extinction coefficient of 1.56 × 105 $M^{-1}cm^{-1}$.

Estimation of catalase

Catalase activity was assayed by the method of Mohandas et al. (1984). The assay mixture consisted of 1.99 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml of H_2O_2 (0.019 M) and 10 µl PMS (10% w/v) in a total volume of 3.0 ml in a quartz cuvette. Decrease in absorbance due to the disappearance of H_2O_2 was recorded at an interval of 30 s up to 3 min at 230 nm spectrophotometrically.

Glutathione peroxidase

Specific activity was measured according to the procedure described by Stripe and Corta (1969). The reaction mixture in a 3 ml cuvette consisted of 1.53 ml of phosphate buffer (0.05 M, pH 7.0) 0.1 ml of 1 mM EDTA, 0.1 ml of 1 mM NaN_3, 0.1 ml of 1 mM reduced glutathione, 0.1 ml of 0.2 mM NADPH, 0.01 ml of 0.25 mM H_2O_2 and 100 µl PMS in a final volume of 2.0 ml. The activity was measured in terms of decrease in absorbance at 340 nm suggestive of disappearance of NADPH at an interval of 30 s for 3 min at room temperature. The enzyme activity was calculated as nmole NADPH oxidized/min/mg protein by using molar extinction coefficient of 6.22 × 103 $M^{-1}cm^{-1}$.

Xanthine oxidase (XO)

XO catalyses the conversion of xanthine to uric acid, which shows a characteristic absorption peak around 290 nm. The following spectrophotometric method for XO estimation is based on the procedure of Marklund and Marklund (1974) as modified by Ali et al. (2000). Briefly, 0.2 ml of PMS was diluted to 1 ml with Tris buffer (0.5 M pH 8.1) and incubated for 5 min at 37°C. Reaction was started by adding 0.1 ml of 1 mm xanthine (1.52 mg dissolved in 10 ml of 1 N NaOH). The reaction mixture was kept at 37°C for 20 min. The reaction was terminated by the addition of 0.56 ml of ice cold perchloric acid (10% w/v in distilled water). After 10 min, 2.5 ml of distilled water were added to the precipitated mixture, which was then centrifuged at 1200 × g for 10 min. The clear supernatant was decanted and the optical density (OD) was read at 290 nm. The results are expressed as µ moles of uric acid formed/mg protein. The activity of XO was calculated by using a 2 mm stock solution of uric acid to prepare the standard curve.

Superoxide dismutase (SOD)

SOD activity was measured as per protocol adapted from Kun and Abood (1949). Pyrogallol auto oxidation by superoxide radical (O_2^-) generated by univalent reduction of O_2 is inhibited by SOD. SOD converts superoxide radical to H_2O_2 which does not interfere with the auto oxidation process (Marklund and Marklund 1974).

$$2O_2^- + 2H + SOD \rightarrow H_2O_2 + O_2$$

Briefly, two test tubes were taken and marked C and T (control and test). In control tube, Tris buffer (50 mm Tris and 1 mm EDTA) and pyrogallol (20 mm) was added while as in test tube Tris buffer, pyrogallol and test extract (0.1 ml) was added. After an induction period of 90 s, absorbance was recorded first in control and then in tests every 30 s for 3 min at 420 nm. The induction period was allowed to achieve a steady state of auto oxidation and pyrogallol. The rate of change of absorbance per minute of 0.02 to 0.03 was noted between 3½ to 4½ min time interval in the control. The rate of change of absorbance in the sample was noted at this particular time interval to calculate the SOD activity. The increase in absorbance at 420 nm after addition of pyrogallol was inhibited by the presence of SOD.

Succinate dehydrogenase (SDH)

SDH was estimated by the method of Orlowski and Meister (1973). Briefly, 5 ml calibrated centrifuge tubes were pipetted 0.5 ml of 0.1 M phosphate buffer (pH 7.4) added 0.5 ml of 0.2 M sodium succinate, 0.2 ml of 10% tissue homogenate, make of volume up to with distill water and lastly 1.0 ml of freshly prepared 0.1% triphenyl tetrazolium chloride solution, after shaking tubes for a period of 20 min. Immediately after removal of the tubes from the water bath, 7 ml of acetone was added and the tubes were stopped and shaken vigorously, precipitate was centrifuged and clear supernatant was

drawn off for the determination of the OD color is stable for several hours and measured by spectrophotometer using 420 nm wavelength. Triphenyl tetrazoliumchloride is a suitable indicator of the SDH activity of tissue homogenate. Tissue homogenate in the presence of succinate in buffer reduced colourless tetrazolium salt to a red water insoluble formazone. The activity was expressed as µg tetrazolium dye reduced/mg tissue of 38°C for 20 min.

Gamma-glutamyl transpeptidase (GGT) activity

GGT activity was determined by the method of Jollow et al. (1974) using gamma glutamyl p-nitroanilide as substrate. The reaction mixture contains 0.2 ml serum, which was incubated with 0.8 ml substrate mixture (containing 4 mM gamma nitroanilide, 40 mM glycylglycine and 11 mM $MgCl_2$ in 185 mM Tris HCl buffer, pH 8.25) at 37°C. 10 min after initiation of the reaction, 1.0 ml of 25% TCA was added and mixed to terminate the reaction. The solution was centrifuged and the supernatant fraction read at 405 nm. Enzyme activity was calculated as nmol p-nitroanilide formed/min/mg protein using a molar extinction coefficient of 1.74 $\times$ 103/M cm. Protein was estimated by Lowry's method.

Reduced glutathione (GSH)

GSH in the liver was determined by the method of Misko et al. (1991). In this estimation, the acid soluble sulphdryl group (of which more than 93% is reduced glutathione) forms a complex with DTNB. The complex, 5-thio-2-nitrobenzoate is yellow in colour and can be detected at 412 nm. Briefly, 1 ml of the tissue sample was precipitated with 1 ml of sulphosalicyclic acid (4% w/v in distill water). The samples were kept at 40°C for at least 1 h and then subjected to centrifugation at 1200 $\times$ g for 15 min at 40°C. The assay mixture contained 0.1 ml of supernatant, 2.7 ml of phosphate buffer (0.1 M pH 7.4) and 0.2 ml of freshly prepared DTNB (4 mg/ml of 0.1 M phosphate buffer of pH 7.4) in a total volume of 3.0 ml. The colour developed was read immediately at 412 nm in a spectrophotometer. The activity was calculated using GSH as standard and expressed as µ mole of GSH/g tissue.

Nitric oxide (NO)

NO was determined by the method of Ramprasath et al. (2005). In this method, nitrite was measured by using Griess reaction. Briefly, to 50 µl of PMS sample diluted to 1 ml with distill water and 1% sulphanilamide in 1 N HCl, 0.02% naphthalene ethylene diamine dihydrochloride (NEDD) is added and immediately mixed and incubated at 37°C for 30 min. Reaction was read at 540 nm. The activity was calculated using sodium nitrate as standard and expressed as n mole of nitrate /ml of PMS.

Statistical analysis

The level of significance between the two groups was based on the students test followed by the analysis of variance. The t-test was determined with the help of software available on internet (www.graphpad.com). The level of significance was chosen at $P< 0.0001$, $P< 0.001$ and $P< 0.05$.

RESULTS AND DISCUSSION

The results obtained are given in Tables 1 to 3. The condition that arises as a result of the excessive generation of the ROS or due to the depletion of antioxidants is described as the oxidative stress. Many plants and plant products have been attributed with the potential to alleviate the problems of the inflammation through free radical abrogations which were assessed in our present study.

The plant is a wild one in Apharwat area of Gulmarg, Ferozpur and Thajwass glaciers of Sonamarg in the valley of Kashmir. It is abundantly used as a traditional medicine for treating arthritis and related inflammatory disorders by Gujars and nomads inhabiting the higher reaches of the valley of Jammu and Kashmir. In literature, it has been also found to be present in abundance in Muzaffarabad, Kanawar (PAK, 8,500'), and many other places in Baluchistan. During the collection of leaves a portion of stem is also removed. The rest of the plant is then uprooted and dried.

The phenolic content of the aqueous and ethanolic extract of *A. acuminata* has been found to be significant (88.8 and 59.6) µg gallic acid equivalent (GAE) per mg extract as done by Singletons procedure. The later might be responsible to act against an oxidant damage hence, inflammation through their radical abrogating properties.

Acute inflammation induced in rats by injecting carrageenan, has been widely used for understanding the pathophysiological basis of inflammation and to study the effect of traditionally used anti-arthritic medicinal plants. Evaluation of SDH, LPO, NO, glutathione, and SOD etc are among the biochemical changes that have been reported to get altered under inflammatory conditions. Dioclofenac was used as an allopathic control medicine throughout the study, and percent inhibition of inflammation was calculated for various pharmacological and biochemical parameters. All studies were carried out on ethanolic and aqueous extracts.

Various biochemical changes in inflammation have been reported (Naik et al., 1972). SDH, for example, is a key inner mitochondrial membrane enzyme that is linked to the energy yielding citric acid cycle in living cells. An increase in SDH would mean an increased supply of adenosine-5'-triphosphate (ATP) to liver and possibly other tissues including the inflamed tissue. A significant increase in the activity of SDH in animal models of inflammation has been reported (Mary et al., 1982). In consonance with these reports, our study also reflects an increase in the level of SDH in carrageenan induced inflammation model. The test extracts of *A. acuminata* caused the inhibition of SDH but the effect was more pronounced with the ethanolic extracts of *A. acuminata*.

GGT, another enzyme associated with the inflammation is involved in the biosynthesis of leukotrienes (Sing et al., 1986), which act as inflammatory mediators at pico or nanomolar concentration. A significant increase in GGT was observed following carrageenan treatment, consistent with the similar studies reported by others (19, 20). The results with the test extracts of *A. acuminata*

Table 1. Effect of *A. acuminata* on carrageenan induced paw oedema in rats.

Group	1st hour mean ± SD	3rd hour mean ± SD	Percent inhibition
Carrageenan alone	0.345 ± 0.079	0.39 ± 0.014	
Carrageenan + T.E 1	0.173 ± 0.021*	0.128 ± 0.011*	(49.8), (67.l)
Carrageenan + T.E II	0.113 ± 0.022*	0.11 ± 0.05*	(67.2), (71.7)
Carrageenan + diclofenac	0.14 ± 0.07*	0.116 ± 0.03*	(59.4), (70.25)

The table represents the effect of ethanolic and aqueous extracts of *A. acuminata* on carrageenan induced paw oedema in male Wistar rats. Diclofenac is used as a known reference anti-inflammatory drug. Positive control, Diclofenac; T.E I, *A. acuminata* (ethanolic); T.E II, *A. acuminata* (aqueous). Values are expressed as mean ± SEM (n = 6). *P < 0.001 are statistically significant.

Table 2. Effect of test extracts on biochemical parameters in carrageenan induced paw oedema.

Group	SDH (mcg TPT/ mg of tissue)	GGT (nmol p-nitroanilide/ mg protein	XOD (µmol uric acid/ mg protein)	LPO (MDA/gm tissue)
Normal	2.7 ± 0.24	398 ± 13	178 ± 10.2	3915.2 ± 528
Carrageenan alone	5.9 ± 0.33	812 ± 31	213 ± 9.9	7292.5 ± 444
Carrageenan + T.E I	*3.4 ± 0.57 (42.3)	490 ± 44 (39.6)	*142 ± 11.5 (33.3)	*4148.7 ± 382 (43.1)
Carrageenan + T.E II	*4.7 ± 0.29 (20.3)	*587 ± 34 (27.7)	*174 ± 12.3 (18.9)	*4495.2 ± 482 (38.3)
Carrageenan + diclofenac	*2.4 ± 0.41 (59.3)	*423 ± 16 (47.9)	*127 ± 11.7 (40.3)	4150.2 ± 587 (43.0)

SDH: Values are expressed as mean ± SEM (n = 6). The group designed as normal received saline. Enzyme activity is expressed as mcg triphenyltetrazolium chloride reduced per mg of liver for 10 min at 38°C. GGT: Values are expressed as mean ± SEM (n = 6). The group designed as normal received saline. Enzyme activity is expressed as nmole p-nitroanilide/mg protein. XOD: Values are expressed as mean ± SEM (n = 6). Activity is expressed in µmole uric acid/mg protein. LPO: Values are expressed as mean ±SEM (n=6). Enzyme activity is expressed as malonaldehyde formed/gm of tissue. *P < 0.001 are statistically highly significant.

which showed appreciable percentage inhibition comparable to diclofenac, a known anti-inflammatory drug.

An increase in LPO following carrageenan treatment and hence, Inflammation triggers some uncharacterized metabolic reactions in the liver, which increases the output of lipid peroxides. In studies with our extracts, ethanolic *A. acuminata* shows a good percentage of inhibition on LPO, suggesting that the anti-inflammatory effects could be also mediated through modulation of LPO. The activity of XO was measured in this study to ascertain the role of molybdoflavoprotein as a source of free radicals that may contribute to inflammation. In the present study, we observed an increase in the activity of XOD in acute model of inflammation. The extracts however were capable of inhibiting the level of XOD, limiting the generation of ROS and consequent inflammation.

Among the compensatory metabolic responses, catalase and GSH peroxidase (GPx) are the enzymes of particular interest. The level of catalase as measured in acute model of inflammation, decreased when carrageenan was used. The decrease could be due to excessive production of H_2O_2 suggesting that catalase could provide an important line of defense against inflammation. Probably catalase could be considered as protein that can be used for early disease detection. Following the treatments with the test extracts, the activity of the catalase improved suggesting that the extract might be inducing catalase to counteract the damaging effect of peroxides that are produced in higher amounts in inflammation.

In addition to catalase, another protective enzyme, glutathione peroxidase, a selenium containing enzyme catalyzing the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GSH, has been shown to protect mammalian cells against oxidative damage (Mary et al., 1982). During carrageenan induced inflammation, the activity of the GPx is observed to be seriously depressed. In the presence of test extracts, the reversals were observed with test extracts of *A. acuminata*. The effects were comparable with diclofenac known anti-inflammatory drug. The observed effects of test extracts and diclofenac clearly depict the involvement of peroxides in the tissue injury and consequent inflammation.

Another enzyme SOD takes care of the superoxide anion radical that is one of the common radicals

Table 3. Effect of test extracts on biochemical parameters in carrageenan induced paw oedema.

Group	CAT (nmol H_2O_2/min/ mg protein)	GPx (nM NADPH/min/ mg protein)	SOD (units/mg protein)	GSH (µmol GSH/ g tissue)	NO (nmol nitrite/ml)
Normal	718 ± 11	497 ± 15.5	9.58 ± 0.17	654 ± 26.0	298 ± 21.5
Carrageenan alone	528 ± 15.8	298 ± 25	3.79 ± 0.11	573 ± 19.0	484 ± 11.3
Carrageenan + T.E I	*678 ± 9.9 (-28.4)	*412 ± 13 (-38.2)	*6.04 ± 0.06 (-59.3)	*661 ± 21 (-15.3)	*318 ± 19.0 (34.2)
Carrageenan + T.E II	613 ± 17.6 (-16.0)	340 ± 31 (-14.0)	4.67 ± 0.14 (-18.8)	616 ± 32 (-7.5)	*372 ± 21.6 (23.1)
Carrageenan + diclofenac	861 ± 17.7 (-63.0)	*439 ± 21.6 (-47.3)	*5.09 ± 0.11 (-34.3)	*636 ± 11.8 (11.5)	*384 ± 12.0 (20.6)

Values are expressed as mean ± SEM (n = 6). *p < 0.001 are statistically highly significant.

produced in the cell under both normal and diseased conditions. SOD enzymes may include the Mn-containing SOD in mitochondria and Cu/Zn SOD in cytosol or on extracellular surfaces (Boughton-Smith et al., 1993). In acute inflammation, superoxide is produced at rate that overwhelms the capacity of the endogenous SOD enzyme defense system to remove it. Superoxides has been implicated in number of effects which may include release of cytokines (Salvemini et al., 1991) and recruitment of neutrophils at the site of inflammation (Salvemini and Masferrer, 1996; Ali et al., 2001). The activity level of SOD is an important biochemical parameter in inflammation as it modulates superoxide which is known to cause tissue damage and consequent inflammation (Al-Majeed et al., 2003). Our present study suggest that the test extracts show the positive modulation of the SOD following carrageenan treatment indicating that they have a potential to affect free radical scavenging. In particular, the comparable results of ethanolic *A. acuminata* and diclofenac suggest that there is a positive correlation between modulation of SOD and the final inflammation.

Endogenous tripeptide, GSH, constitutes a major defense against radical damage that is of common occurrence in inflammatory conditions (Mary et al., 1982). GSH is the most abundant thiol present in all mammalian cells. GSH serves many functions. It can store cysteine in a nontoxic form and serves as a co-substrate for GPx, wherein GSH is used as a hydrogen donor to reduce hydrogen peroxide and organic peroxide to water and alcohol. Besides, it maintains the sulfhydryl residues of certain proteins and enzymes in the reduced state, and also forms conjugate with exogenous and endogenous toxic compounds in a reaction catalyzed by glutathione-sulphur-transferase. The level of hepatic glutathione has been reported to change under inflammatory conditions (Mary et al., 1982). The results of the present study depicted the status of GSH in inflammation. It is interesting to note that the level of GSH decreased slightly following carrageenan injection. However, following treatment with test extracts, GSH levels improved with ethanolic extracts of both test plants.

The synthesis of NO, an important molecule in the process of inflammation and produced from activated cells and macrophages is basically controlled by inducible nitric oxide synthase (iNOS) and thus, regulation of iNOS is an important feature of acute inflammation. The importance of iNOS for the maintenance of acute inflammation is further supported by the observation that its protein expression is upregulated during the last stage of inflammation in paws and in dorsal horn neurons of the spinal cord after injection of carrageenan. NO is besides a potent vasodilator, and its involvement during an inflammatory response may also be related to its ability to increase vascular permeability and oedema through changes in local blood flow (Salvemini et al., 1991). Furthermore, NO has been shown to increase the production of proinflammatory prostaglandins *in vitro* and *ex vivo*. It has been reported that iNOS selective inhibitor when given to rat, failed to inhibit carrageenan induced oedema during the first 3 h (Salvemini et al., 1991). NO might regulate cyclooxygenase (COX) which appears to be concentration dependent. In the present study, an increase in the level of NO in acute inflammation model was observed which was however reduced in the presence of our test extracts. The results of extracts are comparable to diclofenac.

Conclusion

The plant *A. acuminata* has been found to be a potential candidate as anti-inflammatory agent, hence, the active components of the said plant need to be isolated and evaluated for further study.

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