Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 6, 2014

Original Article

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF RHIZOME PART OF *DRYNARIA QUERCIFOLIA* (L.) J. SMITH.

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Received: 24 Jul 2013 Revised and Accepted: 02 Feb 2014

ABSTRACT

Objective*:* The present investigation was aimed to justify the scientific basis in traditional use of *Drynaria quercifolia* as anti-inflammatory agent in North-East India.

Methods*: In-vitro* antioxidant activities were evaluated by Reducing power method, Superoxide scavenging method and DPPH radical scavenging method. *In-vitro* and *in vivo* anti-inflammatory activity were evaluated using albumin denaturation and membrane stabilizing method and carrageenan induced inflammation method. *In-vitro* cyclooxygenase inhibition was also done to investigate the pathway of anti-inflammatory action.

Result*:* Both methanol (MEDQ) and aqueous (AEDQ) extracts showed significant (**p<0.01) inhibition of rat paw edema in dose dependent manner and the MEDQ was the most active. The MEDQ exhibited highest inhibition of COX-1 and COX-2, protein denaturation and haemolysis at 100µg/ml. The extracts also exhibited antioxidant property against DPPH, super oxide radicals and reducing power activity. The maximum 95.972µg Gallic acid equivalents total phenolic content and 81.03µg Quercetin equivalent flavonoids content in MEDQ were estimated that was 20 fold (phenolic content) and 3 fold (flavonoids) more than AEDQ.

Conclusion: These observations established the traditional claim of usefulness of *D. quercifolia* rhizome against inflammation, which could be due to cyclooxygenase enzyme inhibition and free radical scavenging activities of the extracts.

Keywords: Anti-inflammatory, Antioxidant, Cyclooxygenase and *D. quercifolia*.

INTRODUCTION

Inflammation is the host response to trauma or as the defense mechanism against invasive organisms which eventually lead to redness, pain, swelling and temperature that evokes inflammatory cells (macrophages, neutrophils, monocytes, dendritic and mast cells) to invade the site of infection or wounds establishing an 'inflammatory microenvironment' that leads to the death and degradation on the organism, agent or affected cells and eventual restoration of cellular or organ repair process [1]. In many inflammatory disorders there is excessive activation of phagocytes, production of O_2^-, OH radicals as well as non free radicals species (H_2O_2), which can harm severely tissues either by powerful direct oxidizing action by activating matrix metello protinase damage seen in various arthritic tissues [2,3]. Due to its implication in virtually all human and animal diseases, inflammation has become the focus of global scientific research, more so, since the currently used anti-inflammatory agents both steroidal and non-steroidal are prone to evoking serious adverse reactions [4, 5]. However, antioxidant supplements or antioxidant containing foods may be used to help the human body to reduce oxidative damage. Herbs and spices are, in general harmless sources for obtaining natural antioxidants. It is evident that there is an increasing demand to evaluate the antioxidant properties of direct plant extract. In spite development of new synthetic anti-inflammatory drugs the search of new drugs is necessary from alternative sources. It is well documented that synthetic anti-inflammatory drugs (NSAIDs) produce intestinal tract ulcers (with potential internal bleeding) in 10 – 30% of long term users, and erosions of the stomach lining and intestinal tract in 30 – 50% of cases and also cause liver and kidney damage with long term use [6,7].

Drynaria quercifolia (L.) J. Smith of the family Polypodiaceae of Pteridophyta is distributed widely in the evergreen forests of India. The rhizome is reported to be used by different cultural groups of India for treatment of diarrhea, typhoid, cholera, chronic jaundice, fever, headache and skin diseases [8,9]. Whole plant is anthelmintic, expectorant, tonic and used in the treatment of chest and skin diseases. The fronds have astringent properties and are found to strengthen and promote the repair of sinews, muscles and bones [10 – 13]. They are effective for lower back and ligament injuries *D. quercifolia* rhizome is used topically in traditional Chinese medicine to stimulate hair growth and to treat baldness. *D. quercifolia* along with other combination of herbs is used in pain from traumatic injury, such as sprains and contusions with bruising and swelling [14, 15]. With this background the present study designed with an aim to justify traditional claims of using *D. quercifolia* as source of anti-inflammatory agent with scientific tools.

MATERIALS AND METHODS

Plant materials

Drynaria quercifolia (L.) J. Smith (DQ) rhizomes was collected from Tripura, North-East India in the month of July, 2011 and authenticated by consulting with Assam University Herbarium (AUS/LS/20044). The plant material was cleaned, shade dried and reduces into coarse powder with a electrical blender.

Extraction and sample preparation

The powder of *D. quercifolia* obtained was soaked with the petroleum ether to remove wax and fat materials and then subjected to successive soxhlet extraction with methanol and water one after another as solvents in 1:4 (w/v) ratios which were standardize in laboratory condition before extraction. The respective extracts (methanolic extracts=MEDQ and aqueous extract=AEDQ) were concentrated under reduced pressure in vacuum evaporator (Buchi Rotavapor® R-210) and dried in vacuum desiccators. After drying all products was stored in refrigerator (8 ± 2°C) and same was used for *in vitro* and *in-vivo* studies.

Animals

Albino rats (Wister) weighing 150-200g and albino mice weighing 20-25g of either sexes were used in this study. They were procured

from Regional Institute of Medical Sciences (RIMS), Imphal. The animals were housed in polypropylene cages and maintained at 27°C ± 2°C and standard 12 hr dark / light cycle and acclimatized for one week. They were fed with standard food and water *ad libitum*. The waste in the cages was renewed daily to ensure hygienic condition and maximum comfort for animals. Ethical clearance for handling the animals and experimentation was obtained from the Institutional Animals Ethical Committee (IAEC), IBSD, Imphal (approval No.-IBSD/IAEC/Trainee/Ph.cology/9) prior to the commencement of the experimental works.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out for Saponins, Flavonoids, Steroids, Alkaloids, Phenols, Tannins, Reducing sugar, Carbohydrate, Protein following the methodology of [16, 17].

Determination of Antioxidant Activity

Reducing power activity

Different doses of methanol extract (MEDQ) and aqueous extract (AEDQ) were mixed in 1 ml of distilled water so as to get 10µg, 20µg , 40µg, 80µg and 100µg concentration and a control (without any test sample). L- Ascorbic acid (Sisco Research Laboratories Pvt. Ltd. India) used as positive control. These were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide (2.5ml, 1%) The mixtures were incubated at 50°C for 20min. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture, which were then centrifuged at 3000 rpm for 10min. The upper layer of the solutions (2.5ml) were mixed with distilled water (2.5ml) and ferric chloride (0.5ml, 0.1%), and the absorbance (OD) was measured at 700nm in Thermo Multiscan Spectrum [18]. Increased absorbance of the reaction mixture indicates increase in reducing power. Percentage inhibition was calculated and this activity was expressed as an effective concentration 50 (EC_{50}).

The percentage reducing power was calculated by using the formula.

% Reducing power = [Test $_{OD}$ – Control $_{OD}$ / Test $_{OD}$] X 100

Super oxide anion scavenging activity

100µl riboflavin solution, 200µl EDTA solution, 200µl ethanol and 100µl NBT solution was mixed in a test tube and the reaction mixture were diluted up to 3 ml with phosphate buffer (pH. 7.4). The absorbance of solution was measured at 560nm using phosphate buffer as blank after illumination for 15 min. This was taken as control reading.

Different concentration (10µg, 20µg, 40µg, 80µg and 100µg) of MEDQ and AEDQ and L- Ascorbic acid (Positive control) in 100µl, was mixed with 100µl riboflavin, 200µl EDTA, 200µl ethanol and 100µl Nitro blue tetrazolium (NBT) solution in the test tubes, then the reaction mixtures were diluted upto 3ml with phosphate buffer. The absorbance of solution was measured after illumination for 15min at 560nm in Thermo Multiscan Spectrum [18, 20]. Percentage inhibition was calculated and this activity was expressed as an inhibition concentration 50 (IC_{50}).

The percentage inhibition was calculated by using the formula.

$$\% \text{ Inhibition} = \frac{\text{Control}_{OD} - \text{Test}_{OD} \text{ X } 100}{\text{Control}_{OD}}$$

DPPH Free radical scavenging activity

The free radical scavenging activity of MEDQ and AEDQ were measured by 1,1-Diphenyl-2-picryl hydrazil (DPPH HiMedia laboratories Pvt. Ltd. India) using the method of Gulchin *et al*., (2002). Briefly, 0.1mM solution of DPPH in ethanol was prepared. Then, 1ml of this solution was added to 3ml of MEDQ, AEDQ and L-Ascorbic acid (positive control) solution at different doses (10–100g). The mixture were shaken vigorously and allowed to stand at room temperature for 30min. Then the absorbance was measured at 517nm in Thermo Multiscan Spectrum. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity [19, 20].

Percentage DPPH free radical inhibition was calculated and this activity was expressed as an inhibition concentration 50 (IC 50).

The percentage inhibition was calculated by using the formula.

$$\% \text{ Inhibition} = \frac{\text{Control}_{OD} - \text{Test}_{OD} \text{ X } 100}{\text{Control}_{OD}}$$

Estimation of Total Phenolic Content

Total Phenolic content of extracts was determined by Folin–Ciocalteu method. Extract solution / standard (100µl) mixed with 200µl 10% (v/v) F–C reagent. 800µl, 700 mM Na_2CO_3 was added into each tube and incubate the assay tubes at room temperature for 2h. 200ml sample or blank was transferred from the assay tube to a clear 96 well microplate and absorbance of each well was taken at 765nm. Standard curve was calculated from standard Gallic acid at 765nm in Thermo Multiscan Spectrum and total Phenolics was obtained as Gallic acid equivalents using the regression equation between Gallic acid standards and A_{765nm} [21].

Estimation of Total Flavonoids

The total flavonoid content of the extracts was determined by aluminium chloride ($AlCl_3$) colorimetric method. Extract (0.5 ml) were mixed with 1.5ml methanol, 0.1ml of 10% $AlCl_3$, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415nm with Thermo Multiscan Spectrum. Using quercetin as standard, standard curve was prepared and linearity was obtained in the range of 1-10µg/ml. using the standard curve the total flavonoid content was expressed as quercetin equivalent in percentage w/w of the extracts [21].

***In-vitro* anti-inflammatory activity**

Inhibition of albumin denaturation

Methods of Mizushima and Kobayashi [1968] and Sakat *et al*., (2010) followed with minor modifications. The reaction mixture was consisting of test extracts at different concentration (50 – 400µg) and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. Diclofenac sodium (10mg) was used as a standard drug. The sample extracts and standard were incubated at 37° C for 20min and then heated to 51°C for 20 min. after cooling the samples the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicate.

Percent inhibition of protein denaturation was calculated as fallows,

$$\% \text{ Inhibition} = \frac{\text{Control}_{OD} - \text{Test}_{OD} \text{ X } 100}{\text{Control}_{OD}}$$

Where Control $_{OD}$ is the absorbance without sample, Test $_{OD}$ is the absorbance of sample extract / standard.

Membrane stabilization test

Preparation of red blood cells [RBCs] suspension

Fresh whole human blood (10ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% (v/v) suspension with normal saline [24, 25].

Heat induced haemolysis

The reaction mixture (2ml) consisted of 1 ml of test sample solutions (MEDQ and AEDQ) at different concentration (50 – 400µg) and 1ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium (10mg) was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates for all the test samples [24, 25].

Percentage of inhibition of haemolysis activity was calculated by the following formula,

$$\% \text{ Inhibition} = \frac{\text{Control}_{OD} - \text{Test}_{OD} \times 100}{\text{Control}_{OD}}$$

Where Control $_{OD}$ is the absorbance without sample, Test $_{OD}$ is the absorbance of sample extract / standard.

Determination of acute toxicity (ALD_{50})

The acute toxicity for MEDQ and AEDQ were determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline no. 423, Annexure – 2d) method of CPCSEA, Govt. of India was adopted for toxicity studies. The tested extracts were administrated orally. The mortality and behavioral abnormalities was observed in 48h after administering 2000mg/kg in the all cases [26].

Carrageenan Induced Paw edema

Paw edema was induced by injecting 0.1ml of 1% (w/v) carrageenan sodium salt (Ozone pharmaceuticals and chemicals, Gujarat, India) subcutaneously into the sub-plantar region of the rat right hind paw to group of 6 animals each, which were pre-treated either with normal saline 2 ml/kg (Control) or diclofenac sodium 8mg/kg p.o. (reference) or 150 mg/kg, p.o. or 100 mg/kg, p.o of test samples (MEDQ and AEDQ extract), 30 min before the carrageenan injection. The paw volume was measured plethysmometrically before administering carrageenan and 1, 2, 3, 4 and 6 hours after. Inhibition of Inflammation was calculated as the increase in volume [ml] of the paw after treatment [27].

Cyclooxygenase-1 (COX 1)and Cyclooxygenase-2 (COX 2)Inhibition assay

The potency of the extract for Cyclooxygenase inhibition was assessed by calculating percentage inhibition at the dose of 100µg/ml concentration. COX-1 or COX-2 inhibition was checked using a colorimetric assay kit purchased from Cayman Chemicals, USA. Briefly test samples were (MEDQ and AEDQ) dissolved DMSO and diluted to get 100 µg/ml concentrations for sample. Diclofenac Sodium (Ozone pharmaceuticals and chemicals, Gujarat, India) and Etoricoxib (Etoshine 120, Sun Pharma, India) as positive control solution at different doses [10–100µg] Our assay employed separately for COX-1 and COX-2 inhibition. Percentage inhibition was calculated as per manufacturer instruction and activity was expressed as inhibition concentration 50 [IC_{50}].

Statistical Analysis

Data were expressed as mean ± Standard Error Mean (SEM). Differences were considered significant at ***P<0.001, or **P < 0.01 or * P<0.05 when compared test groups v/s control group. For numerical results, one-way analysis of variance (ANOVA) with Tukey-Kramer Multiple Comparisons post tests were performed using GraphPad InStat Version 3 (GraphPad Software) and all graphs were made by using GraphPad Prism5 software.

RESULTS AND DISCUSSION

The present preliminary phytochemical study shows the presence of flavonoid, phenol, tannin, steroid, protein, carbohydrate in methanol extract and flavonoid, phenol, protein, carbohydrate in aqueous extract. Details of the result illustrated in Table 1.

The antioxidant potentiality of test samples (MEDQ and AEDQ) have been studied by different mechanisms, *viz*. prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity and radical scavenging. Various antioxidant models and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function, reducing power, superoxide scavenging assay, DPPH assay are most commonly used for the evaluation of antioxidant activities of extracts [18].

Table 2 & Fig 1 shows the reducing power, superoxide and DPPH free radical scavenging capacity of MEDQ and AEDQ compared to standard ascorbic acid. For the measurements of the reducing power ability, we investigated the $Fe3^{+}$ – $Fe2^{+}$ transformation in the presence of MEDQ and AEDQ samples. Where, the reducing power of MEDQ and AEDQ showed higher activities than the control in dose dependent manner. The IC_{50} value was 49.83µg for MEDQ and 41.5µg for AEDQ (Table 2, Fig 1). Whereas, IC_{50} value of standard ascorbic acid was 12.39 µg (Table2, Fig 1).

In case of the NBT- superoxide anion system, MEDQ and AEDQ showed higher scavenging activities in dose dependent manner. Where, the decrease of absorbance at 560nm with antioxidants indicates the consumption of superoxide anions generated in the reaction mixture. The IC_{50} values of MEDQ (42.93µg) shows highest superoxide scavenging activity than AEDQ (67.20µg) but their activity was less than standard ascorbic acid (7.60µg) (Table 2, Fig 1).

DPPH in presence of ethanol produces stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Our study illustrates the hydrogen donating ability of MEDQ and AEDQ in various concentrations compare to standard antioxidant (L- ascorbic acid). The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compare to other methods. The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517nm induced by MEDQ and AEDQ when compare to control. The IC_{50} values of MEDQ (53.60µg) shows highest superoxide scavenging activity than AEDQ (66.20µg) but their activity was less than standard ascorbic acid (4.30µg) (Table 2, Fig 1).

It was reported that oxidative stress occurs when free radical formation exceeds the body's capacity to protect itself and contributes different biological chronic conditions such as arteriosclerosis, arthritis, cancer, diabetes and various neuro-degenarations. The primary antioxidants that react with free radicals, which may limit free radical damage occurring in the human body [18]. The observation of present study supported ability of MEDQ and AEDQ in protection against oxidative damage.

It was reported that the flavonoids are a large class of compounds, ubiquitous in plants, and usually occurring as glycosides. They contain several polyphenols or phenol hydroxyl functions attached to ring structures. The cleavage of the glycosidic ring takes place possibly in the gastrointestinal tract releasing of the free polyphenols. The chemical activities of polyphenols components in terms of their reducing properties as hydrogen or electron donating agents predict their potential for action as free radical scavengers (antioxidants). Thus, free radical-scavenging activities of MEDQ and AEDQ may be attributed to the presence of flavonoids and other polyphenols in the extracts.

Flavonoids and other plant phenolics are reported, in addition to their free radical scavenging activity having multiple biological activities including vasodilatory, anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, anti-allergic, antiviral, and estrogenic effects, as well as being inhibitors of phospholipase A_2, Cyclooxygenase (COX), and lipoxygenase (LOX), glutathione reductase and xanthine oxidase [28]. These biological activities are related to their anti-oxidative effects [29].

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is one of the well defined causes of inflammation [30]. In present study, AEDQ and MEDQ have shown inhibition of thermally induced protein (Albumin) denaturation in dose dependent manner, where the IC_{50} values of MEDQ (16.90µg) shows highest inhibition of heat induced heamolysis than AEDQ (20.24µg) but their activity was less than standard diclofenac sodium (8.13µg) (Table 3, Fig 2). Further, the HRBC membrane stabilization has been used as a method to study the *in-vitro* anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membranes its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the

inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes release during inflammation produce a various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [25, 28]. The injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. It is therefore expected that compounds with membrane stabilizing properties, should offer significant protection of cell membrane against injurious substances [31]. In present study, the IC_{50} values of MEDQ (12.63µg) shows highest inhibition of heat induced heamolysis than AEDQ (15.43µg) but their activity was less than standard diclofenac sodium (8.72µg) (Table 3, Fig 2) . These extracts were effective in inhibiting both heat induced albumin Denaturation and heat induces haemolysis indicated usefulness of test extracts against acute inflammation (Table 3 & Fig 2).

The Cyclooxygenase (COX) and 5-lipoxygenase are two important enzymes which catalyze the formation of mediators involved in the inflammatory process. Inhibitors of COX are the main strays of current therapy aimed to modulate pain, inflammation and to control fever [32]. In present study an attempt has be taken to evaluate capability of samples MEDQ and AEDQ on inhibition of both COX-1 and COX-2 in *in-vitro* assay. MEDQ shows the maximum inhibition of COX-1 and COX-2 followed by the AEDQ. The COX-1 and COX-2 inhibition of these extracts was compared with a standard diclofenac sodium and Etoricoxib (Table 4 & Fig 3).

On the basis of effect of test samples in different *in-vitro* assays, AEDQ and MEDQ were evaluated for *in-vivo* anti-inflammatory activity by using carrageenan-induced rat paw edema model. Both extracts were shown a significant (*p<0.05 & **p< 0.01) anti-inflammatory activity in dose dependent manner when compared with the normal control group. The MEDQ exhibited the maximum anti-inflammatory activity at 150mg/Kg (Table 5, Fig 4). The carrageenan-induced rat paw edema assay has frequently been used to evaluate the anti-inflammatory effect of natural products. The induction of edema by using carrageenan is believed to be biphasic in nature. The first phase of inflammation occurs within 1h of Carrageenan injection and is associated with the release of inflammatory mediators such as histamine and serotonin from mast cells. The second phase starts after 1 hr and is illustrated by an increased release of prostaglandins (PGs) at the site of inflammation. During the second phase, the macrophages are believed to release the large amounts of interleukin-1 (IL-1) which led to the augmented gathering of polymorphic nuclear cells (PMNs) to the site of inflammation. The activated PMNs then release the lysosomal enzymes and reactive oxygen species, resulting destruction of connective tissue which induces paw inflammation [33]. In present study, the edema measured after 3 hr of carrageenan injection shows dose dependent inhibition of inflammation by treated groups of animals when compare to control group of animals.

However, detailed studies are necessary to decode the nature of *D. quercifolia* exacts and mechanism of action of the phyto-constituents responsible for the anti-inflammatory effects. The acute toxicity study indicated that *D. quercifolia* is a safe and nontoxic to experimental animals, as *D. quercifolia* is being used extensively by different cultural groups of India to treat various ailments.

Our present investigation was established scientific basis of using *D. quercifolia* in traditional practice for treatment of inflammatory disorder. The anti-inflammatory activity of *D. quercifolia* rhizome could be at least partially due to COX-1, COX-2 enzyme inhibition and free radical-scavenging activities which may be attributed to the presence of flavonoids and other polyphenols in the extracts and thus *D. quercifolia* could be a potent anti-inflammatory agent for use in future.

Table 1: Preliminary phytochemical screening of methanolic and aqueous Extract of *Drynaria quercifolia* (L.) Smith

Sample	Alkaloid	Flavonoid	Phenol	Tanin	Steroid	Saponin	Protein	Carbohydrate	Reducing Sugar
MEDQ	-	+	+	+	+	-	+	+	-
AEDQ	-	+	+	+	-	-	+	+	-

Table 2: IC50 value of *In-vitro* antioxidant activities and total flavonoid and phenolic content Of MEDQ and AEDQ.

S. No.	Sample	IC50 value (µg) of *In-vitro* antioxidant Activities			Total Flavonoids Content (100 µg of Quercetin equivalent)	Total Phenolics Content (100µg of Gallic acid equivalent)
		Reducing power activity	Super oxide anion scavenging activity	DPPH* scavenging activity		
1.	ASCORBIC ACID	12.39	7.6	4.3		
2.	MEDQ	49.83	42.93	53.6	81.03	95.972
3.	AEDQ	41.05	67.2	66.2	4.1	32.60

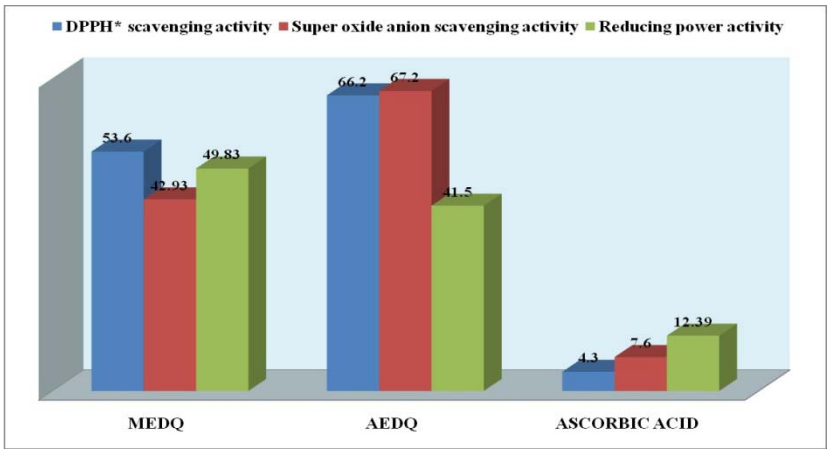

Fig. 1: *In-vitro* antioxidant activities of MEDQ and AEDQ.

Table 3: IC50 value of *In-vitro* anti-inflammatory of MEDQ And AEDQ.

S. No.	Samples	IC50 Value (µg) of *In-Vitro* Anti-inflammatory activity	
		Albumin Denaturation Assay	Inhibition of Heat induced Heamolysis Assay
1	MEDQ	16.90	12.63
2	AEDQ	20.24	15.43
3	DIACLOFENAC SODIUM	8.13	8.72

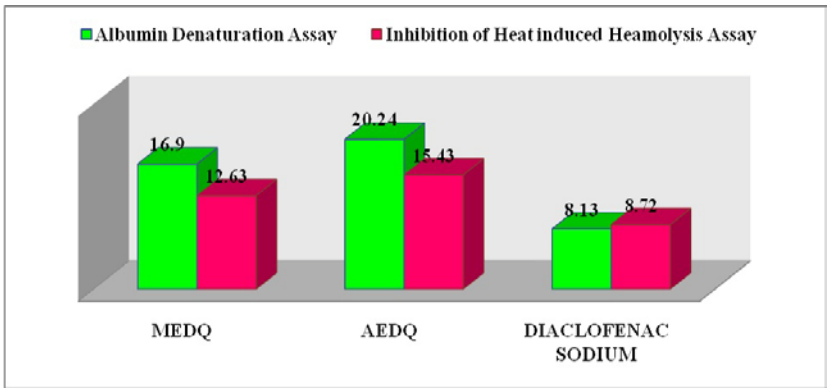

Fig 2: IC50 value of *In-vitro* anti-inflammatory of MEDQ And AEDQ.

Table 4: *In-vitro* Cyclooxygenase-1 and Cyclooxygenase-2 Inhibition assay of MEDQ and AEDQ.

S. No.	Samples	IC50 Value (µg) ± SEM of *In-Vitro* COX inhibition Assay	
		COX - I	COX - II
1	MEDQ	06.66 ± 0.002	06.25 ± 0.008
2	AEDQ	20.00 ± 0.003	12.50 ± 0.005
3	Diclofenac Sodium	03.43 ± 0.002	08.63 ± 0.003
4	Etoricoxib	-	02.34 ± 0.004

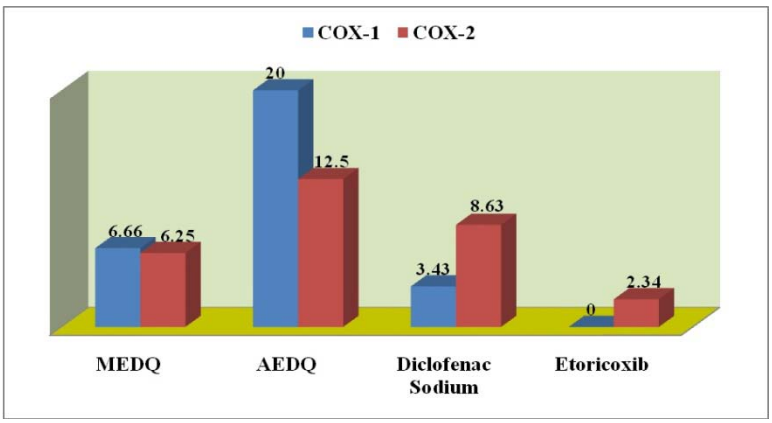

Fig. 3: *In-vitro* Cyclooxygenase-1 and Cyclooxygenase-2 Inhibition assay of MEDQ and AEDQ.

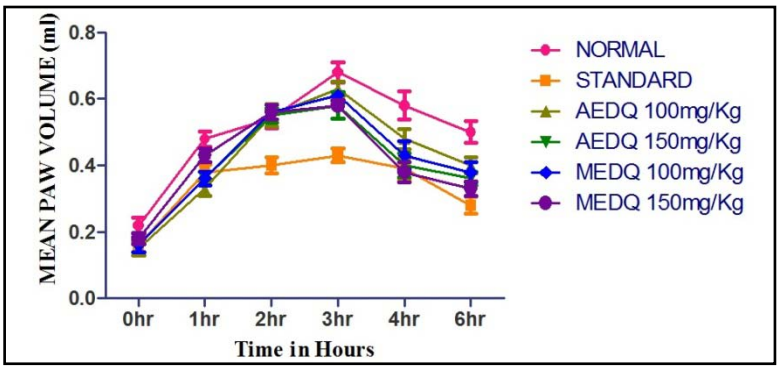

Fig. 4: *In vivo* Anti-inflammatory activity of MEDQ and AEDQ

Table 5: *In vivo* Anti-inflammatory activity of MEDQ and AEDQ

Treatment	Mean paw volume (ml)					
	0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	6hrs
Normal control (1ml normal saline p.o.)	0.22 ± 0.024	0.48 ± 0.023	0.54 ± 0.029	0.68 ± 0.030	0.58 ± 0.043	0.50 ± 0.033
Standard Diclofenace Sodium (10 mg/Kg)	0.16 ± 0.020	0.38 ± 0.024	0.40 ± 0.025	0.43 ± 0.020	0.39 ± 0.033	0.28 ± 0.025**
AEDQ (100 mg/kg)	0.15 ± 0.020	0.33 ± 0.021	0.55 ± 0.034	0.63 ± 0.021	0.48 ± 0.030	0.4 ± 0.025*
AEDQ (150 mg/kg)	0.16 ± 0.021	0.36 ± 0.021	0.55 ± 0.022	0.58 ± 0.040	0.40 ± 0.036	0.36 ± 0.021**
MEDQ (100 mg/kg)	0.16 ± 0.021	0.36 ± 0.021	0.56 ± 0.021	0.61 ± 0.016	0.43 ± 0.042	0.38 ± 0.030*
MEDQ (150 mg/kg)	0.18 ± 0.016	0.43 ± 0.021	0.56 ± 0.021	0.58 ± 0.016	0.38 ± 0.030	0.33 ± 0.021**

(All data were expressed as mean + Standard Error Mean (SEM). Differences were considered significant at *** P<0.001, ** P<0.01, * P<0.05 when compared test groups Vs Control group (n = 6).

ACKNOWLEDGEMENT

I would like to Acknowledge Assam University, Silchar for providing financial assistance. I would like to thank Dr. N. C. Talukdar, Director (i/c) and Scientist – F, Institute of Bioresources and Sustainable Development [IBSD], Dept. of Biotechnology, Govt. of India, Imphal, Manipur and the technical staffs of Pharmacology Laboratory, IBSD, Imphal, Manipur for providing all possible facilities to successfully complete this research work.

Abbreviation

AEDQ - Aqueous extract of *Drynaria quercifolia* (L.) J. Smith.

MEDQ - Methanolic extract of *Drynaria quercifolia* (L.) J. Smith.

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