IJPSR (2021), Volume 12, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 January 2020; received in revised form, 24 May 2020; accepted, 30 May 2020; published 01 March 2021

FRESH JUICE OF *DRYNARIA QUERCIFOLIA* RHIZOME FOR THE MANAGEMENT OF ARTHRITIS

U. M. Dhanalekshmi [1, 2], B. Ramya [1], T. Gowri [1] and R. Srinivasan [* 1]

Organic & Bio-organic Chemistry Division [1], Central Leather Research Institute (Council of Scientific and Industrial Research), Chennai - 600020, Tamil Nadu, India.
College of Pharmacy [2], National University of Science and Technology, Sultanate of Oman.

Keywords:

Drynaria quercifolia rhizome, Anti-arthritic, *In-vitro*, Extract, Toxicity

Correspondence to Author:
Dr. R. Srinivasan

Principal Scientist,
Organic and Bio Organic Chemistry Division, CLRI, Chennai - 600020, Tamil Nadu, India.

E-mail: dhanamum@yahoo.co.in

ABSTRACT: *Drynaria quercifolia* rhizome (DQR) ethnomedicinal information shows the traditional usage of this rhizome for the treatment of body and joint pain, diarrhoea, typhoid, cholera, and skin diseases. The fresh juice of rhizome is still being consumed as an energy drink by tribes and localities of Tamil Nadu and Kerala. However, no investigations have been carried out to claim the anti-arthritic activity of DQR to date. *In-vitro* studies on anti-arthritic effect of the crude extract were performed. *In-vitro* anti-arthritic activities were evaluated by using elastase, PLA2, hyaluronidase, and proteinase and assays like protein denaturation inhibition, membrane lysis, and NO scavenging activity. Based on the promising in-vitro results, the use of DQR as an anti-arthritic agent may be agreed upon, and further establishment towards the safety and efficacy is conceded. Acute toxicity study revealed NOAEL (No Observed Adverse Effect Level) up to 2000 mg/kg body weight. Oral administration of 1000 mg/kg body weight (crude extract) for 14 days led to the well tolerance level in albino rats.

INTRODUCTION: Rheumatoid Arthritis (RA) has a global distribution and affects 0.5-1% (with a female preponderance) of the population [1]. RA is an important cause of disability and mortality and carries a high socioeconomic cost [2]. The goals of management in RA are to control inflammation, prevent progressive joint destruction, preserve and improve activities of daily living and alleviate pain. The conventionally available drugs and their prolonged duration of treatment are associated with many adverse reactions, apart from GI disorders, immune deficiency, and humoral disturbances.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(3).1465-73

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(3).1465-73

Accordingly, reducing the side effects and cost should be considered while designing improved therapeutics for RA, besides enhancing medicinal effectiveness. Alternative treatments based on plant products and herbal mixtures are popular as traditional medicines in India, US, and other countries [3].

According to WHO, still, 80% of the world's population rely on plant-derived drugs and has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe, modern drugs. There are a number of advantages associated with herbal medicines, which include efficacy, tolerability, lower cost, and easy availability [4]. A large number of plant-derived products act as goldmines for the treatment of arthritis because they are inexpensive, lack side effects, and are effective in chronic conditions.

In ancient texts, about 500 plants have been indicated in the treatment of arthritis; however, only a small number of plants have been evaluated scientifically. *Drynaria quercifolia* Rhizome [DQR] (L.) J. Smith. (Polypodiaceae) is an epiphytic medicinal pteridophyte, distributed widely in the evergreen forests of the Western Ghats of Kerala, locally called 'Marappanna-kizhangu' or 'Attukalkizhangu'.

The rhizome is reported to be used by tribal communities of Tamil Nadu and Kerala to cure various diseases and still in the regular practice of the local people of Kolli hills by consuming it as a soup for health benefits [5]. Ethnomedicinal information shows that the rhizome of plant DQR was traditionally used for the treatment of body pain, joint pain, dyspepsia, diarrhoea, typhoid, cholera, chronic jaundice, fever, headache, and skin diseases [5, 6].

However, no systematic investigations have been carried out to analyze the anti-arthritic activity of DQR. Based on the medicinal value of DQR, we are interested in screening the rhizome of DQR for its anti-arthritic property using *in-vitro* models.

MATERIALS AND METHODS:

Plant Collection and Identification: Fresh rhizome of DQR was collected from Kolli hills, Namakkal District, Tamil Nadu (INDIA) by our research group. The plant material was authenticated by Prof. V. Chelladurai, Research Officer (Retd), Government Siddha College, Tirunelveli. A voucher specimen (CLRICSIR/ 2011/09) has been preserved at our laboratory for future reference.

Preparation of Plant Extract and Preliminary Phytochemical Analysis: The rhizome was thoroughly washed with running tap water and then with distilled water. The fur portion was removed from the rhizome. The flesh part was isolated, sliced and minced, and press filtered. The resulting juice was centrifuged at 4000 rpm for 10 min.

The supernatant liquid was concentrated under reduced pressure and lyophilized to obtain a brown-colored free flow residue. The residue was stored in a refrigerator at 4-5 ºC until use (2 kg flesh part gave 1000 ml of juice which gave 27.87 g of crude extract. The percentage yield was 2.8%). Phytochemical constituents of the extract were tested preliminarily for alkaloids, saponins glycosides, cardiac glycosides, flavonoids, anthra-quinones, terpenoids, phytosterols, proteins, and aminoacids [7].

***In-vitro* Anti-arthritic Evaluation:** The different concentrations of crude extract of DQR were studied for *in-vitro* anti-arthritic activity.

Elastase Inhibition Assay: Elastase, an enzyme that belongs to the chymotrypsin family of proteases. It is a chief protein present in the extracellular matrix (ECM) of joints. For elastase inhibition assay, N-Suc-(Ala) 3-nitroanilide was used as the substrate [8, 9]. The release of γ-nitro aniline was measured spectrophotometrically at 410 nm in a 96-well reader [10, 11]. The reaction mixture (1 ml) consisting of 0.7 ml of 200 mMTris-HCl buffer (pH 8.0), 0.1 ml of 0.2 mM substrate, 0.1 ml of 0.104 unit/mL elastase and 0.1 ml of plant extract (100/ 250/ 500/ 1000 µg/ml) was incubated at 25 ºC for 15 min. Dexamethasone was used as the standard drug. The percentage inhibition of elastase was calculated as follows:

Percentage inhibition = (O.D of control - O.D of test) / (O.D of control) × 100

Phospholipase A2 Inhibitory Assay: Phospholipases A2 (PLA2) enzymes play an important role in the initiation and amplification of the inflammatory reaction by the liberation of arachidonic acid from the membrane phospholipids and in the biosynthesis of eicosanoids from various lipid mediators. 0.1 ml of enzyme solution (0.02 mg/ml Bee Venom PLA2 in DMSO) was added with 0.1 ml plant extract (100/ 250/ 500/ 1000 µg/ml in DMSO), incubated at room temperature for 90 min. Then 2 ml of substrate solution was added (3.5 mM Lecithin in 7.0 mM Triton in a solution of 100 mM NaCl, 5.0 mM $CaCl_2$, and 0.055 mM Phenol red), and incubated at room temperature for 5 min. The optical density was measured at 550 nm [12]. The percentage inhibition was calculated as follows:

Percentage inhibition = (O.D of control - O.D of test) / (O.D of control) × 100

Hyaluronidase Inhibitory Assay: Hyaluronidase (HAses) is the endoglycosidase that degrades the hyaluronic acid. Hyaluronidase activity has been

found to be elevated in sera of patients with RA. The method used for hyaluronidase inhibition assay measures the amount of terminal N-acetyl glucosamine liberated from hyaluronic acid (HA) [13]. HA is dissolved in water (8 mg/ml) by sonication and was stored at -20 ºC. Agarose was dissolved in 0.3 M sodium phosphate buffer at pH7.0 in a beaker and then placed on a hot plate and maintained at 55 ºC before use. HA solution was preheated to 55 ºC and mixed with the agarose to give a final concentration of 0.8 mg/ml of HA and 0.8% (w/v) of agarose. Warm HA-agarose mixture (100 µl) was dispensed into each well of the micro-plate. Once the gel had been set, each well was filled with 100 µl of the HAse (in 0.3M sodium phosphate buffer) and 100 µl of plant extract (100/ 250/ 500/ 1000 µg/ml) and incubated at 37 ºC. After incubation, the enzyme samples were removed and each well was filled with 100 µl of 10% cetylpyridinium chloride. After incubation at room temperature for 30 min, the transmittance at 595 nm was measured by using automatic plate reader. The percentage of inhibition was calculated using the following formula:

Percentage inhibition = (O.D of control - O.D of test) / (O.D of control) × 100

Proteinase Inhibitory Action: Neutrophils are known to be a rich source of proteinases. It was previously reported that leucocyte proteinases play an important role in the development of tissue damage during inflammatory reactions [14]. The reaction mixture (2.0 ml) contains 0.06 mg trypsin, 1.0 ml of 25 mMtris-HCl buffer (pH 7.4) and 1.0 ml aqueous solution of extract (100/ 250/ 500/ 1000 µg/ml) and was incubated at 37 ºC for 5 min. Then 1.0 ml of 0.8% (w/v) casein was added and incubated for 20 min. 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. The optical density of the supernatant was read at 280 nm against buffer as blank [15]. The percentage of inhibition was calculated using the following formula:

Percentage inhibition = (O.D of control - O.D of test) / (O.D of control) × 100

Inhibition of Protein Denaturation: Protein denaturation is a process in which proteins lose their secondary and tertiary structure by application of external stress or by a compound such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat [16].

The reaction mixture (5 ml) consists of 0.05 ml of test extracts (100/ 250/ 500/ 1000 µg/ml), and 4.5 ml bovine serum albumin (5% aqueous solution) and pH of the reaction mixture was adjusted to 6.3 by using a small amount of 1N HCl. The above mixture was incubated at 37 ºC for 20 min and then heated to 51 ºC for 20 min. After cooling the samples, and was measured spectrophotometrically at 660 nm [17]. Control represents 100% protein denaturation. The results were compared with the standard (Diclofenac sodium 250 mcg/ml) sample. The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (O.D of control - O.D of test) / (O.D of control) × 100

Membrane Stabilizing Activity: The principle involved in this assay is the stabilization of rat red blood cell membrane against hypotonicity-induced membrane lysis [18]. The reaction mixture (4.5 ml) consisted of 2 ml hypotonic saline (0.25% NaCl), 1 ml 0.15 M phosphate buffer (pH 7.4) and 1 ml test solution (100/ 250/ 500/ 1000 µg/ml) in normal saline. 0.5 ml of 10% rat RBC in normal saline was added. For the control sample, 1 ml of isotonic saline was used instead of the test solution and sample solution without RBC act as product control. The mixture was incubated at 56 ºC for 30 min. The tubes were cooled under running tap water for 20 min. The mixtures were centrifuged, and the optical density of the supernatant was read at 560 nm [19]. The result was compared with the standard (acetylsalicylic acid 250 mcg/ml) sample. Percentage membrane stabilizing activity was calculated as follows:

Percentage inhibition = (O.D of control - O.D of test) / (O.D of control) × 100

Nitric Oxide Scavenging Assay: Nitric oxide was generated from sodium nitroprusside, which at physiological pH liberates nitric acid. This nitric acid gets converted to nitrous acid and further forms nitrite ions (NO2-) which diazotize with sulphanilic acid and couple with naphthyl-ethylenediamine (Griess reagent), producing pink colour, which can be measured at 546 nm [20].

Sodium nitroprusside (10 mM, 2 ml) in phosphate buffer saline was incubated with the test compounds in different concentrations (100/250/ 500 µg/ml) at room temperature for 30 min.

After 30 min, 0.5 ml of the incubated solution was added with 1 ml of Griess reagent, and the absorbance was measured at 546 nm.

The nitric oxide radicals scavenging activity was calculated according to the following equation:

Percentage inhibition = (O.D of control - O.D of test) / (O.D of control) × 100

Experimental Animals: The present study was conducted after obtaining approval from the Institutional Animal Ethical Committee [PROPOSAL NO: IAEC No. 15/01/2011 dated 21.01.2011], and this protocol met the requirements of national guidelines of CPCSEA.

Wistar female albino rats (150-200 g) used for this study were procured from King Institute Guindy, Chennai, India, and housed in the Institutional animal house under standard environmental conditions (23 ± 1 °C, 55 ± 5% humidity and 12 h/12 h light/dark cycle)and maintained with free access to a standard diet (Hindustan Lever, Bangalore, India) and water *ad libitium*.

Acute and Sub-acute Toxicity Study: Acute toxicity study was designed as per the OECD guidelines- 423. 3 healthy adult, non-pregnant female Wistar albino rats weighing between 150-200g were selected for the study.

Being a traditional herbal medicine, the mortality was unlikely at the highest starting dose level (2000 mg/kg body weight). Hence, an acute toxicity test at one dose level of 2000 mg/kg body weight was conducted in all three animals. General clinical observations were done on the same day.

The observation period was 14 days for sub-acute toxicity studies. Animals were divided into three groups, and each group containing six animals. First group received extract of *Drynaria quercifolia* (1000 mg/kg b.w) dissolved in water; second group received fresh juice of *Drynaria quercifolia* (30 ml/kg b.w) orally, third group is the control group.

The health conditions of the animals were recorded. At the end of the study (after 14 days), blood was collected from the orbital sinus for haematological and biochemical estimation. Then the animals were sacrificed to collect the pancreas, liver, lung, and kidney for histopathological analyses.

Statistical Analysis: The statistical package used was Graph Pad Prism version 8. The data were analyzed by Analysis of Variance (ANOVA). Values were expressed as mean ± S.E.M, and those values with $P< 0.05$ were considered significant.

RESULTS AND DISCUSSION: The crude extractof *Drynaria quercifolia* rhizome was evaluated for the phytochemical constituents. Results showed that crude extract was positive for alkaloids, proteins and aminoacids, glycosides, flavonoids, tannins and terpenoids. An anti-arthritic evaluation was performed by various *in-vitro* assays. **Fig. 1A** shows that DQR extract has elastase inhibition activity (A), the maximum inhibition of 93%was observed, whereas standard drug (dexamethasone) showed inhibition of 97% at a concentration of 500 µg/ml. DQR extract has shown maximum (89%) PLA2 inhibition activity at the concentration 1000 µg/ml as displayed in **Fig. 1B**. **Fig. 1C** depicts the maximum (78.5%) hyaluronidase enzyme inhibition at the concentration of 1000 µg/ml. The maximum inhibition (66%) of proteinase enzyme was observed at the concentration 500 µg/ml, which is lesser than the standard (73%) drug aspirin at a concentration of 500 µg/ml as represented in **Fig. 1D**.

The maximum inhibition (70%) in protein denaturation was observed at the concentration 500 µg/ml, which is nearly equal to the standard (75%) drug aspirin at the same concentration and the results are displayed in **Fig. 2E** at the concentration 500 µg/ml, which is nearly equal to the standard (75%) drug aspirin at the same concentration. Membrane stabilization 53% and 52% were observed at the concentration 250 µg/ml and 500 µg/ml, respectively, but this effect is lesser than that of standard (74.5%) drug aspirin at the concentration of 500 µg/ml and the results are shown in **Fig. 2F** DQR extract has good nitric oxide radical inhibition activity, the maximum inhibition (86%) was observed at the concentration of 1000 µg/ml and are depicted in **Fig. 2G**.

To assess the safety of the DQR extract toxicological studies were carried out using Wistar albino rats. The results of the acute toxicity study confirm that there were no changes in body weight, behaviour, and physical performance of the rats. No toxic signs were observed, and also there was no mortality at the dose level of 2000 mg/kg b.w.

In a sub-acute toxicity study for a period of 14 days, there was no significant ($P<0.05$) difference in body weight **Table 1**, difference in body weight, feed and water consumption in the treatment groups when compared to control animals and the results are tabulated in **Table 1** treatment groups when compared to control animals.

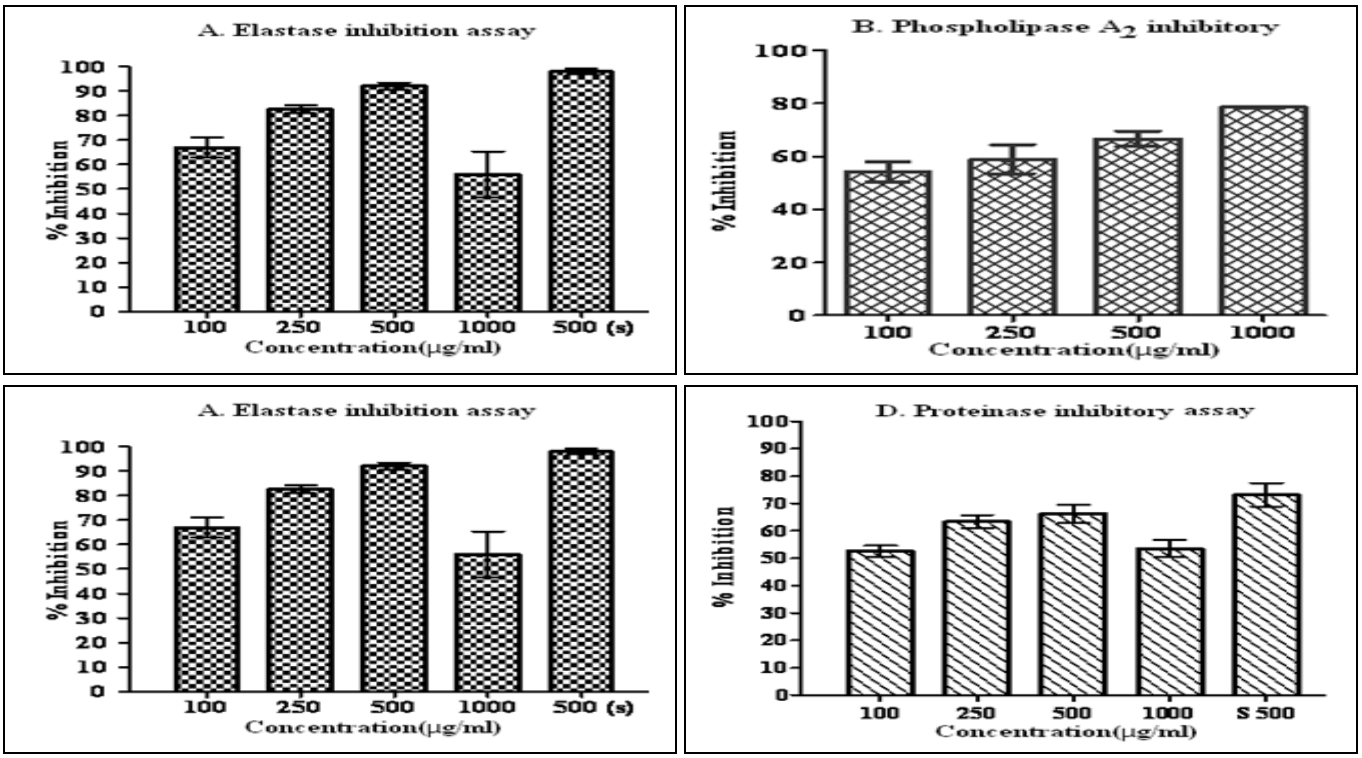

FIG. 1: ***IN-VITRO*** **ANTI-ARTHRITIC EFFECTS OF DQ RHIZOME CRUDE EXTRACT.** Results are expressed as mean ± SD, (n=6).

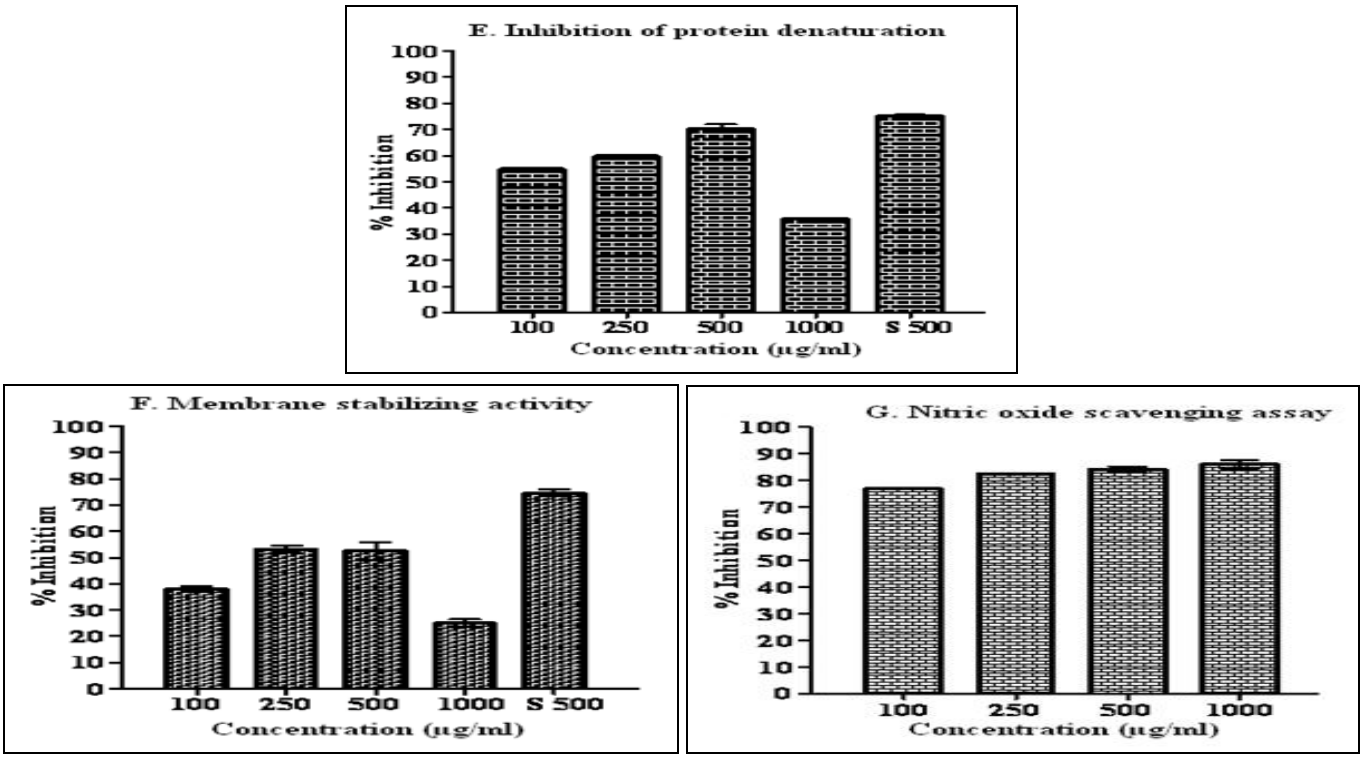

FIG. 2: ***IN-VITRO*** **ANTI-ARTHRITIS EFFECTS OF DQ RHIZOME CRUDE EXTRACT.** Results are expressed as mean ± SD, (n=6).

There was no significant difference in haemoglobin level, total count; differential count, RBC, PCV and platelet count in the treated groups when compared with the control group mentioned in **Table 2**. All the biochemical parameters were within the respective laboratory reference range. There was no significant difference ($P<0.05$) in the glucose, cholesterol, creatinine, TGL, ALP, SGOT, SGPT, and bilirubin levels in the treatment groups in comparison with the control group mentioned in **Table 2**.

TABLE 1: BODY WEIGHT OF EXPERIMENTAL ANIMALS

Groups	DAYS				
	0	4th	7th	10th	14th
Group I	154 ± 18.8	153.16± 6.24	154.5 ± 18.5	157.5 ± 18.8	166.2 ± 18
Group II	145 ± 16.66	145.16 ± 6.58	148.5 ± 6.25	155.33 ± 6.4	160.5 ± 6.0
Group III	145.1 ± 7.0	144.66 ± 5.60	150 ± 6.84	156.16 ± 6.7	172.6 ± 7.2

The values are expressed as mean ± SEM, (n=6).

TABLE 2: HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

Parameters	Group I	Group II	Group III
Hb(g/dl)	11.31 ± 0.4	11.03 ± 2.3	10.78 ± 1.5
TC (cells/cumm)	8250 ± 288	8350 ± 244.5	8280 ± 311
DC- Polymorphs (%)	34.5 ± 2.9	32.8 ± 2.5	33.2 ± 3.1
DC- Lymphocytes (%)	64 ± 2.9	63.4 ± 3.6	65.4 ± 2.6
DC- Eosinophil (%)	3 ± 0.8	3.2 ± 0.5	2.8 ± 0.83
RBC (million/cumm)	4.41 ± 0.2	4.2 ± 0.2	4.5 ± 0.4
PCV (%)	32.83 ± 4.8	33.84 ± 1.8	35.2 ± 2.3
PC (lakhs/cumm)	2.21 ± 0.2	2.18 ± 0.1	2.2 ± 0.2
Glucose (mg/dL)	134 ± 2.4	143.40 ± 11.6	139.50 ± 22
Cholesterol (mg/dL)	32.66 ± 2.3	33.20 ± 2.5	50.167 ± 16.7
Creatinine (mg/dL)	0.483 ± 0.1	0.546 ± 0.1	0.53 ± 0.1
TGL (mg/dL)	93.50 ± 2	95.60 ± 4.6	89.00 ± 20.1
SGPT (U/L)	36.167 ± 1.5	48.80 ± 11	54.167 ± 21
SGOT (U/L)	55.50 ± 2.0	68.00 ± 6.2	50.500 ± 15.3
ALP (U/L)	113.66 ± 3.6	142.0 ± 27.9	142.33 ± 33.9
Bilirubin Total(mg/dL)	0.650 ± 0.10	0.70 ± 0.15	0.85 ± 0.06

The values are expressed as mean ± SEM, (n=6). Comparison of GP 1 with all other groups, does not shown a significant value ($P<0.05$).

Histopathological studies reports in **Fig. 3** showed that Group I (control) showed no abnormality in liver (A), kidney (D), pancreas (G), and lungs (J). Group II (DQR extract-treated group) showed no abnormality in the kidney (E) and pancreas (H), but very mild biliary hyperplasia in liver(B) and pulmonary congestion in lungs (K), whereas Group III (DQR fresh juice group) showed no abnormality in the kidney (F) and pancreas (I).

Rheumatoid arthritis (RA) is very well known as it leads to chronic inflammation of the joint, and there is no known cure [1]. Optimal treatment for the disease involves a combination of medications, rest, joint strength-ening exercises and protection, and patient education. In the case of RA, many of the commonly used drugs are becoming less acceptable because their prolonged duration of treatment is associated with many adverse reactions, apart from GI disorders, immuno-deficiency, and humoral disturbances.

Agents derived from the plants can modulate the expression of pro-inflammatory signals which have the potential to treat arthritis. *Drynaria quercifolia* rhizome is one of such plant that is claimed to possess anti-inflammatory, anthelmintic, and astringent properties [5, 6]. Therefore this study was undertaken to evaluate the anti-arthritic activity of *Drynaria quercifolia* rhizome. Apart from the regular identification of phytochemical constituent, systematic *in-vitro* evaluation assays were conducted in a sequential manner like elatase inhibition assay, PLA2 inhibition assay, hyaluronidase inhibition assay, inhibition of protein denaturation, the effect on membrane stabilization, proteinase inhibition, and nitric oxide scavenging effect. Toxicological studies were then conducted to estimate the maximum tolerable dose and to assess the safety of the plant by using an animal model. From the maximum tolerable dose, the sub-acute study dose was finalized.

The result of the present phytochemical study is supported by other researchers [21]. Our study showed the presence of alkaloids in addition to the other phytochemicals. This difference may be explained by the fact that variations may sometimes occur in bioactive compounds of the same plant in a different environment and by the solvent's nature for extraction [22].

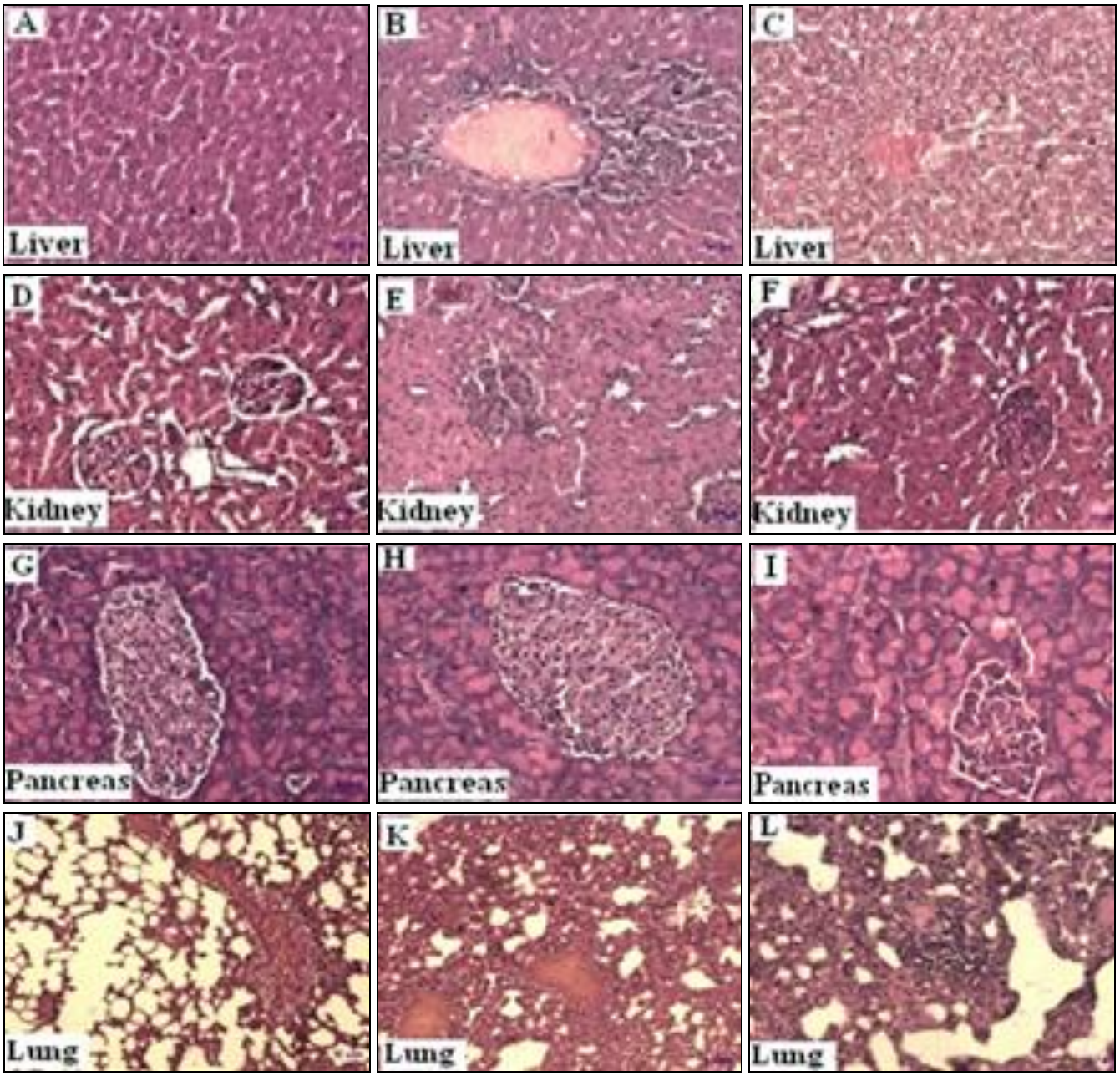

FIG. 3: HISTOPATHOLOGICAL SLIDES IN MICROSCOPIC PICTURE (MAGNIFICATION × 100) OF ORGANS OF RAT TREATED WITH DQR EXTRACT AT 2000 mg/kg BODYWEIGHT AND DQR FRESH JUICE AT 30 ml/kg BODY WEIGHT COMPARED WITH CONTROL GROUP RAT TREATED WITH NORMAL SALINE. FIRST LANE CONTROL GROUP, SECOND LANE DQR EXTRACT AT 2000 mg/kg BODYWEIGHT GROUP, THIRD LANE DQR FRESH JUICE AT 30 ml/kg BODY WEIGHT GROUP

The maximum elastase inhibition (93%) was observed at the concentration of 500 µg/ml. Human neutrophil elastase has broad substrate specificity enzymatic cleavage of elastin and ECM proteins such as collagen, hyaluronan, laminin *etc*. Under normal physiological conditions, endogenous inhibitors protect healthy tissues from damage when it get disturbed; many diseases such as lung disorder, cardiovascular disorder, arthrosclerosis, arthritis may be observed [23, 24]. Our DQR extract-based results showed a very good inhibition capacity of human neutrophil elastase, and hence it

can act as a potent endogenous inhibitor. Standard drug (dexamethasone) showed inhibition of 97% at a concentration of 500 µg/ml.

In the present study, the PLA2 inhibition was maximum at 1000 µg/ml (89%). The PLA2 plays an important role in the initiation and amplification of the inflammatory reaction by the liberation of arachidonic acid from the membrane phospholipids and in the biosynthesis of eicosanoids from various lipid mediators produced elsewhere [12]. The crude extract of DQR exhibited maximum hyaluronidase inhibition (78.5%) at the concentration of 1000 µg/ml. Hyaluronidase contributes in the destruction of ECM, and also plays a vital role in many biological and physiological processes like inflammation, angiogenesis, disease progression *etc*. 25 From our results, it is evident that DQR extract exhibits good antagonist action against hyaluronidase enzyme, and the effect is concentration-dependent.

Proteinase inhibition was maximum (66%) at the concentration of 500 µg/ml, which was slightly lower than the standard drug (aspirin). It was reported that leucocyte proteinases play an important role in developing tissue damage during inflammatory reactions, and a significant level of protection was provided by proteinase inhibitors [26].

Protein denaturation inhibition (70%) was observed to be maximum at a concentration of 500µg/ml, which was slightly lower than that of the standard drug (aspirin) at the concentration of 500 µg/ml. The production of auto-antigens in certain rheumatic diseases may be due to *in-vivo* denaturation of proteins [15]. Hence the results of the present study indicate that DQR extract has good inhibition on protein denaturation, but the effect is almost equal to that of standard.

Membrane stabilization property was maximum (53.1%) at the concentration of 250 µg/ml, which was lower than the standard drug (aspirin).This shows that the DQR extract has anti-inflammatory activity. Also, DQR exhibited the maximum (86%) NO scavenging activity at the concentration of 1000 µg/ml. The scavenging activity of reactive nitrogen species seems to be important in determining the effect of drugs under inflammatory conditions like RA [27]. *In-vitro* results showed that DQR extract has an anti-arthritic activity which is useful in the management of RA.

Based on the promising *in-vitro* results, the use of DQR as an anti-arthritic agent may be agreed upon, and further establishment towards the safety and efficacy is conceded. The acute toxicity study results showed that DQR extract was well tolerated in the dose up to 2000 mg/kg b.w. The results of the sub-acute toxicity study showed that no significant difference between the control and treatment groups in body weight, haematological and biochemical parameters. This shows that DQR extracted in the dose of 1000 mg/kg b.w and was well tolerated, and no toxic signs were observed. There was no mortality. Histopathological examination of liver, kidney, lungs, and pancreas did not show any significant abnormality. This shows that the DQR is safe in dose of 1000 mg/kg b.w administered orally over the period of 14 days.

CONCLUSION: Our preliminary results revealed the very good potential of *Drynaria quercifolia* rhizome extract and also lent scientific validation to the traditional use of DQR in arthritis treatment. Further studies are warranted to assess the *in-vivo* pharmacological action and the exact mechanism behind the activity. Isolation and identification of active component(s) study are also being continued by our research group, which will help in finding new anti-arthritic lead compounds.

ACKNOWLEDGEMENT: RS, UMD, and BR, TGare thankful to Mr. V. Elango, Animal House, CLRI for assistance in the maintenance of experimental animals.

CONFLICTS OF INTEREST: The author declares no conflict of interest.

REFERENCES:

1. Scott DL and Steer S: The course of established rheumatoid arthritis. Best Practice and Research: Clinical Rheumatology 2007; 21: 943-67.
2. Parveen K and Michael C: Kumar & Clark's Clinical medicine. Elsevier, edition 9th, 2016.
3. Mangesh SB, Virendra GK and Rohini RP: Therapeutic effects of polyherbal preparation on adjuvant induced Arthrtis in Wistar rats. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3: 58-60.
4. Dharmasiri MG, Ratnasoorya WD and Thabrew MI: Anti-inflammatory activity of decoctions of leaves and stems of *Anisomeles indica* at pre-flowering and flowering stages. Pharmaceutical Biology 2011; 40: 433-439.

5. Karthik V, Raju K, Ayyanar M, Gowrishankar K and Sekar T: Ethnomedicinal uses of pteridophytes in kolli hills, Eastern Ghats of Tamil Nadu, India. Journal of Natural Product and Plant Resources 2011; 2: 50-55.
6. Selvan T, Thirunavukkarasu T, Shyamala P and David E: A review on some poisonous plants and their medicinal values. Journal of Acute Disease 2014; 3: 85-90.
7. Trease GE and Evans WC: Text book of Pharmacognosy. ELBS Baillere Tindall University press, London. Edition 16th 2009.
8. Baylac S and Racine P: Inhibition of human leukocyte elastase by natural fragrant extracts of aromatic plants. International Journal of Aromatherapy 2004; 14: 179-82.
9. Genc Y, Dereli FTG, Saracoglu I and Akkol EK: The inhibitory effects of isolated constituents from *Plantago major* subsp. major L. on collagenase, elastase and hyaluronidase enzymes: Potential wound healer. Saudi Pharmacology Journal 2019; 28: 101-06.
10. Siedle B, Cisielski S, Murillo R and Loser B: Sesquiterpene lactones as inhibitors of human neutrophil elastase. Bioorganic and Med Chem 2002; 10: 2855-61.
11. Monica BPC, Maria B, Tiziana B and Luca M: Anti-Inflammatory Activity of Thymol: Inhibitory Effect on the Release of Human Neutrophil Elastase. Pharmacology. 2006; 77: 130-36.
12. Maher K, Sonia M, Yassine BA and Mohamed H: *In-vitro* study of the PLA2 inhibition and antioxidant activities of Aloe vera leaf skin extracts. Lipids in Health and Disease 2011; 10: 30.
13. Karolina G, Dagmara W, Paweł Z and Irma P: Anti-inflammatory activity of saponins from roots of *Impatiens parviflora* DC, Natural Product Research 2018; DOI: 10.1080/14786419.2018.1519708.
14. Oyedapo OO and Famurewa AJ: Antiprotease and Membrane Stabilizing Activities of Extracts of *Fagara Zanthoxyloides*, *OlaxSub scorpioides* and *Tetrapleura Tetraptera*. Pharmaceutical Biology 2008; 33: 65-69.
15. Kesharwani D, Paliwal R, Satapathy T and Paul DS: Rheumatiod Arthritis: An Updated Overview of Latest Therapy and Drug Delivery. Journal of Pharmacopuncture 2019; 22: 210-24.
16. Verdoliva V, Senatore C, Polci ML, Rossi S, Cordella M, Carlucci G, Marchetti P, Antonini-Cappellini G, Facchiano A, D'Arcangelo D and Facchiano F: Differential denaturation of serum proteome reveals a significant amount of hidden information in complex mixtures of proteins. PLOS One 2013; 8(3): e57104.
17. Radibratovic M, Minic S, Stanic-Vucinic D, Nikolic M, Milcic M and Velickovic CT: Stabilization of Human Serum Albumin by the Binding of Phycocyanobilin, a Bioactive Chromophore of Blue-Green *Alga Spirulina*: Molecular Dynamics and Experimental Study. PLOS One 2016; 11: e0167973.
18. Omale J and Okafor PN: Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. African Journal of Biotechnology 2008; 7: 3129-33.
19. Chaity FR, Khatun M and Rahman MS: *In-vitro* membrane stabilizing, thrombolytic and antioxidant potentials of *Drynaria quercifolia* L., a remedial plant of the Garo tribal people of Bangladesh. BMC Complementary and Alternative Medicine 2016; 16: 184.
20. Adebayo SA, Ondua M, Shai LJ and Lebelo SL: Inhibition of nitric oxide production and free radical scavenging activities of four South African medicinal plants. Journal of Inflammation Research 2019; 12: 195-03.
21. Prasanna G and Anuradha R: A comprehensive review on phytopharmacological activities of *Drynaria quercifolia* L. International Journal of Phytochemistry and Pharmacognosy Research 2016; 8: 1304-13.
22. Couceiro MA, Afreen F, Zobayed SMA and Kozai T: Variation in concentrations of major bioactive compounds of St. John's wort: Effects of harvesting time, temperature and germplasm. Plant Science2006; 170: 128-34.
23. Natacha J, Pierre V, Abderrazzak B and Philippe M: Neutrophil elastase activity imaging: recent approaches in the design and applications of activity-based probes and substrate-based probes. *In-vivo* Imaging of Inflammation and Infection 2019; 2019: 1-12.
24. Kobayashi S, Uyama H and Kim Y: Inhibition effect of (+) catechin-aldehyde polycondensates on proteinases causing proteolytic degradation of extracellular matrix. Biochem and Biophy Research Communications 2004; 320: 256-61.
25. Grish K and Kemparaju K: The magic glue hyaluronan its eraser hyaluronidase: A biological overview. Science 2007; 80: 1921-43.
26. Deshpande V, Varsha MJ and Kadam J: *In-vitro* anti-arthritic activity of *Abutilon indicum* (Linn.) Sweet. Journal of Pharmacy Research2009; 2:644-645.
27. Helieh O, Chen T and Villiers W: Green tea polyphenols and sulfasalazine have parallel anti-inflammatory properties in colitis models. Frontiers in Immunology 2013; 4: 132-39.

How to cite this article:
Dhanalekshmi UM, Ramya B, Gowri T and Srinivasan R: Fresh juice of *Drynaria quercifolia* rhizome for the management of arthritis. Int J Pharm Sci & Res 2021; 12(3): 1465-73. doi: 10.13040/IJPSR.0975-8232.12(3).1465-73.