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EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *RUMEX VESICARIUS LINN*

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ABSTRACT

The aim of this study is to establish the anti-inflammatory activity and analgesic activity by using carrageenan induced paw edema in rats for ethanolic extract of leaves of *Rumex vesicarius Linn*. Anti inflammatory activity effect was assessed by difference in paw edema volume, before & after the dose administration of the extracts in rats, Analgesic activity was assessed by Tail immersion method and the effects were compared with Standard drug Diclofenac (6mg./3/ml.). These observations helped us to conclude that ethanolic extract of *Rumex vesicarius Linn*, is endowed with both Anti inflammatory and Analgesic activity.

KEYWORDS: Carrageenan, Anti-Inflammatory, *Rumex Vesicarius Linn*.

INTRODUCTION

Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells and irritants. It is the protective attempt by the organism to remove the injurious stimuli as well as initiate healing process for the tissue and considered to be the major cause of rheumatoid arthritis. Recent studies indicate that the mediators and cellular effectors of inflammation are important constituents of the local environment of tumors.

An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses. It is a part of the host defense mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin & prostaglandins. The development of non-steroids in overcoming human sufferings such as Rheumatoid arthritis has evoked much interest in the extensive search for new drugs with this property. The complex events and mediators involved the inflammatory reaction can induce maintain or aggravate many diseases. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary which may be achieved by the aid of plant extracts.[1] Rumex vesicarius Linn is a widely used traditional medicine in many parts of the world for the treatment of various diseases viz. Helminthes inhibitory activity, Anti-oxidant, Anti-inflammatory & Anti-diuretic activity.

MATERIALS AND METHODS

Plant material and extraction

Rumex vesicarius Linn., (Family: Polygonaceae) known as "Bladder dock" or "Chukkakura" is an annual, glabrous herb, 15-30 cm in height, branched from the root, with long, elliptic, ovate or oblong leaves and monoecious flowers. The aerial parts are powered and subjected to soxhlation using Hexane, Benzene, Chloroform, Ethyl acetate, Ethanol and Water by successive solvent extraction method based on the increasing order of polarity of solvent. Initially 25gm of crude aerial parts of the plant powder was taken and packed in a packing paper. This pack was placed in a soxhlet extractor for 24 hrs (approx) with different solvents i.e. (Hexane, Benzene, Chloroform, Ethyl acetate, Ethanol and Water) and the temperature was adjusted as per the solvent been used in the extraction. The extract was distilled, evaporated and dried in vacuum. The resulted extract yield was 3.8g and the chemical constituents of the extract of *Rumex vesicarius*. Linn contain Carbohydrates, Fats and oils, Steroids, Glycosides, Anthraquinones, Flavonoids and Saponins.[2] Wistar Albino rats (110-240g, fasted) of either sex were used and grouped and housed in polyacrylic cages (six animals per cage) and maintained under standard laboratory conditions (temp 24-28ºc, relative humidity 60-70% and 12hr dark light cycle). They were fed commercial rat feed and drinking water were provided ad libitum throughout the experimental period. The animals were acclimated to laboratory conditions one week prior

to the initiation of experimental work. All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institutional Animal Ethics Committee Reg.No. (1217/PO/RE/S/CPCSEA).

Preliminary Phytochemical Screening[3,4]

The extracts obtained from crude drugs i.e. (Hexane, Benzene, Chloroform, Ethyl acetate, Ethanol and Water). These extracts were subjected to qualitative chemical test for the identification of various chemical constituents.

After six successive extractions the extracts were subjected to a vacuum rotary evaporator and concentrated extracts were obtained along with solvent recovery.

TESTS FOR CARBOHYDRATES

- Molish's test (General test): To 2-3ml aqueous extract, add few drops of alpha naphthol solution in alcohol shake and add conc sulphuric acid from sides of the test tube. Violet ring is formed at the junction of two liquids.

- Fehling's test: The extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. Heat in boiling water bath for 5-10min. The solution was observed for a color reaction.i.e. Yellow, brick and red precipitate.

- Benedicts test: Mix the equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath for 5min. Solution appears green, yellow, or red depending on amount of reducing sugar present in test solution.

TEST FOR GYCOSIDES

Tests for cardiac glycosides

- **Baljet's test:** A yellow to orange color is observed with sodium picrate.
- **Legal's test:** To aqueous solution add 1ml pyridine and 1ml sodium nitroprusside.
- **Keller kilani test:** to 2ml of extract, add glacial acetic acid, one drop 5% $FECL_3$ and conc. sulphuric acid.

TEST FOR ANTHRAQUINONE GLYCOSIDES

- Borntrager's test: 0.5 g of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

TEST FOR FLAVONOIDS

Three methods were used to test for Flavonoids.

- First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow coloration that is appearing on standing indicates the presence of Flavonoids.
- Second, a few drops of 1% aluminum solution were added to a portion of the filtrate.
- Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution.

TEST FOR SAPONINS

- Foam test: To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.
- Haemolytic test: Add drug extract or dry powder to one drop of blood placed on glass slide. Haemolytic zone appears.

TEST FOR TANNINS AND PHENOLIC COMPOUNDS

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered; add few drops to the following reagents

- 5%$FECL_3$ solution: A few drops of 0.1% ferric chloride was added and observed for deep blue- black color.
- Lead acetate solution: A few drops of lead acetate was added and observed for white precipitate.
- Acetic acid solution: A few drops of acetic acid was added and observed for red color solution.

TEST FOR ALKALOIDS

To residue add dilute hydrochloric acid. Shake well and filter. With filtrate perform following tests.

- **Dragendroffs test:** to 2-3ml filtrate add few drops Dragendroffs reagent. Orange brown precipitate is formed.
- **Mayer's test:** 2-3ml of filtrate add few drops of mayers reagent and observe the precipitate.
- **Hager's test:** 2-3ml filtrate with Hager's reagent gives yellow precipitate.
- **Wagner's test:** 2-3ml filtrate with few drops Wagner's reagent gives reddish brown precipitate.

TESTS FOR PROTEINS

- **Biuret test:** To 3ml of test solution add4%sodium hydroxide and few drops of 1% copper sulphate solution. Violet or pink color appears.

TESTS FOR STEROIDS

- **Salkowski Reaction:** To 2ml of extract, add 2ml of chloroform and 2ml of conc. sulphuric acid. Shake well. Chloroform layer appears red and acid layer appears greenish yellow in color.

Lieberman burchard reaction: Mix 2ml extract with chloroform. Add 1-2ml of acetic anhydride and 2 drops

concentrated sulphuric acid from the side of test tube. First red, then blue and finally green color appears.

Acute toxicity test: The acute toxicity studies of EERV were found to be non lethal up to dose of 2000mg/kg body weight of animals. The acute toxicity studies were performed according OECD guideline 423. The selected doses were 500mg/kg & 750 mg/kg relative to in vitro anti-inflammatory activity by HRBC method.

METHODS

ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced paw edema method in Albino rats: Albino rats of either sex weighing 150-300 gm were randomly distributed into four groups of five animals each. The first group as Control, second group served as Standard while third and fourth group as test. They were marked and numbered for identification. Animals were deprived of food for 18 hours before the experiment. On the day of experiment they were assigned into 4 groups. The first group control has received 0.05ml sterile saline containing 1% Carrageenan solution. The paw edema was induced in the sub-plantar region of the rat. The standard group has received Diclofenac – orally and test group has received the ethanolic extract of *Rumex vesicarius Linn* by oral administration.

The standard and test group were concomitantly administered with saline containing Carrageenan injection in left paw. Control groups of animals received the same volume of vehicle instead of tested agents in right paw.[5] The volume of paw was measured by Plethysmometer immediately after injection. Before measuring the paw volume Plethysmometer has to be adjusted. The mercury level in the two arms of Plethysmometer should be at same level .Increase in the volume can be determined by rise in the water level in the opposite arm of Plethysmometer.[6] Readings of same paw were carried out at 1hour interval up to 3hour and compared with the initial readings. Increase in paw volume was considered as edema volume and measured in mm. The readings obtained were subjected to statistical one way ANOVA (Analysis Of Variance).

ANALGESIC ACTIVITY

Tail immersion test

The rodent tail withdrawal reflex can be elicited by immersion of the tail in hot water at 5ec. This test is specific for opioids like central analgesics and is used to differentiate them from peripheral analgesics.[7] Young female Wistar rats weighing between 150 to 200 gm are used. They are placed into individual cylindrical rat holders leaving the tail hanging out freely. The animals are allowed to get acclimatized to rat holders for 30min before testing. The lower 5cm portion of the tail is immersed in a cup of freshly filled water at exactly 55'c temperature. The reaction time is recorded using a stop-watch of 0.5 sec accurately after each determination the tail is carefully dried. The reaction time is determined before and periodically 0.5, 1, 2, 3, 4 and 6h after the oral or s.c administration of the test substance. The cut off time of immersion is 15sec and withdrawal time of control rats usually lies between 1 and 5.5 s. a withdrawal time more than 6s is regarded as a positive analgesic response. ED50 values can be calculated for each compound and response curves (onset peak, and duration of the action) can be plotted.

RESULTS

Preliminary Phytochemical screening of the various aerial parts extracts of *Rumex vesicarius.*Linn

TEST	HEXANE	BENZENE	CHLOROFOM	ETHYL ACETAE	ETHANOL	WATER
Carbohydrates		-	+	-	-	+
Proteins	-	-	-	-	-	-
Aminoacids	-	-	-	-	-	-
Fats and Oils	+	+	+	-	-	-
Steroids	+	+	-	-	-	-
Glycosides	+	+	+	+	+	-
Anthraquinone	+	+	+	+	+	+
Flavonoids	-	-	-	-	+	-
Alkaloids	-	-	-	-	-	-
Saponins	-	-	-	-	+	+
Tannins	-	-	-	-	-	-

The individual extracts after extraction was then subjected to qualitative chemical test to find out the presence of various phytoconstituents

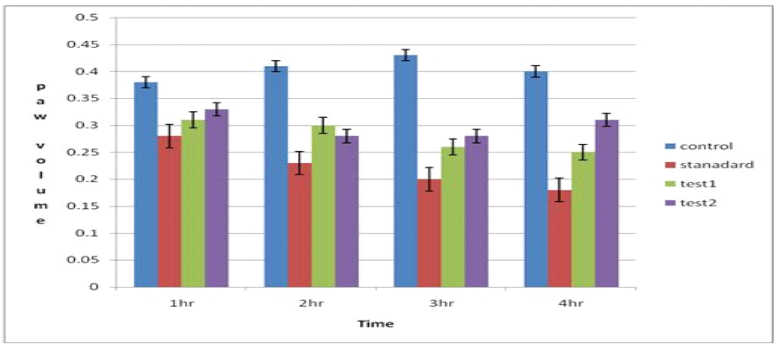
The results from the above table showed that the aerial parts extracts of *Rumex vesicarius*. Linn contains Carbohydrates, Fats and oils, Steriods, Glycosides, Anthraquinones, Flavonoids and Saponins

Anti-inflammatory activity of EERV using Carraegenean induced paw edema

S.No	Treatment	Dose (mg/kg/ml)	Mean difference in Paw volume (ml.) ± S.E.M				% inhibition
			1h	2h	3h	4h	
1	Control	0.05ml	0.38± 0.017	0.41± 0.17	0.43± 0.021	0.40± 0.026	--
2	Standard	6mg/3ml	0.28± 0.17**	0.23± 0.021*	0.20± 0.026 ***	0.18± 0.017***	55%
3	Test(low)	500mg/ kg	0.31± 0.017*	0.30± 0.026**	026± 0.021 ***	0.25± 0.022**	37.5%
4	Test(high)	750mg/ kg	0.33± 0.021	0.28± 0.17***	0.28± 0.017***	0.31± 0.017*	22.5%

- ***=p<0.0001, **=p < 0.001, *=p<0.05, done by stastical analysis of ANOVA followed by student t-test,
- EERV= Ethanolic Extract of *Rumex vesicarius Linn*

Effect of EERV at various time intervals using carreagenan induced paw edema method

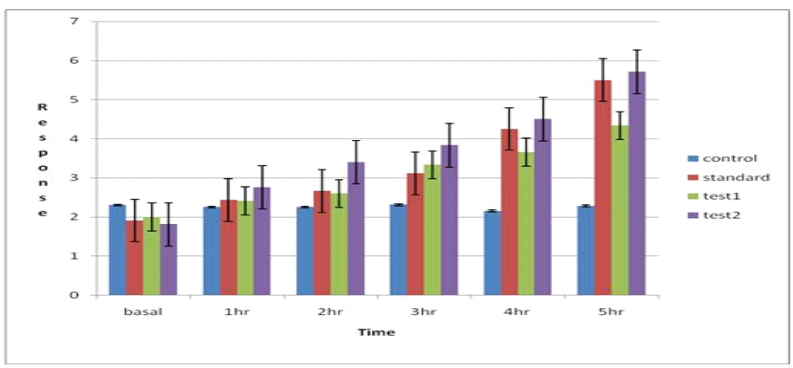


EERV= Ethanolic Extract of *Rumex vesicarius Linn*

Analgesic activity of EERV

S.No	Treatment	Dose (mg/kg/ ml)	Basal Reaction Time	Mean difference in Paw volume (min.) ± S.E.M				
				1h	2h	3h	4h	5h
1	Control	0.05ml	2.3± 2.44	2.25± 2.46	2.25± 2.46***	2.31± 2.53	2.15± 2.35	2.28± 2.44 ***
2	Standard	6mg/3ml	1.91± 2.09	2.43± 2.66	2.66± 2.92***	3.11± 3.39	4.25± 4.65	5.5± 6.04 ***
3	Test (low)	500 mg/kg	2±2.1	2.41± 2.6	2.6± 2.92***	3.33± 3.6	3.66±4.01	4.3± 4.69 ***
4	Test (high)	750 mg/kg	1.81± 1.98	2.76± 3.03	3.4± 3.79***	3.83± 4.25	4.5± 4.92	5.71± 6.26***

EFFECT OF EERV AT VARIOUS TIME INTERVALS BY TAIL IMMERSION



DISCUSSION

This study is the first report related to anti-inflammatory and analgesic activity of *Rumex vesicarius Linn* leave extracts. The analgesic activity was evaluated through tail immersion assays in mice, whereas anti-inflammatory activity was performed through Carrageenan induced paw edema in rats.

Carrageenan induced edema has been commonly used as an experimental model for acute inflammation and is believed to be biphasic. The early phase (1-2hour) of Carrageenan model is mainly mediated by Histamine, serotonin and increased synthesis of Prostaglandins in the damaged tissue surroundings. The late phase is sustained by Prostaglandin release and mediated by bradykinin, leukotrienes, polymorph nuclear cells and prostaglandin by tissue macrophages.[7]

The significant inhibitory activity shown by the extract of EERV 500mg/kg over a period of 3hours in Carrageenan induced inflammation was quite similar to that exhibited by the groups treated with Diclofenac sodium. The highest percentage inhibitory activity was found in the dose 500mg/kg.

The brain and spinal cord play an important role in central pain mechanism. The dorsal part of the spinal cord is rich with substance P, endogenous opioids, somatostatine, and other inhibitory hormones which are the targets of pain and inflammation.[8] It is also established that tail clip, tail flick, and tail immersion models are the well-established methods for measuring the central analgesic effects of drugs through opioid receptor .Our present study demonstrated that both chloroform and methanol extracts were effective against all these models at 200 mg/kg doses which were comparable with standard drug dextropropoxyphene. Narcotic analgesics are active against both peripheral and central pain, while nonsteroidal anti-inflammatory drugs inhibit peripheral pain.[9, 10]

These results indicate that the extract was in later phase in dose dependent manner probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophil mobilization. This anti-inflammatory effect of EERV extract may be due to presence of diterpenoid in the plant Statistical Analysis:

All values obtained were expressed as Mean + SEM. Statistical significances between control and treated groups were calculated by one way Analysis of Variance (ANOVA) test and the P<(0.01) was considered to be highly significant. Standard deviation of control vs. test of low dose (50mg/kg) was found to be 0.16642. Standard deviation of control vs test high dose (200mg/kg) was found to be 0.15119.

It can be concluded from present study that (500mg/kg), (750mg/kg) leaf extract showed Anti inflammatory and Analgesic activity. Further research, to isolate anti-inflammatory principle & exact mechanism involved, is needed can be used for the development of herbal drug for anti-inflammatory conditions and warrants further studies to decipher its exact mechanism of action.

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REFERENCES

1. Fayez A, Rafeeq A.K and Shahid R, preliminary screening of Methanolic extracts of Celastrus paniculatus and Tecomella undulate for analgesic and anti-inflammatory activities. J. Ethanopharmacol, 1994; 42: 193-198.
2. Frank tutin and William Bentley clewer the constituents of rumex ecklonianus", j. chem. soc., tra
3. MOHMMED a. Alfawaz, 2006" chemical composition of hummayd (Rumex vesicarius) grown in Saudi Arabia".
4. Irfan Corch, Arzu Gormez, Sezai Ercisli, Memnune Sengul. 2008" Total phenolic content, antioxidant and antibacterial activity of Rumex cripsus l.,
5. Antarkar SS, Chinwalla T, Bhatt N, Antiinflammatory activity of Rubia cordifolia Linn. In Rats, Indian Journal of Pharmacology, 1994; 15(3): 185-188.
6. Winter CA, Risley EA, and Nuss GW. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs, Proc soc.Exp.Bio.Med.1962:11; 544- 547.ns., 1910; 97: 1-11.
7. Collier HO, Kinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br. J. Pharmacol. 1968; 32: 295–310.
8. Crunkhon P and Meacock SER, mediators of the inflammation induced in the rat paw by carrageenan. Brit. J. pharmacol. 1971; 42: 392- 402.
9. R. McCurdy and S. S. Scully, "Analgesic substances derived from natural products (natureceuticals),"Life Sciences, 2005; 78(5): 476–484.
10. Elisabetsky, T. A. Arnador, R. R. Albuquerque, D. S. Nunes, and A. Do CT Carvalho, "Analgesic activity of Psychotria colorata (Willd. ex R. and S.) Muell. Arg. alkaloids," Journal of Ethnopharmacology, 1995; 48(2): 77–83.
11. Janssen, C. J. E. Niemegeers and J. G. H. Dony, "The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats," Arzneimittelforschung, 1963; 13: 502–507.