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ASSESSMENT OF ANTI-INFLAMMATORY ACTIVITY OF *ZIZIPHUS JUJUBE* LEAVES AND BARK EXTRACTS ON FORMALIN - INDUCED RAT PAW EDEMA ANIMAL MODEL

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ABSTRACT

Objective: The aim of present study was evaluation of anti-inflammatory activity of the various extracts of *Ziziphus jujube* leaves and barks in animal models by mercury displacement volume method. **Materials and methods:** Leaves and barks of *Ziziphus jujube* were powdered, sieved and extracted separately with petroleum ether, chloroform, 90% ethanol and distilled water. Paw edema in animals was produced individually by injecting a 0.1ml volume of formalin into the sub - plantar tissue of the rat hind paw foot. Test drugs petroleum ether extract, chloroform extract, alcoholic extract and aqueous extracts were given orally to all test groups individually at a dose of 200mg/kg according to the body weight. Analgin was used as a standard reference drug. % inhibition produced by *Ziziphus jujube* leaves and bark extract was comparable with Analgin. **Result:** The experiment result showed statistically significant anti-inflammatory activity in albino rats against formalin-induced rat paw edema. Study indicates that *Ziziphus jujube* leaves and bark extract possess significant anti-inflammatory.

KEYWORDS

Ziziphus jujube, Anti-inflammatory activity, Extracts and Mercury displacement volume.

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INTRODUCTON

Today, scientists have made tremendous progress, in the field of medical research, but still the subject of core research to development of new herbal medicines by using current technology. Nociception and chronic diseases remain one of the world's major health problems of the adult age above 45 years[1-3]. Inflammation is a process by which human body's white blood cells and the other things they make sure to protect you from infection from outside, invaders like bacteria and viruses. In

some cases, like arthritis, autoimmune diseases trigger inflammation when there are no invaders to fight off by the immune system. Inflammation is mainly concerned about the response of living tissues to injury, which involves a complex array of enzyme activation, mediator release and extravasations of fluid, cell migration, tissue breakdown and repair[4,5]. Anti-inflammatory activity is important for wound healing procedure. In this phenomenon, immune responsive compounds like cytokinins and interleukins are produced by keratinocytes, B lymphocytes, T lumphocytes and macrophages[6-11]. Jujube is the common name of *Ziziphus jujube* and it is also called red date, Chinese date and Ber in Chhattisgarh[12-14]. Phytoconstituents of this plant contain ziziphussaponin I, II and III, betulinic acid, aiphitolic acid, betulonic acid, oleanolic acid, ursolic acid, jujubosides A, B, B1, XI jujubogenin, spinosins, sanjoinines, swertish etc[15-21]. This plant also contains cyclic peptide alkaloids, which are sativanine C, G, E, H, F, D and K, sanjooinenine, franguloine and amphibine, jubanine- C, scutianine-C and zizyphine -A were isolated[22-24]. Phytoconstituents are present including saponins, flavonoids, sugars, mucilage, vitamin A, vitamin B2, Vitamin C, minerals such as calcium, phosphorus and iron are found in fruits[25].

Ziziphus jujube is used to cure chronic hepatitis, chest and rib pain, reduce blood pressure, laxative agent, antitussive, cure wounds, aphrodisiac, digestible, used in vomiting, thirst, treat tuberculosis, cure blood sickness, leucorrhea and eye ailments. Pharmacologically it is used as an antidiabetic, sedative, antioxidant, anxiolytic, sweetness inhibiting properties, anticancer, anti-obese effect, hypolipidemic, wound healing activity, CNS depressant, myocardium protecting activities, antimicrobial, immunostimulant, antinephritic, antiulcer, antifertility, anxiolytic, anti-inflammatory, permeability enhancement activity, anti-proliferative and apoptotic effect etc[27-34].

The plant parts were collected from the Jashpur District of Chhattisgarh, India. A plant specimen was identified and authenticated by a Botanist from Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India. The voucher number is SLT / Med. Plant/03/2009 was deposited in the Department of Pharmacy (Pharmacognosy), Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India.

Preparation of extracts

Plant parts were cleaned by removing all unwanted particles, dried under shade, and powdered by the electric grinder. 100 gm of powdered plant parts were extracted by solvents of increasing polarity with petroleum ether, chloroform, 90% alcohol, and distal water by using Soxhlet apparatus successively. All extracts were filtered separately, concentrated by vacuum pressure and dried. All physical characteristics and yields of all extracts were noted.

Phytochemical screening

All extracts were dissolved in suitable solvents and various chemical tests were carried out for phytoconstituents present in the plant part. The phytoconstituents present in all leaf and bark extracts were confirmed by qualitative analysis.

Animal procurement of animals

Animals were obtained from the department of Pharmacy. Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh. Albino rats of both sexes weighing between 200 and 250 gm were taken for the experiment. They were housed in an animal house under standard environmental conditions. Standard pellets and water were given to animals for free access. All procedures and protocols were approved by the Institute of Animal Ethics Committee (IAEC) and the registration number is 994/a/06/CPCSEA. All animal experiments were carried out strictly according to the guidelines of CPCSEA.

Experimental design

The rat hind paw oedema method is the simplest and most widely used model for studying the anti-inflammatory activity of new phytoconstituents or extracts. The paw oedema induced by histamine 5HT, bradykinin, dextran, hyaluronidase, formalin, and prostaglandin E1 has been used for studying the antagonism to these mediators. These agents can be injected in a 0.1 ml volume of suitable concentration in sterile saline into the subplantar

tissue of the rat hind paw foot, and the paw volume can be measured immediately by the plethysmometric method[35-36].

Determination of anti-inflammatory activity

In the present study, the formalin induced rat hind paw oedema method was used to determine anti-inflammatory activity. Analgin was used as a standard drug.

Albino Wister rats (200-250 g) were used for the study. The animals were divided into seven groups, with six rats in each group. Inflammation was produced in animals by injection of 0.1 ml of 1% w/v formalin into the subplantar region of the left hind paw. This experimental method was applied to all extracts of *Ziziphus jujube* leaf and bark in their respective anti-inflammatory activity.

Group 1 (normal control): Received sterile saline, to serve as control.

Group 2 (formalin control): Received formalin (0.1ml).

Group 3 (standard): Received formalin (0.1 ml) and Analgin (30 mg/kg) as standard.

Group 4 (test group): Received formalin (0.1 ml) and petroleum ether extract at a dose of 200 mg/kg.

Group 5 (test group): Received formalin (0.1 ml) and chloroform extract at a dose of 200 mg/kg.

Group 6 (test group): Received formalin (0.1 ml) and alcoholic extract at a dose of 200 mg/kg.

Group 7 (test group): Received formalin (0.1 ml) aqueous extract at a dose of 200 mg/kg.

The paw volume was measured after formalin injection. The average paw swelling in the group of extract treated rat was compared with the control group and the percentage change in oedema was calculated.

Statistical analysis

Values are expressed as the mean ± SEM of six observations. Data was analyzed by one way analysis of variance (ANOVA) followed by Dunnett's comparison test, using the computerized software GraphPad in stats. $P \leq 0.05$ and $P \leq 0.001$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening

Qualitative analysis of various extracts revealed that alkaloids, glycosides, saponins, flavonoids and phenolic compounds are present in plant parts.

Physical characteristic and yield

Physical characteristics and yield of all extracts are given in Table No. 1.

Anti-inflammatory activity

Injection of formalin inflammagens into the subplantar surface of rat hind paw produced a marked increase in paw volume as compared to the control group. The results of the anti-inflammatory effect of *Ziziphus jujube* are shown in Table No.1. Significant activity was shown by *Ziziphus jujube* leaf and bark extracts as compared to the control group. But 200 mg/kg leaf alcoholic extract showed maximum anti-inflammatory activity as compared to control. Results are represented in Table No.2 and Table No.3, Figure No.1 and Figure No.2.

Discussion

Inflammation is a natural, complex, protective biological response in living organisms. In this process, pathogens, damaged cells, and irritants initiate the healing process for the inflammatory sites. When the tissues are damaged, then tissue mediated responses and neurogenic responses occur that release histamine, 5HT, prostaglandins, cyclooxygenase, bradykinin and serotonin[39]. Some other chemicals, like interleukin, interferon, tumor necrosis factors and granulocytes macrophage colony stimulating factors, are also released. The liberation of these chemicals is responsible for the pain and inflammation of tissue[40]. Formalin induces paw edema, which closely resembles human arthritis. The Inflammatory effect of formalin is given by tissue mediated and neurogenic responses. The plant species used in the study was selected based on ethnomedicinal use in the treatment of inflammation in Chhattisgarh. In the present study, all the extracts exhibited significantly reduced paw edema as compared to the standard drug. Plant extracts reduce inflammation by inhibiting the release of chemical mediators at inflamed sites. Recent research has revealed thatbnumerous flavonoids contributed substantially to several

plants anti-inflammatory properties[41]. Thus, the present experiment clarifies that the bioactive substance flavonoids may influence the anti-inflammatory activity. However, animal studies and other studies are required to identify and isolate the active constituents that are responsible for the anti-inflammatory activity.

Table No.1: Physical characteristics and yield of various extracts

S.No	Extracts	Nature	Colour	Yield (gm)	%Yield (w/w)
1	Pet. ether ext. of *Ziziphus jujube* leave powder	Semi Solid	Blackish green	1.52	1.52
2	Chloroform extract of *Ziziphus jujube* leave powder	Semi Solid	Black	0.42	0.42
3	Ethanolic extract of *Ziziphus jujube* leave Powder	Semi Solid	Reddish black	5.62	5.62
4	Aqueous extract of *Ziziphus jujube* leave powder	Solid	Chocolate red	5..68	5.68
5	Pet. ether ext. of *Ziziphus jujube* bark powder	Solid	Green	1.18	1.18
6	Chloroform extract of *Ziziphus jujube* bark powder	Solid	Black	0.62	0.62
7	Ethanol extract of *Ziziphus jujube* bark powder	Solid	Black red	5.62	5.62
8	Aqueous extract. of *Ziziphus jujube* bark powder	Solid	Blackish red with shining	6.12	6.12

Table No.2: Anti-inflammatory activity of *Ziziphus jujube* leaves and bark extracts

S.No	Group	Dose	Paw edema volume (ml)			
			***Ziziphus jujube* leaf extract**		***Ziziphus jujube* stem bark extract**	
			30 min	**60 min**	**3 0 min**	**60 min**
1	Control (distal water)		0.50±0.03	0.51±0.002	0.50±0.003	0.50±0.002
2	Formalin (1%) control	0.1ml	1.45±0.02	1.45±0.012	1.65±0.05	1.65±0.006
3	Formalin and standard (analgin)	30mg	1.06±0.043**	0.92±0.004**	1.09±0.002**	1.12±0.03**
4	Formalin and petroleum ether extract	200mg	1.35±0.006**	1.30±0.002**	1.60±0.062**	1.55± 0.06**
5	Formalin and chloroform extract	200mg	1.40±0.032**	1.24±0.032**	1.35±0.02**	1.±0.002**
6	Formalin and alcoholic Extract	200mg	1.09±0.005**	0.93±0.0036**	1.52±0.01**	1.50± 0.004**
7	Formalin and aqueous Extract	200mg	1.32±0.001**	1.15±0.006**	1.45±.03**	1.35± 0.002**

Mean ±SEM, (n=6) followed by Dunnett's test, Significant at **P‹0.01,*P‹0.05, ns P›0.05. Ns - Not significant.

Table No.3: % inhibition of Anti-inflammatory activity of *Ziziphus jujube* leaves and bark extracts

S.No	Group	Dose	% inhibition of paw edema			
			Ziziphus jujube leaf extract		*us jujube* stem barkextract	
			30 min	60 min	3 0 min	60 min
1	Control (distal water)		-	-	-	-
2	Formalin (1%) control	0.1ml	-	-	-	-
3	Formalin and standard (analgin)	30mg	25%	35.5%	33%	32%
4	Formalin and petroleum ether extract	200mg	6.3%	13.3%	3%	6%
5	Formalin and chloroform extract	200mg	3.4%	14.4%	13%	15%**
6	Formalin and alcoholic extract	200mg	24%	35.8%	7%	9.3%
7	Formalin and aqueous extract	200mg	8.3%	23%	12%	13.1%

Note: % Inhibition = D0-Dt/D0×100 where D0 was the average inflammation of the group of rats after 30 min treatment of formalin; and Dt was the average inflammation of the drug treated rats after 30 and 60 min.

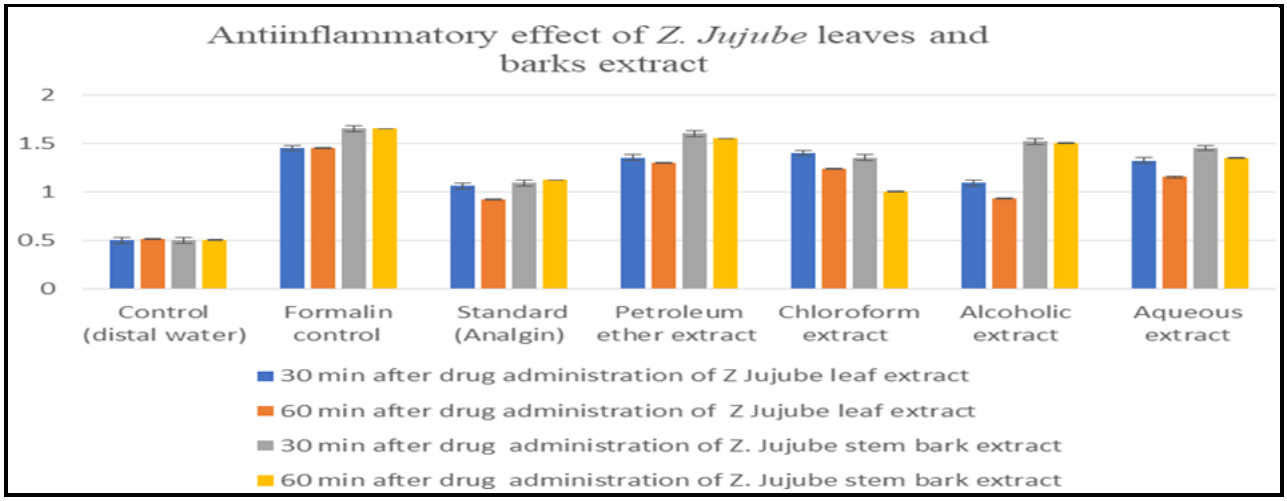

Figure No.1: Anti-inflammatory effect of *Ziziphus jujube* leaves and bark extracts on formalin induced rat paw oedema

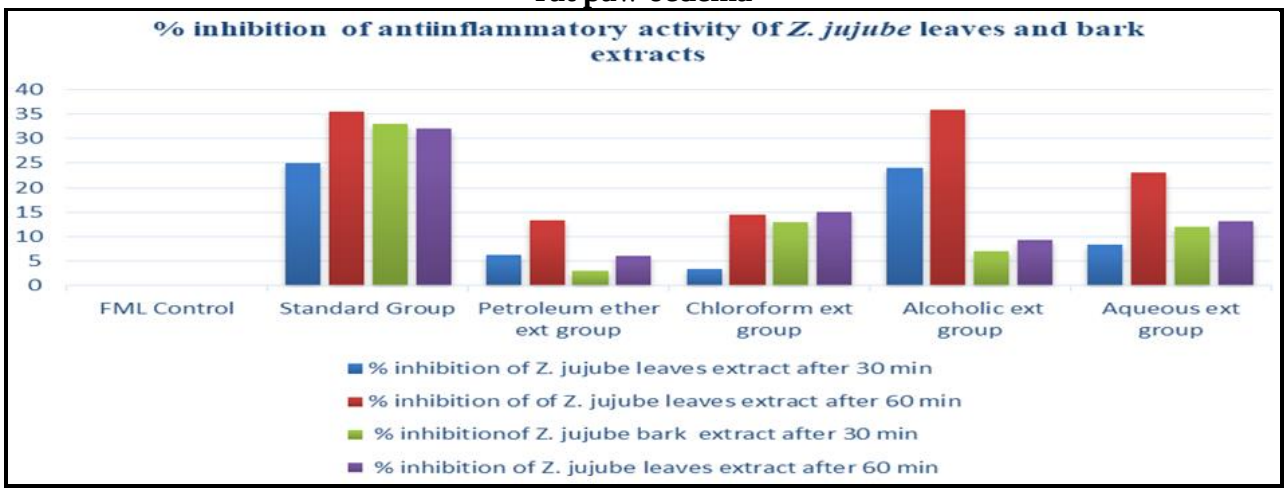

Figure No.2: Percentage inhibition of paw edema volume with rats after drug administration

CONCLUSION

In conclusion, the results of the present investigation that *Ziziphus jujube* leaf and bark extracts exhibit anti-inflammatory activities. This activity is due to the presence of flavonoids and phenolic substances present in the plant. Flavonoids are known to target prostaglandins, which are involved in the late phase of acute inflammation. Hence, the presence of flavonoids may be to anti-inflammatory activity. Further studies may reveal the exact bioactive substances by isolation and structural elucidation and well as the mechanism of action responsible for the anti-inflammatory action. This study may be helpful for the development and formulation of new herbal drugs for the management of inflammation. The present study may also support the fact that this medicinal plant is traditionally used in treating diseases associated with inflammatory pain.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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