Asian Journal of Pharmaceutical Education and Research
Vol -8, Issue-3, July-September 2019
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

PHYTOCHEMICAL SCREENING INVESTIGATION OF ANTI-INFLAMMATORY EFFECT OF HYDROALCOHOLIC EXTRACTS OF *ZIZIPHUS XYLOPYRUS* (RETZ.) WILLD

Divya Prajapati*[1], C.K. Tyagi[1], Hemant Kumar Sharma[1], Prabhakar Budholiya[1], Geeta Parkhe[2]

[1]College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.)

[2]Scan Research Laboratory, Indrapuri, Bhopal (M.P.)

*Corresponding Author's E mail: Divyansha95@gmail.com

Received 24 May 2019; Revised 10 June 2019; Accepted 18 June 2019; Available online 10 July 2019.

ABSTRACT

Ziziphusxylopyrus (Retz.) willd known as katber is used traditionally in the treatment of various diseases like Bronchial Asthma, Thirst, Diarrhoea and as Aphrodisiac, Antimicrobial, Anti-inflammatory, Antinoceceptive and as Anticonvulsants. The present work showed phytochemical screening, *invivo* anti-inflammatory activities and sub-acute toxicity of hydroalcoholicextract of twigs of *Ziziphusxylopyrus* (retz.) Willd (*Z. xylopyrus*). The anti-inflammatory activity was evaluated by Carrageenan-Induced Rat Paw Edema method. Acute toxicity of the extract (2000 mg/kg) was examined in Swiss albino mice for 14 days. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by FolinsCiocalteau reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids. The total phenolics content of *Z. xylopyrus* extract was (2.56 mg/100mg), followed by flavonoids (1.25mg/100mg) respectively. Hydroalcoholic extract up to 2000 mg/kg did not produce any toxic effects. The hydroalcoholic extract of *Z. xylopyrus* (200 and 400 mg/kg) inhibited the inflammation induced by carrageenan in rats in a dose dependent manner. The hydroalcoholic extract of *Z. xylopyrus* possesses a strong anti-inflammatory activity and may be considered an interesting source of effective anti-inflammatory compounds.

Keywords: *Ziziphus xylopyrus* (Retz.) willd, Sub-acute toxicity, anti-inflammatory effect, phytochemical screening.

INTRODUCTION:

Inflammation refers to body's normal protective response to tissue injury caused by physical trauma, toxic chemicals or microbiological agents [1]. The classical signs of inflammation are skin redness, swelling, pain, heat, and loss of function [2]. The process of inflammation involves changes in blood flow, destruction of tissues, increased vascular permeability and the synthesis of pro-inflammatory mediators[3]. The injured cells, lymphocytes, phagocytes, mast cells and blood proteins are the sources of inflammatory mediators. The most important inflammatory mediators include bradykinins, serotonins,

histamine, tumor necrosis factor-α, interleukin-6, interleukin-1β, leukotrienes, phospholipase A2, nitric oxide (NO), lipoxygenases and cyclooxygenase 2 (COX-2) [3,4]. Inflammatory process has two phases: acute and chronic. The acute inflammation occurs a few minutes after tissue damage. It is characterized by an increase in permeability of blood vessels, extravasation of fluid and proteins and accumulation of white blood cells for a short period [5]. The primary mediators of acute inflammation include histamine, serotonin, and C0X-2 [6]. The failure of the management of acute inflammation and an autoimmune response to a self-antigen lead to chronic inflammation and disease [7]. Chronic inflammation is mediated by inflammatory mediators such as PGE2, nitric oxide and lipoxygenases. Chronic inflammation may results in ailments such as chronic peptic ulcers, rheumatoid arthritis, systemic lupus, asthma, chronic periodontitis and cancer [8]. During the inflammatory response, the PGE2 are at low levels in tissues with no inflammation and increase immediately in acute inflammation. As immune cells infiltrate the tissues, further increases in PGE2 levels is observed [9]. The non-steroidal anti-inflammatory drugs (NSAIDs) such as naproxen, indomethacin, ibuprofen, diclofenac, and ketoprofen are the most commonly used conventional medicinal products in the treatment of inflammation [10]. The NSAIDs inhibit the expression of cyclooxygenase 2 (COX-2) enzyme responsible for the production of PGE2 which induces pyrexia [11]. However, the prolonged use of NSAIDs is linked with severe effects on the gastrointestinal tract, kidney, and cardiovascular system [12]. The demand for herbal medicine is increasing due to the growing recognition of natural products having fewer side effects, easily available, better cultural acceptability and being comparatively affordable [13]. *Ziziphusxylopyrus (Retz.)* willd (Family: Rhamnaceae) *is* distributed in North Western India, Uttar Pradesh, Bihar and Central South India. In Hindi it is known as katber, in Tamil -kottei, and in Telugu-Gotte. This Plant is a large shrub or small tree, having spines and about four meter in hight. Root bark and fruit of this plant, traditionally used to treat Bronchial Asthma, Thirst, Diarrhoea and as Aphrodisiac. Fruit and bark is used as Antimicrobial. Bark is used as an Anti-inflammatory, Antinoceceptive and as Anticonvulsants[14]. Therefore, the present study was designed to investigate anti-inflammatory activities of hydroalcoholicextract of twigs of *Z. xylopyrus*by using Carrageenan-Induced Rat Paw Edema model.

Materials and Methods

Plant material

Fresh leaves of *Ziziphusxylopyrus*were collected from area adjoining forests of Bhopal in the month of March.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction of plant material

The correctly identified leaves of *Z.xylopyrus*are dried and coarsely powdered. They should be extracted with Hydro alcoholic solvent in order to their increasing polarity to get correct and dependable retention factor to get significant results.Plant material was subjected to hot continuous extraction with (500 ml) 80% methanol (30-40^{o}C) in a Soxhlet apparatus for 24 hours. The extraction procedure was ensured by pouring a few drops of extract from thimble left no residue on evaporation. After complete extraction the solvent was evaporated and concentrated to dry residue. % yield was calculated for each extract after drying under vacuum.

Qualitative phytochemical analysis of plant extract

The leaves of *Z.xylopyrus* extract obtained were subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate[15, 16]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from twigs of *Z.xylopyrus* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total Phenol Determination

The total phenolic content was determined using the method of Olufunmiso et al[17]. A volume of 2 ml of twigs of *Z.xylopyrus* extracts or standard was mixed with 5 ml of FolinCiocalteau reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total Flavonoids Determination

The total flavonoid content was determined using the method of Olufunmiso et al[17]. 1 ml of 2% AlCl3 methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible

spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Animals

In the present investigation the Swiss albino rats of either sex, weighing between 150-250 g were used. Animals were allowed to acclimatize for two weeks before commencing the study and maintained under standard laboratory conditions (25±2ºC temperature, 45-65% relative humidity and 12 h light and 12 h dark cycle). The animals were fed with standard laboratory animal feed and water *ad libitum* throughout the study. The animal experimental protocol was duly approved by the Institution Animal Ethical Committee, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.)(IAEC No. COPSSUTMS/ANIL/19-06).

Acute oral toxicity

Acute oral toxicity was performed according to Organization for Economic Co-operation and Development (OECD) guideline No. 420**(OECD 420, 2011)**. Swiss albino rats fasted overnight, accessing water *ad libitum* were used in this study. The extract was administered orally at a dose of 2000 mg/kg body weight and the animals were observed for mortality or any abnormal behavior for first 24 h, then for next 14 days. Further behavioral responses, neurological responses as well as autonomic responses were observed[18].

Anti-inflammatory activity

Carrageenan-induced paw edema model

Rats were divided into four groups; each group consisting of six animals. Paw edema was induced by injecting 0.1 ml of 1% *w/v* carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat.

Group	Treatment
Group I (Negative control)	Carrageenan (0.1 ml of 1% *w/v*)
Group II (Standard control)	Carrageenan (0.1 ml of 1% *w/v*) + Diclofenac sodium (10 mg/kg, p.o.) as standard reference
Group III (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (200 mg/kg, p.o.) of *Z. xylopyrus*
Group IV (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (400 mg/kg, p.o.) of *Z. xylopyrus*

The paw thickness was measured before injecting the carrageenan and after 60, 120, 180 min. using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group.

The percentage (%) inhibition of edema is calculated using the formula:

$$\% \text{ Inhibition} = \frac{To - Tt}{To} \times 100$$

Where T_t is the thicknessof paw of rats given test extract at corresponding time and To is the paw thickness of rats of control group at the same time.

Data Analysis

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Dunnet's test. Differences were considered as statistically significant at $P < 0.05$, when compared with control.

Results and Discussions

The crude extracts so obtained after the hot continuous extraction, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using hydro alcohol as solvents are depicted in the Table 1.

Table 1: % Yield of plant material

S. No.	Solvents	*Z. xylopyrus*
1	Hydroalcoholic	11.92

Preliminary phytochemical screening of twigs of *Z. xylopyrus* extracts revealed the presence of various components such as phenolic compounds, carbohydrates, flavonoids, saponins and diterpins among which flavones were the most prominent ones and the results are summarized in table 2.

Table 2: Result of Phytochemical screening of hydroalcoholic extracts

S. No.	Test	Extracts Hydro alcoholic extract
1	Alkaloids	+ ve
2	Carbohydrates	-ve
3	Glycosides Anthraquinones Saponins Flavonoids Cardiac	-ve -ve -ve -ve
4	Proteins and amino acids	+ve
5	Sterols	+ve
6	Tannins	+ve
7	Phenolic compounds	-ve
8	Acidic compounds	-ve
9	Resins	+ve
10	Fates and oils	-ve

+ ve – **Present, - ve – Absent**

The content of total phenolic compounds (TPC) and total tannin content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.0002X+ 0.025, R^2= 0.980,** where × is the absorbance and y is the tannic acid equivalent (GAE). Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: **Y=0.004 X+0.001, R^2=0.996**, where X is the absorbance and Y is the quercetin equivalent (QE). Results was shown in table 3 and fig 1& 2

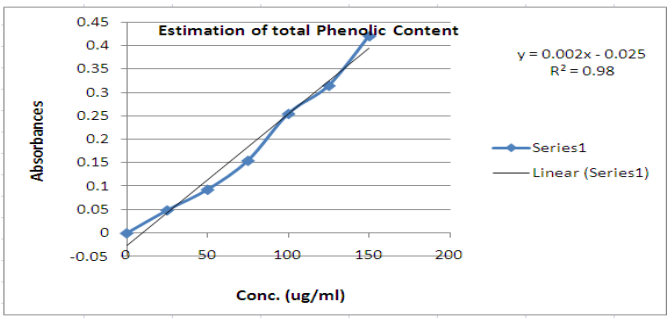

Figure 1: Graph of Estimation of Total Phenolic content

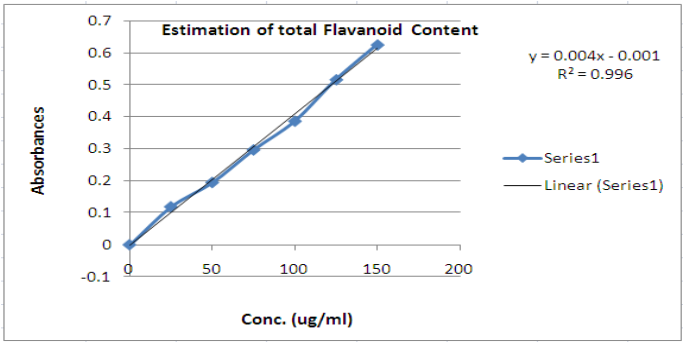

Figure 2: Graph of Estimation of Total flavonoid content

Table 3:Total Phenolic and Total flavonoid content

S. No	Extracts	Total phenolic content	Total flavonoids content
1	Hydroalcoholic	2.56	1.25

Acute oral toxicity

No adverse changes and mortality were observed in animals, which orally received hydroalcoholic extract (2000 mg/kg) of *Z. xylopyrus*. This indicates that 2000 mg/kg is maximum safe dose. So 1/10th and 1/5th*i.e.* 200 and 400 mg/kg of body weight, of the maximum safe dose were selected for studying *in vivo* anti-inflammatoryeffects. Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve [19]. The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to histamine and serotonin component. Table 4 & Fig 3 shows the effect of hydro-alcoholic extract of Z. xylopyrus and diclofenac sodium (standard drug) as compared to carrageenan control at different hours in carrageenan-induced paw edema model using vernier caliper. Hydro-alcoholic extract administered at a dose of 200 mg/kg p.o prevented carrageenan-induced paw edema with a percentage inhibition of 23.64%, 35.73%, 41.24%, and 59.60% at 1, 2, 3,and 4 hour, respectively, while 29.84%, 44.76%, 59.78%, and 74.78% at a dose of 400 mg/kg p.o. at 1, 2, 3.and 4 hour, respectively. Diclofenac sodium at a dose of 10 mg/kg p.o. prevented carrageenan-induced paw edema with a percentage inhibition of 55.40%, 59.81%, 68.32%, and 76.40% at 1, 2, 3.and 4 hour, respectively.

Table 4*In vivo* anti-inflammatory activity by using Carrageenan-induced paw edema model

Group	Dose of extract (mg/kg, p.o.)	Change in paw thickness (mm)±SD (% inhibition)			
		1 hr	2hr	3hr	4hr
Group I (Negative control)	Carrageenan (0.1 ml of 1% *w/v*)	3.28±0.14	4.28±0.19	5.74±0.15	6.23±0.16
Group II (Standard control)	Carrageenan (0.1 ml of 1% *w/v*) + Diclofenac sodium (10 mg/kg, p.o.)	1.46±0.18** (55.40%)	1.72±0.12** (59.81%)	1.82±0.14*** (68.32%)	1.47±0.18*** (76.40%)
Group III (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (200 mg/kg, p.o.)	2.50±0.11* (23.64%)	2.75±0.12* (35.73%)	3.37±0.11** (41.24%)	2.51±0.17*** (59.60%)
Group IV (Treatment Control)	Carrageenan (0.1 ml of 1% *w/v*) + Hydro-alcoholic extract (400 mg/kg, p.o.)	2.30±0.07* (29.84%)	2.36±0.19** (44.76%)	2.31±0.17*** (59.78%)	1.57±0.09*** (74.78%)

Each values represents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with toxicant control group (one-way ANOVA followed by Dunnett's test).Values in parentheses indicate percent inhibition activity (H), calculated as 100 x (value of negative control – value of treatment) / value of negative control

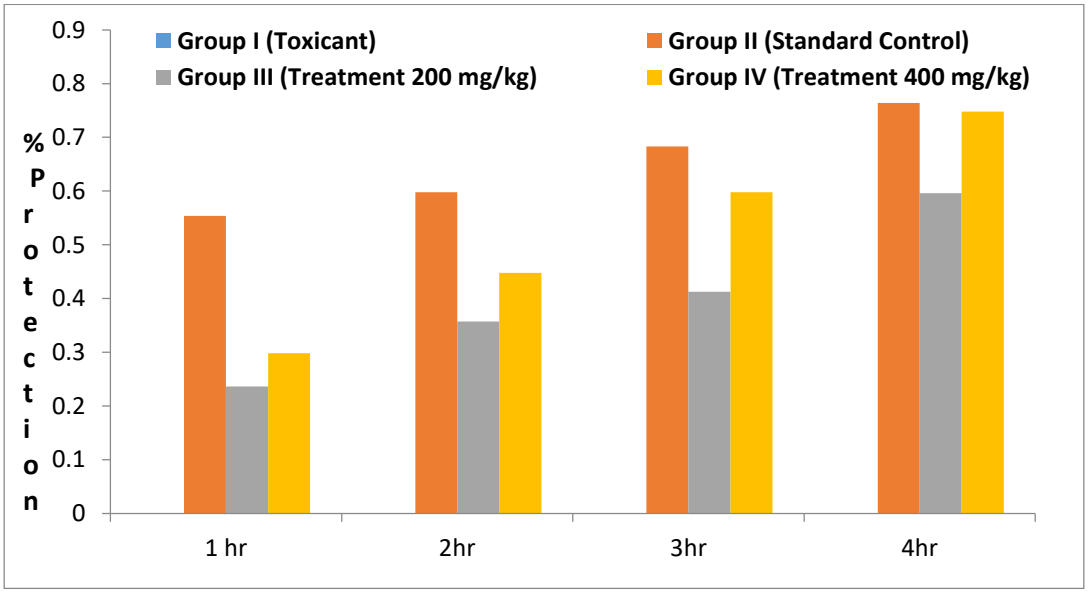

Figure 3 Percentage protection against inflammation induced by Carrageenan-induced paw edema on treatment with hydro-alcoholic extract of *Z. xylopyrus*

Conclusion

The Hydroalcoholic extract of *Z. xylopyrus*showed potent anti- inflammatory activity on carrageenan induced paw edema in rats. The anti-inflammatory activity of twigs extract of *Z. xylopyrus* demonstrated a dose-dependent response and was comparable to diclofenac (reference drug). The extracts were most active at the dose level of 400mg/kg body weight in the fourth hour of treatment. The extracts of *Z. xylopyrus*could, therefore, be an alternative bio-resource for generating anti-inflammatory agents. However, further studies are necessary to elucidate the mechanism behind this effect and their active compounds. The present study, therefore, scientifically confirms and supports the traditional use of *Z. xylopyrus*in the management of inflammation.

References

1. Calixto JB, Campos MM, Otuki MF, Santos ARS. Antiinflammatory compounds from plant origin. Part II. Modulation of proinflammatory cytokines, chemkines and adhesion molecules. Planta Medica 2004; 70: 93-103.
2. Hurley JV. Acute inflammation. Edinburgh, London: Churchill Livingstone.1972.
3. Shah BN, Patel NP, Pandya P. Role of leukotriene in inflammation and antileukotriene therapy. Journal of Pharmacy Research. 2008; 1: 113-123.
4. Williams JG, Maier RV. The inflammatory response. Journal of Intensive Care Medicine. 1992; 7: 53-66.
5. Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, et al. Carrageenan-induced mouse paw edema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. British Journal of Pharmacology. 2004; 142: 331-338.
6. Ravi V, Saleem TM, Patel SS, Raamamurthy J, Gauthaman K. Antiinflammatory effect of methanolic extract of Solanumnigrum Linn berries. International Journal of Applied Research in Natural Products. 2009; 2: 33-36.
7. Recio MC, Andujar I, Rios JL. Anti-inflammatory agents from plants: progress and potential. Curr Med Chem. 2012; 19: 2088-2103.
8. Nordqvist C. Inflammation: Causes, Symptoms and Treatment. Medical News Today. Tilley L, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. Journal of Clinical Investigation.2015; 108: 15-24.
9. Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. Journal of Clinical Investigation. 2001; 108: 15-24.

10. Warden SJ. Prophylactic use of NSAIDs by athletes: a risk/benefit assessment. PhysSportsmed. 2010; 38: 132-138.
11. Vane J, Botting R. Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J. 1987; 1: 89-96.
12. Traversa G, Walker AM, Ippolito FM, Caffari B, Capurso L, et al. Gastroduodenal toxicity of different nonsteroidal anti-inflammatory drugs. Epidemiology. 1995; 6: 49-54.
13. Kamboj VP. Herbal medicine. Current Science Bangalore. 2000; 78: 35-38.
14. Nair NC, Henry AN. Flora of Tamil Nadu, India, Vol.1, Bot. Sur. India, Southern Circle, Coimbatore, (India), 1983:pp184.
15. Khandelwal, KR. Ed. Practical Pharmacognosy Technique and Experiments, 23rdEdn: 2005; 15,29, 149,56.
16. Kokate CK. Ed. Practical Pharmacognosy, 4thEdn., VallabhPrakashan: 1994; 112,120.
17. Olufunmis O, Olajuyigbe, O. and Anthony AJ. Phenolic Content and antioxidant property of the bark extract of *Ziziphusmucronata* wild. Subsp. Mucronata wild, BMC, Complementary and alternative medicine. 2011; 11: 130.
18. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs.
19. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema in rats. J Pharmacol ExpTher. 1969;166:96–103.