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Original Article

ANTI-INFLAMMATORY AND ANTI-PYRETIC ACTIVITY OF *VITIS VINIFERA* LEAVES EXTRACT

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ABSTRACT

Leaves of *Vitis vinifera* were extracted with methanol. The methanolic extract (100, 200 and 400 mg/kg *p.o.* of MEVV) was studied for anti-pyretic and anti-inflammatory activity. The anti-pyretic were evaluated by using Brewer's yeast-induced pyrexia in rats and rectal temperature was recorded by digital thermometer (HICKS, digital thermometer with beeper). The antipyretic effect was started at 1h and extended for at least 4h after the drug administration. The MEVV was also investigated for anti-inflammatory activity by using carrageenin-induced hind paw oedema in rats and the paw volume was measured plethysmometrically at 0, 1 and 3 h after carrageenan injection. MEVV showed significant ($p<0.01$) and dose dependent anti-pyretic and anti-inflammatory activity in comparison to control group. The potential anti-pyretic and anti-inflammatory by MEVV was comparatively less than that of Paracetamol (150 mg/kg *p.o.*) and diclofenac (10 mg/kg *p.o.*) respectively. No acute toxicity was observed in rats after oral administration of the MEVV at the dose of 2000 mg/kg. These results suggest that the MEVV possess anti-pyretic and anti-inflammatory action.

Keywords: ***Vitis Vinifera,*** **Anti-Pyretic, Anti-Inflammatory, Paw Oedema, Pyrexia.**

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0.0 INTRODUCTION

Grape (*Vitis vinifera)* from *Vitaceae* is a perennial woody vine native to Asia Minor and then introduced in Europe and other continents. [1] *V. vinifera* a large deciduous climber, climbing by means of intermittent, leaves opposed, large, often bifid tendrils, cultivated in many part of India. [2] The ripe fruit is cooling, laxative and purgative, fattening, diuretic, aphrodisiac, appetizer, and the throat; cures thirst, asthma, "vata" and "vatarakta", jaundice, strangury, blood disease. The ashes of stem are good for pains in joints, swelling of the testicle, and piles. [3] The flowers are expectorant, emmenagogue and haematinic, and are useful in bronchitis.[4] Its leaves are consumed in some traditional foods (Dolmathes) and used for diarrhoea, vomiting and varicose treatment.[1] The chemical analysis has shown the presence of procyanidins, anthocyanins, Flavanoids, hydroxylcinnamic acid derivatives, triterpenes, sterols, tannins, polysaccharides, monosaccharide's and non-alkaloid nitrogen containing compounds. [5] The stilbene groups, as resveratrol and viniferins, have also been isolated from leaves. [6] 3-oxo-a-ionol, vomifoliol and dehydrovomifoliol were identified for the first time in fruit from *Vitis vinifera.* [7] Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. It is the body's natural defence to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines like interleukin 1β, α, β and TNF-α), which increase the synthesis of prostaglandin E2 (PGE2) near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature [8]. The inflammatory process is the response to an injurious stimulus evoked by a wide variety of noxious agents (*e.g.*, infections, antibodies, or physical injuries). Inflammatory responses occur in three distinct temporal phases, each apparently mediated by different mechanisms: (a) an acute phase characterized by

transient local vasodilation and increased capillary permeability; (b) a delayed, subacute phase characterized by infiltration of leukocytes and phagocytic cells; and (c) a chronic proliferative phase, in which tissue degeneration and fibrosis occur. [9]

1.0 MATERIALS AND METHODS

2.1 Collection and authentication of plant:

The leaves of the plant were collected from the Balaji nursery, Jagatpura, Jaipur District, Rajasthan state, India in month of March 2009. The identity of the collected plant was confirmed by P.J. Parmar, Joint Director in Botanical Survey of India (BSI), Jodhpur (Rajasthan, India). The Herbarium of the plant was deposited in the BSI against voucher specimen no. JNU/JPR/PC/ JS-1.

2.2 Preparation of methanolic extract of *Vitis vinifera* (MEVV):

The leaves of *Vitis vinifera* were washed, shade dried and powdered. The powdered material was defatted with petroleum ether and then extracted with methanol by cold maceration process. The MEVV was concentrated for further studies at reduced pressure and temperature in a rotary evaporator.

2.3 Experimental animals:

Healthy Wistar rats (150-200 g) of either sex were used for anti-pyretic and anti-inflammatory studies. They were housed at the temperature 25 ± 2^{O}C with 12 h light/dark cycles in polypropylene cages in groups of six animals each. The animals were fasted overnight before the experiment and given water *ad libitum*. The studies conformed to the guiding principles of Institutional Animal Ethics Committee (IAEC) and were duly approved by IAEC (002/2009/IAEC/jnu.)

2.4 Drugs and Chemicals used:

Brewer's yeast, Paracetamol (RDPL Jaipur, Raj.), Carrageenan, Commercial Grade, Type I (Sigma Aldrich, Co.), Diclofenac (RDPL Jaipur, Raj.), Sodium chloride (Quqligens Fine Chemicals A division of GlaxoSmithKline pharmaceutical Ltd. Mumbai) were used in this study. All other chemicals used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd.

2.5 Acute toxicity test: [10]

Acute oral toxicity study for the test extract of the plant was carried out using OECD/OCED guideline 425. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Healthy, young adult albino Wistar rats of either sex (200 -250 g) were used for this study. Animals should be fasted prior to dosing (food but not water should be withheld Overnight). The fasted body weight of each animal is determined and the dose is calculated according to the body weight.

Limit Test at 2000 mg/kg: Dose 2000mg/kg body weight was administered orally to one animal. This first test animal survived. Since, four other animals were dosed (orally) sequentially, so that a total of five animals were tested. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days. No animals were died. So the LD50 is greater than 2000 mg/kg.

2.6 Anti-pyretic activity: [11, 12, 13]

2.6.1 Brewer's yeast-induced pyrexia in rats:

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats. Fever was induced by injecting 20 ml/kg (s.c.) of 20 % aqueous suspension of Brewer's yeast in normal saline below the nape of the neck and rectal temperature was recorded by digital thermometer (HICKS, digital thermometer with beeper) immediately before (-18 h) and18 h after Brewer's yeast injection. Rectal temperature was determined by digital thermometer prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. Only rats that showed an increase in temperature of at least 0.7 ^{O}C were used for experiments. Test agent was administered orally and the temperature was measured at 1, 2, 3 and 4 hr after drug administration. Group I received vehicle (10

ml/kg water) i.e. negative control, Group II served as positive control i.e. Paracetamol (150 mg/kg, p. o.) whereas Group III, IV, V received per oral route methanolic extract of 100, 200 and 400 mg/kg b.w.

2.7 Anti-inflammatory activity: [14, 15, 16, 17]

2.7.1 Carrageenin-induced pedal oedema:

Rats were divided into 5 groups consisting of 6 animals each group. Group I received vehicle (10 ml/kg water) i.e. negative control, Group II served as positive control i.e. Diclofenac (10 mg/kg, p. o.) whereas Group III, IV, V received per oral route methanolic extract of 100, 200 and 400 mg/kg respectively and sixty minute later all the animals were challenged by injecting of 0.1 ml of 1% freshly prepared carrageenan suspension into the sub plantar region of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in water up to this mark. The paw volume was measured by plythesmographic method before injection, immediately after injection (at 0 hour) and again at 1 & 3 hours after challenge with carrageenan. The percentage inhibition of paw volume was also calculated for each group recording as follows:

2.0 RESULTS

No acute toxicity was observed in rats after oral administration of the methanolic extract of *Vitis vinifera* at the dose of 2000 mg/kg.

2.1 Brewer's yeast-induced pyrexia in rats:

The results showed that the MEVV at doses of 100 mg/kg caused less significant lowering of the body temperature. While maximum lowering of body temperature was noticed at 400 mg/kg, as the mean temperature of 37.91 was reduced to 36.95 ^{O}C within 4 h period in a dose-dependent manner. (Table 1)

2.2 Carrageenin-induced pedal oedema:

The MEVV as well as paracetamol showed antiphleogestic activity (Table 2). This anti-inflammatory activity was dose-dependent and found to be statistically significant ($P<0.01$) as compared to the control rats.

Table 1:

Effect of MEVV on Brewer's yeast-induced pyrexia in rats:

Drug	Dose (mg/kg)	Rectal temperature in °C at time (h)					
		-18[a]	0[b]	1	2	3	4
Control	……	36.93±0.27	37.88±0.22 (0.95)[C]	37.91±0.03	37.88±0.03	37.84±0.01	37.85±0.02
Extract	100	36.85±0.2	37.92±0.22 (1.07)[C]	37.78±0.02[*]	37.77±0.03[*]	37.73±0.03[NS]	37.71±0.04[NS]
	200	36.6±0.17	37.93±0.17 (1.33)[C]	37.59±0.03[**]	37.55±0.03[**]	37.21±0.04[**]	37.93±0.13[**]
	400	36.93±0.18	37.91±0.22 (0.98)[C]	37.4±0.03[**]	37.2±0.03[**]	37.05±0.03[**]	36.95±0.08[**]
Paracetamol	150	36.97±0.1	37.89±0.99 (0.92)[C]	37.19±0.04[**]	37.02±0.02[**]	36.99±0.04[**]	36.97±0.05[**]

n: six animals in each group; Values are mean + SEM. [NS]P>0.05, *P<0.05, **P<0.01, when compared to control., a: temperature just before yeast injection, b: temperature just before drug administration, c: change in temperature following yeast injection

Table 2: Anti-inflammatory effect of methanolic extract in carrageenan-induced paw oedema in rat

Group(s)	Dose (mg/kg)	Paw volume after 1 h (ml)	Inhibition (%)	Paw volume after 3 h (ml)	Inhibition (%)
Control	10 ml/kg	0.19±0.024	-	0.21±0.025	-
Extract	100	0.15±0.021[NS]	21.05	0.17±0.02[NS]	18.26
	200	0.11±0.012[**]	42.10	0.08±0.013[**]	58.65
	400	0.09±0.01[**]	52.63	0.068±0.01[**]	67.30
Diclofenac	10	0.06±0.007[**]	68.42	0.04±0.007[**]	76.92

Values are expressed as mean±SEM; from 6 animals in each group. [NS]P>0.05, [**]P<0.01.

3.0 DISCUSSION

The results presented in this study show that a methanolic extract has antipyretic effects and anti-inflammatory properties in rats when administered MEVV at doses of 100, 200 and 400 mg/kg. Carrageenan-induced acute footpad oedema in laboratory animals was first introduced by Winter et. al [14] It has been widely used to screen new anti-inflammatory drugs and remains an acceptable preliminary screening test for anti-inflammatory activity. [18] The development of the carrageenan-induced paw oedema derives from the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin in the inflammatory area. In particular, the initial phase of inflammation (0 – 2 h) has been attributed to the release of histamine, and kinins, followed by a late phase (2.5 – 6 h) mainly sustained by prostaglandin release and more recently have been attributed to the induction of cyclooxygenase-2 in the tissue. [19, 20, 21]

In this study, when the increases dose (100, 200 and 400 mg/kg) of the extract was administered, the anti-inflammatory effect of the extract respectively increased. This could be explained by the presence of possible anti-inflammatory compounds in the crude extract which might have become predominant as the concentration of the extract was increased and thus producing anti-inflammatory activity. This is possible because the crude extract comprises of several chemical constituents which could be acting via various mechanisms. Although the actual mechanism of action of MEVV is not known, it is possible that, the anti-inflammatory activity exhibited by the extract could be attributed to the inhibition of the synthesis, release or action of Inflammatory mediators that are known to be involved in carrageenan-induced inflammation which include cytoplasmic enzymes and serotonin from mast cells and also bradykinin, prostaglandins and other cyclo-oxygenase products. Moreover, flavonoids, which were identified in this study as one of the constituents of the plant extract, are known to target prostaglandins which are involved in the late phase of acute inflammation. Hence, the presence of flavonoids may contribute to the ant-inflammatory activity of the MEVV.

In addition to its anti-inflammatory properties, the extract exhibited antipyretic activity in yeast-induced pyrexia in rats. It is currently accepted that prostaglandin E2 (PGE2) is the final fever mediator in the brain, specifically in the preoptic area of the anterior hypothalamus [22], thus it may be plausible to conclude that the extract inhibits the synthesis of prostaglandins. However, it must be noted that several biochemical events occur leading ultimately to the synthesis of PGE2. Fever is believed to result from a finely tuned, complex event that involves both the peripheral immune system and the brain, through which a series of inflammatory and metabolic processes are regulated. It is established that there are two pathways leading to the transcription and induction of cyclooxygenase (COX)-2, the rate limiting enzyme for prostaglandin (PGE2) synthesis. Both pathways are activated by cytokines e.g. IL-1_, IL-6 and tumor necrosis factor (TNF) which trigger central mechanisms that act via the transcription factors such nuclear factor (NF)_B and signal transducer and activator of transcription (STAT-3) [23] . It may therefore be worthwhile to investigate the exact point in the biochemical events where the extract exerts it antipyretic effect.

5.0 CONCLUSION

In conclusion, we have demonstrated that the methanolic extract from the leaves of *Vitis vinifera* has anti-inflammatory activity in the carrageenan-induced paw oedema in rat model of acute inflammation as well as an antipyretic effect in Brewer's yeast-induced pyrexia in rats comparable to the NSAIDs diclofenac and paracetamol respectively and hence may be potentially useful in the management of inflammatory conditions in humans, a validation of its traditional use as anti-inflammatory agent.

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REFERENCES

6. Gharib MKN, Heidari A. Bronchodilatory Activity of Vitis vinifera Leaf Hydro alcoholic Extract in Rat. Iranian Biomedical Journal 2006; 10 (2):79-83.
7. The Wealth of India: "A Dictionary of Indian raw materials & industrial products first supplement series" Volume-10: (sp W) National Institute of Science Communication and Information Resources (CSIR) New Delhi .527.
8. KR Kirthikar, BD Basu. Indian Medicinal Plants, 2nd edition. Dehra Dun, Bishen Singh Mahendra Pal Singh, 1993; 607-608.
9. Prajapati ND, Kumar U. Agro's dictionary of medicinal plants. Jodhpur India: Dr. Updesh Purohit for agrobios; 2003; 372-373.
10. Aziz R. Popescu ML. Mihele D. Contributions to The Pharmacognostical Study On Grapes Hamburg Cultivar. Farmacia 2008; 5:571-576.
11. Felicio JD, Santos RDS, Goncalez E. chemical constituents from Vitis vinifera (Vitaceae). Arq. Inst. Biol., Sao Paulo 2001; 68(1):47-50.
12. Strauss CR, Wilson B, Williams PJ. 3-oxo-a-ionol, vomifoliol and roseoside in Vitis vinifera fruit. phytochemistry 1987; 26(7):1995-1987.
13. Spacer CB, Breder CD. The neurologic basis of fever. New England J Med 1994 330: 1880-1886.
14. Roberts II LJ, morrow JD. Analgesic-antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. In, Hardman JG (ed). Goodman & Gilman's the pharmacological basis of therapeutics, 10th edition. New York, McGraw-hill, 2001; p. 688.
15. OECD guidelines for the testing of chemicals (Acute oral toxicity – up and down procedure). Adopted 23rd march 2006. [Cited 2008 Mar 20]; Available from: URL: www.oecd.org
16. Hajare SW, Chandra S, Tandan SK, Sarma J, lal J, Telang AG. Analgesic and antipyretic activities of dalbergia sissoo leaves, indian journal of pharmacology 2000; 32: 357-360.

17. Reanmongkol W, Itharat A, Antipyretic activity of the extracts of Hibiscus sabdariffa calyces L. in experimental animals, Songklanakarin J. Sci. Technol., March 2007; 29(1): 29-38.

18. Gupta M, Mazumder UK, Kumar RS, Thangavel SK, Studies on Anti-inflammatory, Analgesic and Antipyretic Properties of Methanol Extract of Caesalpinia bonducella leaves in Experimental Animal Models, IJPT 2003; 2(2):30-34.

19. Winter CA, Risley E, Nuss G, Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962; 111:544-7.

20. Arul V, Miyazaki S, Dhananjayan R. Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of Aegle marmelos corr. J Ethnopharmacol 2005;96:159-63.

21. Banerjee S, Sur TP, Mandal S, Das PC, Sikdar S. Assessment of the anti-inflammatory effects of Swertia chirata in acute and chronic experimental models in male albino rats. Indian J Pharmacol 2000; 32:21-4.

22. Venkataranganna MV, Gopumadhavan S, Mitra SK, Anturlikar SD. Anti-inflammatory activity of JointCare B, a polyherbal formulation, Indian Drugs 2000; 11(37): 543-546.

23. Singh H, Kumar S, Dewan S, Kumar VL. Inflammation induced by latex of aleotropis procera - a new model to evaluate anti-inflammatory drugs. J. Pharmacol. Toxicol. Methods. 2000; 43: 219 - 224.

24. Vinegar R, Truax JF, Selph JL, Johnston PR, Venable AL, McKenzie KK. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed. Proc. 1987; 46: 118-126.

25. Muniappan M, Sundararaj T. Anti-inflammatory and antiulcer activities of Bambusa arundinacea. J. Ethnopharmacol. 2003; 88: 161-167.

26. Di RM. Biological properties of carrageenan. J. Pharm. Pharmacol. 1972; 24: 89-102.

27. Li S, Dou W, Tang Y, Goorha S, Ballou LR, Blatteis CM. Acetaminophen: antipyretic or

hypothermic in mice? In either case, PGHS-1b (COX-3) is irrelevant. Prostaglandins Other Lipid Mediat. 2008; 85: 89-99.

28. Inoue W, Somay G, Poole S, Luheshi GN. Immune-to-brain signaling and central prostaglandin E2 synthesis in fasted rats with altered lipopolysaccharide-induced fever. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2008; 295: 133 – 143.