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PHYTOCHEMICAL SCREENING AND ANTI INFLAMMATORYACTIVITY OF ETHANOLIC LEAF EXTRACT OF *VITIS VINIFERA*

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ABSTRACT

Vitis is the genus in which the Indian medical shrub Grapes (*Vitis vinifera*) is included, which have been reported to have Phytochemicals and anti-inflammatory effect. *Vitis vinifera* is hermaphrodite in nature. In the present study the effects of ethanolic extract of Vitis vinifera on pharmacological changes of Albino rats in normal and pain and inflammation induced rats was investigated. The animals were divided into 3 group's control, low dose (75mg/kg) and high dose (150mg/kg). Rats in each of these groups were sub divided into 2 groups i.e., with inflammation and without inflammation. The rats of each group were tested by injection of Carrageenan and inflammation in rats treated with Vitis vinnifera extract when compared with control group.

KEYWORDS: Vitis vinnifera, phytochemical screening, ethanolic extract, Inflammation, carrageenan.

INTRODUCTION

Most Vitis varieties are wind-pollinated with hermaphroditic flowers containing both male and female reproductive structures. These flowers are grouped in bunches called inflorescences. In many species, such as Vitis vinifera, each successfully pollinated flower becomes a grape berry with the inflorescence turning into a cluster of grapes, Grapevines usually only produce fruit on shoots that came from buds that were developed during the previous growing season. The search for new pharmacologically active agents obtained by

screening natural sources such as microbial fermentations and plant extracts has led to the discovery of many clinically useful drugs. The world health organization (WHO) is fully aware of the importance of herbal medicines to the health of many people throughout the world as stated in a number of resolutions adopted by the world health assembly. Thus, herbal medicines have been recognized, valuable and readily available resources for primary health care and WHO has endorsed their safe and effective use.

Grapes (*Vitis vinifera)*

II MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL

The flowers of the plant Vitis vinifera were collected at college premises of Aditya college of pharmacy Surampalem, East Godavari district of Andhra Pradesh India in the month of February 2018**.**

EXTRACT PREPARATION

The flowers of the plant Vitis vinifera was taken and air shade dried. The shade dried plants were chopped to small pieces and ground well to coarse powder. The dried powder was macerated with ethanol (95%) and extracted by hot percolation. The obtained extract was concentrated and dried by placing in desiccators.

EXPERIMENTAL ANIMALS

Albino rats (80-120g) of either six were used in the study. Animals were housed in the colony cages at ambient temperature 25 ± 2° c, 12h light or dark cycle and 50±5 % relative humidity with free access to food and water ad libitum. Food but not water deprived during experiment. All the experiments were carried out during the light period. Animals were divided into 2 groups of 3 animals each. (one group induced with inflammation and one without inflammation).

Procedure methodology

Phytochemicals in Vitis vinifera

A. Phenolics: V. vinifera contains many phenolic compounds. Anthocyanins m be found in the skin of the berries, hydroxycinnamic acids in the pulp and condensed tannins of the proanthocyanins type in the seeds. Stilbenoidscan be found in the skin and in wood.

B.Stilbenoids: Trans-resveratrol is a phytoalexin produced against the growth of fungal pathogens such as Botrytis cinerea and delta-viniferin is another grapevine phytoalexin produced following fungal infection by Plasmoparaviticola.

C. Anthocyanins: Vitis vinifera red cultivars are rich in anthocyanins that impart their colour to the berries (generally in the skin). The 5 most basic Anthocyanins found in grape are.

1. Cyanidin-S-O-glucoside
2. Delphinidin-3-O-glucoside
3. Malvidin-3-O-g1ucoside
4. Petunidin-3-O-glucoside
5. Peonidin-3-O-g1ucoside

D. Other chemicals

Isoprenoid monoterpenes' are present in grape, above all cyclic linalool,geraniol, nerol, citronellol, homotrienol and monocyclic a-terpineol, mostly occurring as glycosides. Carotenoids accumulate in ripening grape berries. Oxidation of carotenoids produces volatile fragments, C13-norisoprenoids. These are strongly odoriferous compounds, such as Beta-Ionone (aroma of viola), damascenone (aroma of exotic fruits), Beta-damasoone (aroma of rose) and piano] (aroma of flowers and fruits). Tetrahydro-Beta-carbolines are indole alkaloids occurring in grape to a low amount and contributing to its antioxidant power. Melatonin, an alkaloid, hasbeen identified in grape.In-addition, seeds are rich in unsaturated fatty acids, which helps lowering levels of total cholesterol and LDL cholesterol in the blood.

INFLAMMATION

Inflammation is a part of the complex biological response of vascular tissues to harmfulstimuli, such as pathogens, damaged cells or irritants (Ferrero-Malian et aL, 2007). Inflammation is a protective attempt by the organism to remove the injurious stimuliand to initiate the healing process. Inflammation is not a synonym for infection even in cases Where inflammation is caused by infection. Without inflammation Wounds and infectionswould

never heal similarly progressive destruction of the tissue would compromise thesurvival of the organism. However chronic inflammation can also lead to a host of diseasessuch as atherosclerosis, rheumatoid arthritis and even cancer (e.g.; gall bladder carcinoma). Acute inflammation is the initial response of the body to harmful stimuli and is achieved bythe increased movement of plasma and leukocytes from the blood into the injured tissues Prolonged inflammation known as chronic inflammation leads to progressive shift in the typeof cells present at the site of inflammation and is characterized by simultaneous destructionand healing of the tissue from the inflammatory process. inflammation may be triggered bymany causes some of these include burns, chemical irritants, toxins, infection by pathogens, physicalinjury, immune reactions due the hypersensitivity, ionizing radiation, trauma.

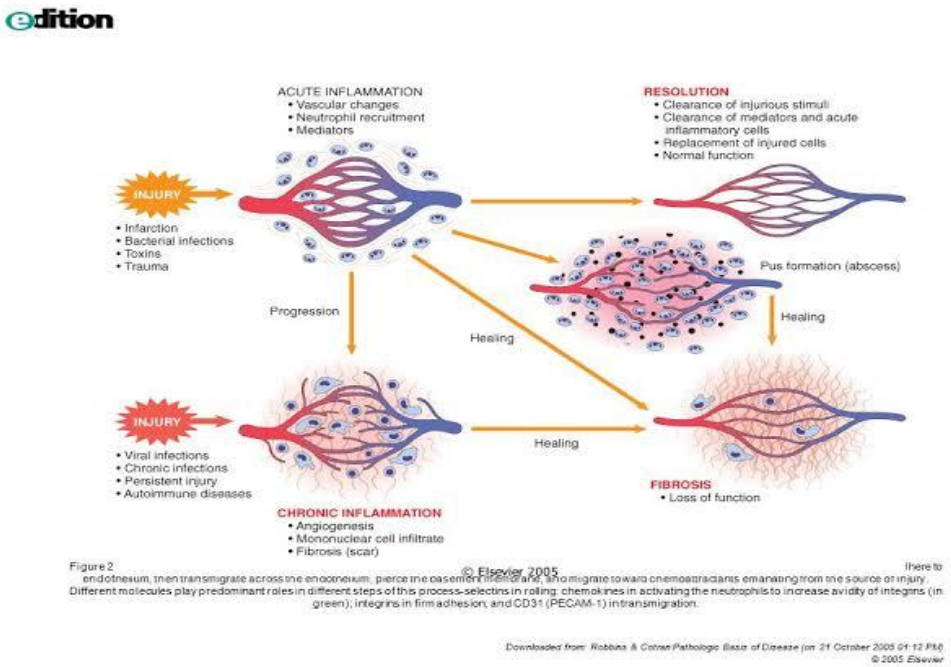
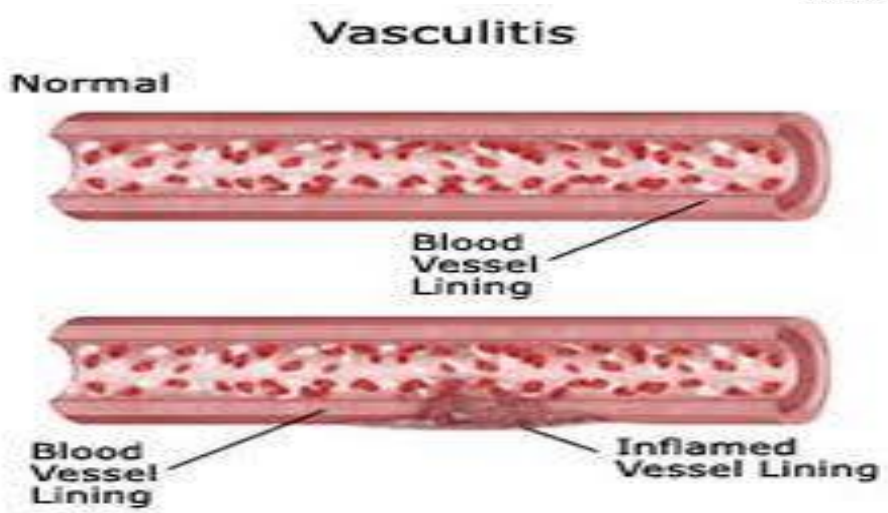


Figure 2 © Elsevier 2005 here to endothelium, then transmigrate across the endothelium, pierce the basement membrane, and migrate toward chemoattractants emanating from the source of injury. Different molecules play predominant roles in different steps of this process-selectins in rolling, chemokines in activating the neutrophils to increase avidity of integrins (in green), integrins in firm adhesion, and CD31 (PECAM-1) in transmigration.



INFLAMMATION PROCEDURE

The screening methods for the evaluation of potential anti-inflammatory agents are also aimed to counter the three phases of inflammation. The tests for anti-inflammatory activity are designed to measure acute inflammation, subacute inflammation and the chronic repair process. These methods are developed to find new drugs against rheumatoid arthritis and other allied diseases. The inflammatory process can be triggered by a number of stimuli like an infectious agent, ischaemia, antigen-antibody interactions and chemical, thermal or mechanical injury. The response is accompanied by signs of erythema, oedema, hyperalgesia and pain.

Methods for testing acute and sub-acute inflammation

CARRAGEENAN INDUCED PAW OEDEMA

Carrageenan Was used to induce oedema in this study. The animals were pretreated animals received Dose (100 mg/kg and 200 mg/kg) suspended in 2%acacia positive control Volume of 20/ Diclofenac (10mg/kg) i.e. negative control group received asimilar volume of 2% Acacia After 30 min 0.1m of 1%w/v suspension of Carrageenan in distilled Welter Was injected subcutaneously on to the sub plantar region of the left hind paw of the animals. Measurement of paw size was carried out with plythesmometer Paw Sizes were measured Immediately before and 1 hr. after Carrageenan injection. Oedema inhibitory activity was calculated using the following formula.

% Inhibition = (1-Vt/Vc)100

Whereas Vt = oedema volume of control animal, Vc= oedema volume of treatment

I. RESULT

PHYTOCHEMICAL SCREENING: Following Phytochemicals are found in vitis vinifera.

Table no 1: Shows following phytochemicals present in Vitis vinifera.

PHYTOCHEMICALS	CHEMICAL TESTS	RESULTS
flavanoids	lead acetate test	
	zinc chloride test	positive
	NaoH test	positive
saponins	froth formation test	positive
tannins	ferric chloride test	positive
carbohydrates	Molischs test	positive
	benedicts test	positive
	Fehlings test	positive
alkaloids	dragendroffs test	positive
	mayers test	positive

	hagers test	positive
terpenoids	salkowski test	positive
	libermannbuchartds test	positive
proteins	biurets test	positive
	ninhydrin test	positive
	millons test	positive
glycosides	legals test	positive
	kellerkilliani test	positive

Table no 2: In-Vivo Anti-Inflammatory Effect Of Ethanolic Leaf Extract Of Vitis vinifiera In Carrageenan Induced Paw Oedema In Rats.

Treatment	Mean increase in paw diameter mm				
	0hr	1hr	2hr	3rd hr	4th hr
Control	1.8±0.015	2.5±0.179	3.9±0.088	4.0±0.208	3.5±0.208
Diclofenac Sodium(10mg/kg)	1.9±0.145	1.9±0.208 50.0%	2.1±0.11 57.1%	2.3±0.96 65.0%	2.2±0.81 25.0%
Low Dose Of Extract of Vitis vinifera (75mg/kg)	1.74±0.135	2.0±0.133 33.3%	2.3±0.176 52.3%	2.6±0.43 60.0%	2.4±0.54 25.0%
High Dose Of Extract of Vitis vinifera(150mg/kg)	1.750.24	1.8±0.185 50.0%	2.0±0.233 66.6%	2.4±0.120 75.0%	2.0±0.145 33.3%

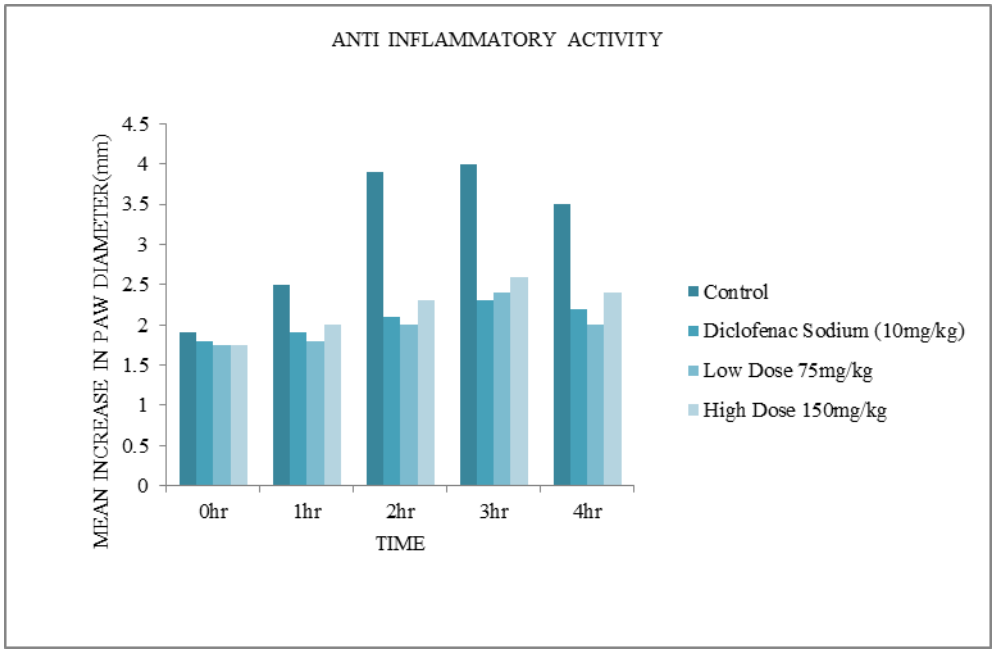


Figure 1.1: Anti-Inflammatory Effect Of Ethanolic Leaf Extract Of Vitis Of Vitis vinifera and Diclofenac Sodium.

Figure 1.2: Carragenan Induced Paw Odema In Rat (100mg/kg).

Low Dose Of Vitis vinifera(75mg/kg)

High Dose Of Vitis vinifera(150mg/kg)

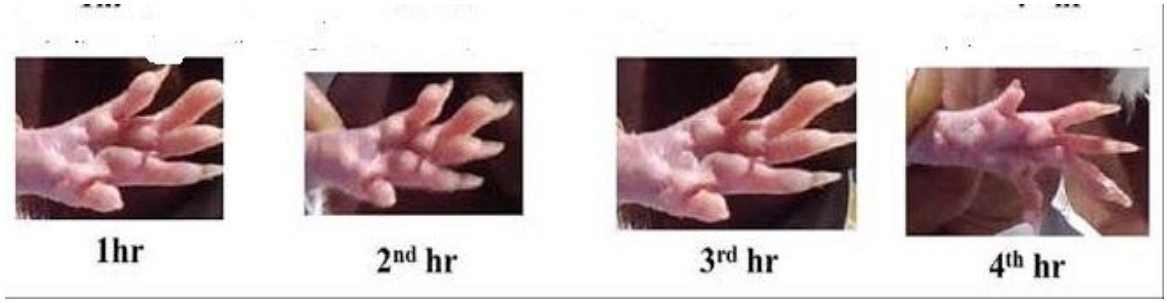
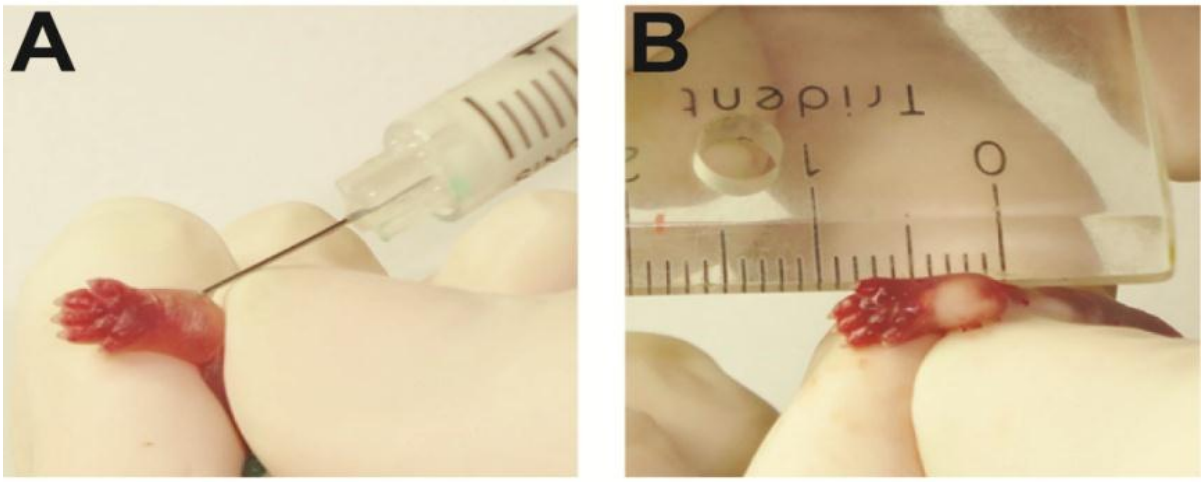


Figure 1.3 Anti Inflammtory Effect Of Vitis vinifera (Low Dose 75mg/kg And High Dose 150 mg/kg).



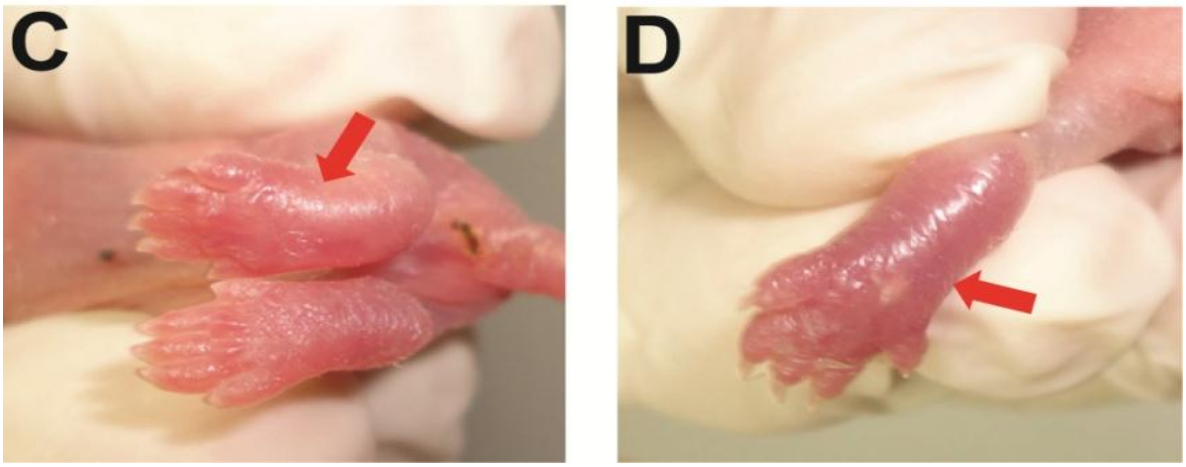


Figure 1.4 anti inflammatory effect of in Rat using Diclofenac Sodium (10mg/kg).

II. DISCUSSION

In the anti inflammatorystudy the Vitis vinifera Leaf extract produced significant inhibition of paw inflammation induced by Carragenan. Histamine, serotonin and prostaglandins play 'a major role in the development of Carragenan induced paw oedema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non steroidal anti-inflammatory agents which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis. The time course of oedema development in Carragenan induced paw oedema model in rats is generally represented by a biphasic curve. The first phase of inflammation within a hour of a Carrageenan injection and is partly due to the trauma of injection and also to the second phase of inflammatory reaction which is measured at 3rdhr.The presence of PGE2 in the inflammatory exudates from the injected foot can be demonstrated at 3hr and period thereafter. Therefore, it can be interfered that the inhibitory effect of ethanolic extract of Vitis vinifera Leaf on Carrageenan induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Significant inhibition of paw oedema in the early hours of study by Vitis vinifera Leaf could be contributed to the inhibition of histamine and or serotonin.

The Anti-inflammatory Activity was investigated using In-vivo models.Anti-inflammatory activity was evaluated using carragenan induced paw odema method. In the anti-inflammatory study the Vitis vinifera Leaf extract produced significant inhibition of paw inflammation induced by Carrageenan**.**

III. CONCLUSION

Since ancient times people have been using plants in various ways as a source of medicine. In conclusion, the data obtained from this study has established the significant analgesic and anti-inflammatory effects of Vitis vinifera Leafextract in laboratory animals and thus justify the local uses of the plant for the treatment of these conditions in humans.

In further studies we can formulate a new methodology for isolation and purification of the compounds present in Vitis vinifera Leaf which may act as a drug to control a broad range of inflammation, These studies will attempt to look into the identification, purification and characterization of specific phytochemical agents that are responsible for the observed biological effects.

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