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Anti-Oxidant and Anti-Nociceptive Activity of Ethanolic Leaf Extract of *Vitis vinifera*

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Abstract

Aim: Evaluation of Antioxidant and Anti nociceptive activity of ethanolic leaf extract of *vitis vinifera*. **Materials and methods:** In the present study the effects of ethanolic extract of *Vitis vinifera* on pharmacological changes of Albino rats in normal and pain 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 ie. with pain and without pain. The rats of each group were tested on Eddy's hot plate apparatus. **Results:** The results showed reduced pain in rats treated with *vitis vinifera* extract when compared with control group. The *in vitro* antioxidant activity was carried out to correlate its protective effect against oxidative stress, significant inhibition against DPPH and Nitric oxide radicals were observed with extract in dose dependent manner and the results were compared with that of standard Gallic acid.

Keywords
Vitis vinnifera, Oxidative stress, Nociceptive

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INTRODUCTION

Shrubs are typically defined as woody plant arising from multiple stems at the base. Species that grow into a shrubby habit may be either deciduous or evergreen. It is economically important as the source of grapes, both for direct consumption of the fruit and for fermentation to produce wine. The study and cultivation of grapevines is called viticulture.

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. It has been realized that medicinal plants are a valuable resource for new pharmaceutical products and thus a potential source of new drugs as well as for economic development (WHO, 2010).

PLANT PROFILE
Botanical name: Vitis vinifera
Common name: Grapes
Location: Asia, North America and Europe
Family: Vitaceae
Genus: Vitis
Species: vinifera
Kingdom: Plantae

MATERIALS 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 ± 2̊ 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 painand one without pain).

EXPERIMENTAL DESIGN

Anti-oxidant activity

A free radical is a molecule with one or more unpaired electrons in its orbital. Many of these molecule species are oxygen centered. These highly unstable molecules tend to react rapidly with adjacent molecules, often generating additional free radicals or other reactive oxygen species(ROS).Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system line, and brain dysfunction. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. The components include:

A. Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols (vitamin E),
carotenoids, and other low molecular weight compounds such as glutathione and lipoic
acid.

B. Antioxidant enzymes, e.g., superoxide dismutase. glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.

C. Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.

D. Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

PRINCIPLES OF INVITRO ANTIOXIDANT METHODS:

DPPH METHOD:

The molecule (1-diphenyl-2-picryl hydrazyl (a-Diphenyl-B-Picryl hydrazy1; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as A Whole. Delocalization of electron also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 517 nm. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. According to (manzocco et al.,1998) the sample extract (0.2 ml) is diluted with methanol and 2 ml of DPPH solution (0.5 mm) is added. After 30 min, the absorbance is measured at 517 nm. The percentage

of the DPPH free radical scavenging is calculated using the equation as given below:

DPPH Scavenged (%) = {(A_o-A_1)/A_o} X100

Where A_o is the absorbance of the blank (containing all reagents except the test 88mph), and A1is the Absorbance of test sample. The antioxidant activity of test sample was expressed as IC_{50}. The IC_{50} value is defined as concentration in (µg/ml) of test sample that sample that scavenges free radicals by 50%.

NITRIC OXIDE SCAVENGING ACTIVITY:

NO is generated in biological tissue by specific nitric oxide synthases, which metabolizes arginine to citrulline With the formation 0f NO via a five electron oxidative reaction (David eta/., 1999) The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO'. Under aerobic conditions, NO. Reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using Griess reagent (Mar cocci et al., 1994). Two (2) ml of 10 mM sodium nitroprusside dissolved in0.5 m1 phosphate Buffer saline (pH 7.4) is mixed with 0.5 ml of sample at various concentrations (0.2-0.8mg/ml). The mixture is then incubated at 25°C. After 150 min of incubation, 0.5 ml of the incubate solution is withdrawn and mixed with 0.5m] of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthyl ethylenediamine dichloride (0.1% w/v)]. The mixture is then incubated at room temperature for 30min and its absorbance pouring into a cuvette measured at 546 nm. The amount of nitric oxide radical inhibition is calculated following this equation:

Nitric oxide Scavenged (%) = {(Ao-A1)/Ao} x100

Where A0 is the absorbance of the blank (containing all reagents except the test sample), and A1 is the Absorbance of the test sample.

The present study demonstrates scientific support for the protective effect of ethanolic leaf extract of *vitis vinnifera* to produce anti-inflammatory and anti-nociceptive activity and lends some credence to traditional claims of its therapeutic benefits in pain and inflammation related disorders.

SCREENING OF ANTI NOCICEPTIVEACTIVITY

TAIL FLICK METHOD:

1.Grouping:

Albino rats weighing between 150-200 gms were divided into 4 groups of 3 rat's each.3 animals being housed in a labeled cage each. Animals were given a period of time to adjust to the new environment. provided with food and water libitum.

Group 1: Animals were administered 0.1m1 normal saline p.o

Group 2: Animals were administered standard reference drug paracetamol (500mg/kg) I.p

Group 3: Animals were administered *Vitis vinifera* ethanolic leaf extract (100mg/kg)

Group 4: Animals were administered *Vitis vinifera* ethanolic leaf extract (200mg/kg)

2. Procedure:

In this method adult albino rats of either sex were selected. The basal reaction time to radiant heat by placing the tip of the tail on the radiant heat source was recorded using stopwatch. The basal reaction time was observed at 0, 15, 30, 60, 120 mins, the analgesic effect of ethanolic leaf extract was assessed using this method.

EDDY'S HOT PLATE METHOD

1. Grouping:

Albino rats weighing between 150-200 gms were divided into 4 groups of 3 rat's each.3 animals being housed in a labeled cage each. Animals were given a period of time to adjust to the new environment provided with food and water libitum.

Group 1: Animals were administered 0.1m] normal saline p.o

Group 2: Animals were administered standard reference drug Tramadol (5mg/kg) I.p)

Group 3: Animals were administered Vitis vinifera ethanolic leaf extract (100 mg/kg).

Group 4: Animals were administered Vitis vinifera ethanolic leaf extract (200mg/kg).

2. Procedure:

In this method adult albino rats of either sex were selected. The animals were placed individually on the hot plate maintained between 55°C. The time taken for each rat to respond to the thermal stimulus by paw licking and jumping off the hot plate was recorded. The reaction time was observed at 0, 15, 30, 60,120 mins, the anti-nociceptive effect of ethanolic Met extract was assessed using this method.

Percentage inhibition = [Rt/Rc-1] 100

Where Rt = reaction time in treated group; and Rc = reaction time in control group

RESULTS

ANTIOXIDANT ACTIVITY

1.1 : DPPH And NITRIC OXIDE Methods of Test Sample and GALLIC ACID

Sample	Concentration(µg/ml)	DPPH Method		Nitric Oxide Method	
		% Inhibition ±SEM	IC_{50} (µg/ml)	%Inhibition ± SEM	IC_{50}(µg/ml)
Test Sample	50	63.9±0.63	1.77	44.8±0.202	24.76
	100	71.1±0.80		57.4±0.305	
	300	80.0±0.41		60.5±0.708	
	500	83.92±0.20		68.8±O.112	
Gallic Acid	1	79.2±0.52	0.53	55.3±0.531	0.97
	2.5	85.1±0.14		60.3±0.302	
	5	87.2±0.24		72.0±0.112	
	10	90.2±0.12		85.4±0.821	

Values are expressed as mean ± SEM, n=4

Figure 1.2: DPPH Test Sample Graph

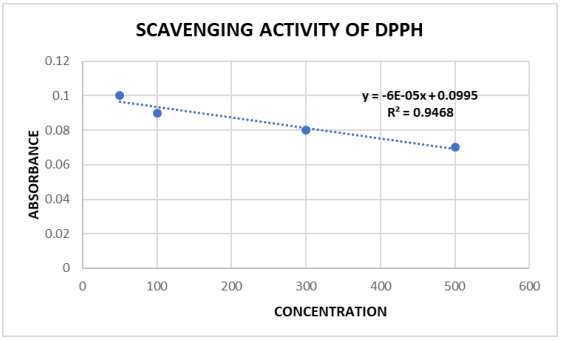
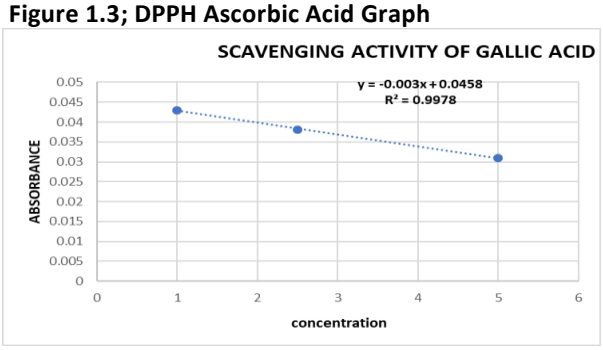


Figure 1.3; DPPH Ascorbic Acid Graph



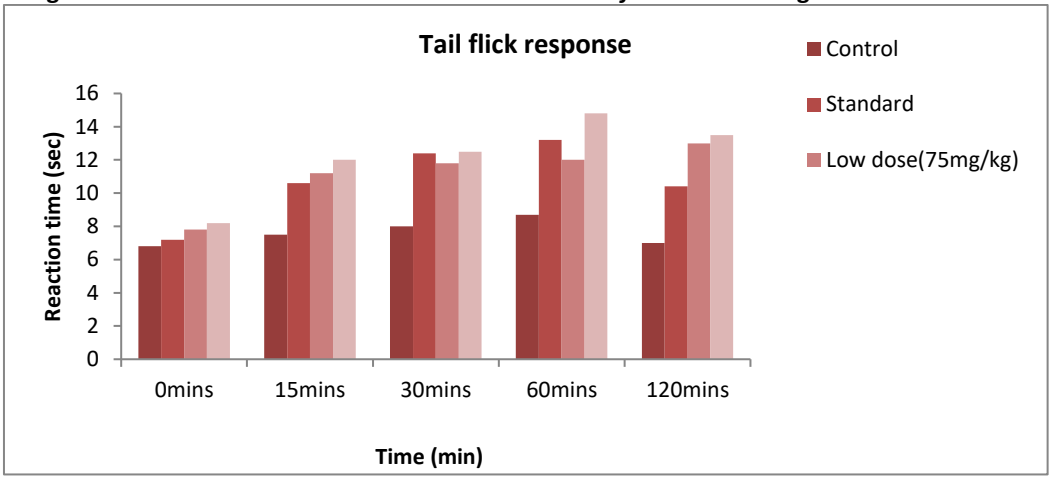
1.4 effect of Ethanolic Leaf Extract of *Vitis vinifera* in Rats Using Tail Flick Method:

Treatment Group	0min		15min		30min		60min		120min	
	B.R.T	% inh	B.R.T	% Inh	B.R.T	% Inh	B.R.T	% Inh	B.R.T	%inh
Control	5.0±0.67	---	6.0±0.32	---	6.5±0.97	---	7.0±0.30	---	5.2±0.81	---
GroupI Tramadol (10mg/kg)	6.0±0.5	30%	13.0±0.61	49.5%	15.0±0.12	80.5%	18.0±0.45	74.7%	17.5±0.98	65.5%
GroupII Extract of Vitis vinifera (75mg/kg)	6.0 ±0.82	25%	11.0±0.82	48.3%	14.0±0.67	75.3%	16.0±0.21	68.5%	6.5±0.65	55.0%
GroupIII Extract of Vitis vinifera (150mg/kg)	7.5±0.94	50%	14.0±0.12	63.3%	18.0±0.92	85.9%	18.5±0.39	75.5%	17.5±0.21	66.5%

Note: B.R.T =Basal Reaction Time (Sec); % inh = % Inhibition

Data analyzed using one-way analysis of variance (ANOVA)and expressed as a mean considered as significant at p<0.01. extract = extract of Vitis vinifera.

Figure 1.5Effect Of Ethanolic Leaf Extract of *Vitis vinifera* In Rat Using Tail Flick Method:



EDDYs HOT PLATE

TAIL FLICK RESPONSE IN RAT

2.1 EDDYS HOT PLATE METHOD

In this method adult albino rats of either sex were selected. The animals were placed individually on the hot plate maintained between 55°C. The time taken for each rat to respond to the thermal stimulus by paw licking and jumping off the hot plate was recorded. The reaction time was observed at 0, 15, 30, 60, 120 mins, the anti-nociceptive effect of ethanolic Met extract was assessed using this method.

Percentage inhibition = [Rt/Rc-1] 100

Where Rt = reaction time in treated group

R_c = reaction time in control group

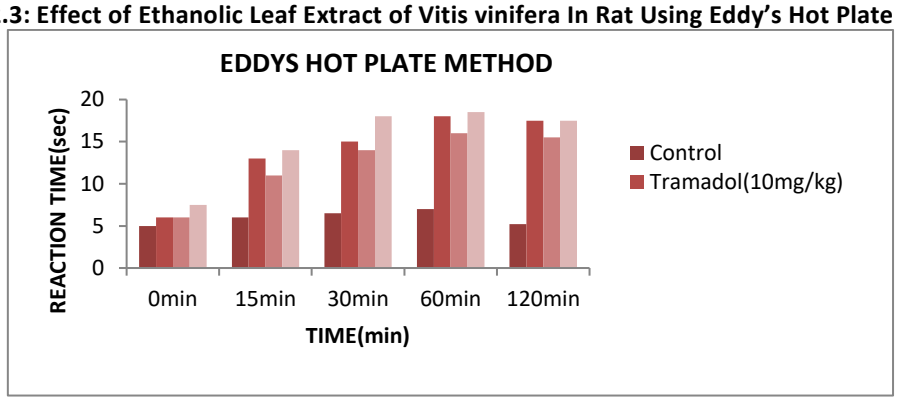
Table 2.2 Effect of Ethanolic Leaf Extract of *Vitis vinifera* In Rat Using Eddy's Hot Plate Method

Treatment Group	0min		15min		30min		60min		120min	
	B.R. T	% inh	B.R. T	% inh	B.R. T	% inh	B.R. T	% inh	B.R. T	%inh
Control	6.8±0.08	-----	7.5±0.20	---	8.0±0.15	---	8.7±0.17	---	7.0±0.26	---
Group I Tramadol10mg/kg	7.2±0.08	55.88%	10.6±0.98	61.3%	12.4±0.34	72.2%	13.2±0.20	80.0%	10.4±0.15	78.5%
Group II Extract of Vitis vinifera 75mg/kg	7.8±0.20	52.7%	11.2±0.20	60.3%	11.8±0.17	51.2%	12.0±0.29	79.5%	13.0±0.23	77.7%
Group III Extract of Vitis vinifera 150mg/kg	8.2±0.17	60.5%	12.0±0.17	69.2%	12.5±0.92	65.8%	14.8±0.12	86.1 %	13.5±0.14	75.7%

Note: B.R.T =Basal Reaction Time (Sec); % inh = % Inhibition

Data analyzed using one-way analysis of variance (ANOVA)and expressed as a mean considered as significant at p<0.01. extract = extract of Vitis vinifera.

Figure 2.3: Effect of Ethanolic Leaf Extract of Vitis vinifera In Rat Using Eddy's Hot Plate Method



PAW LICKING RESPONSE

PAW JUMPING RESPONSE

DISCUSSION

This study demonstrated that ethanolic leaf extract of *Vitis vinifera* exhibited significant analgesic effect against nociceptive stimulus generated by eddy's hot plate method, tail flick method and anti-inflammatory activity against acute inflammatory responses like, carrageenan induced paw oedema. Furthermore, clinical and pathological studies should be conducted to investigate the active potentials of bioactive compounds present in this study.

CONCLUSION

This study demonstrated that ethanolic leaf extract of *Vitis vinifera* exhibited significant analgesic effect against nociceptive stimulus generated by eddy's hot plate method, tail flick method and anti-inflammatory activity against acute inflammatory responses like, carrageenan induced paw oedema. Furthermore, clinical and pathological studies should be conducted to investigate the active potentials of bioactive compounds present in this study.

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