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SUBACUTE TOXICITY STUDIES OF METHANOLIC FRUIT EXTRACT OF *VITIS VINIFERA L.* IN ALBINO RATS

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ABSTRACT

In the present study, phytochemical screening was conducted to find out the secondary metabolites present in the fruit extract. The presence of phytochemicals was well found in methanolic extract when compared to others (ethanol, petroleum ether, chloroform, water). In acute toxicity study, the oral dose upto 2000 mg/kg of fruit extract was administered to three groups in single dose and general behaviour, adverse effects and mortality were determined up to 72hr. and compared to normal group. In sub-acute toxicity study, the methanolic fruit extract of *Vitis vinifera L.*, in male albino rats at the dose levels of 150,250,350,450 and 550 mg/b.w were given at a rate of 1.0ml/rat/day to different sets of animals for 90 days. The result showed that there were no significant signs of toxicity at any dose level used in the study. Physical, biochemical as well as haematological parameters was unaltered throughout the study.

KEYWORDS: *Vitis vinifera L.*, methanolic extract.

INTRODUCTION

Medicinal plants can also be called as herbal remedies that have been used for the treatment of several diseases. Herbal extracts are safer than chemicals for the treatment of ailments. Administration of these extract over a long period of time without proper dosage, may result in toxic effects. The risk associated with the potential toxicity based on therapy demands that the practitioners should be kept reported incidence of renal and hepatic toxicity which results from the ingestion of herbal medicine. (Said *et al.*, 2002).

In a number of studies the herbal preparations have recently received considerable attention and also found to be a promising choice over modern medicines. All over the world, 80% of population continues to use traditional medicines for the primary medical problems in developing countries. Herbal drugs are used as an alternative to clinical therapy and the demand for these herbal remedies has increased recently. In the use of herbal remedies in the last two decades, there is need for a thorough scientific evaluation of these medicinal plants. (Sathya *et al.*, 2012).

Phytochemicals are the chemical components present in plants. In plant metabolism, these phytochemicals can be classified as primary and secondary constituents. Primary constituents consist of common sugars, amino acids, proteins, purines and pyrimidine of nucleic acids, chlorophyll's etc. Secondary constituents include the remaining plant chemicals which are alkaloids (derived from amino acids), terpenoids (a group of lipids) and phenolic (derived from carbohydrates). Even though these herbal drugs have an exclusive use in managing a variety of infirmity, certain studies also have reported the toxic effects of herbal medicines (Krishnaiah *et.al.*, 2007).

Grape plant (*Vitis vinifera* L.) which belong to vitaceae family, is a commonly food and also an economically important plant. The plant is distributed and cultivated in normal climatic patterns that is the regions where sufficient rain, warm and dry summers as well as relatively mild winters are available. The fruit is mainly used for the production grape juice, wine and other food products. The quality of grape products are characterized by their metabolic composition. (Kawakib *et al.*, 2017).

Vitis vinifera L. and its bioactive compounds have several pharmacological activities such as anti-oxidative, anti-inflammatory and antimicrobial activities, as well as in vitro activity against several cancer cell lines and hepatoprotective and cardio protective effects. It seems that grape seed extract and its active components such as proanthocyanidins, resveratrol, and quercetin are potent antioxidants. The intake of grapes and grape juice is likely to have positive effects on human health and especially in postmenopausal women. (Marjan *et al.*, 2009).

Traditionally the leaf of grape is used for diarrhoea, hepatitis and stomach-aches. Grapes, seeds and leaves have been used for preventing heart and blood vessels diseases, varicose veins, haemorrhoids, "hardening of the arteries" (atherosclerosis), high blood pressure,

swelling after injury or surgery, heart attack and stroke. Grape leaf has been used for attention deficit-hyperactivity disorder (ADHD), chronic fatigue syndrome (CFS), diarrhoea, heavy menstrual bleeding, uterine bleeding and cancer. It has been also used as a mild laxative for constipation. (Mansoura *et al*., 2012).

MATERIALS AND METHODS

Vitis viniferaL fruits were collected from chemmad, Malapuram district, Kerala, India during the month of June 2017.

Material processing

The fruits were cleaned and rinsed with distilled water by removing the seeds. The fruits alone were placed in trays, and dried in a circulating forced air incubator at 55 and 60 °C for 48 hours. The dry fruits were crushed using a blender and stored in clear polyethylene bags at 10°C prior to use.

Qualitative phytochemical screening of the fruit extract

The phytochemical screenings with methanol, ethanol, petroleum ether, chloroform and aqueous extracts of *Vitis vinifera L* were done by modern method of Peach and Tray (1955) to identify the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides and phenols.

Preliminary animal studies

Selection of animal for toxicity studies

Healthy adult male albino rats weighing about 150 to 200 g were purchased from Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. The animals were maintained in 12 hr. light and dark cycle at 28°C ±2°C in a well-ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for days prior to the commencement of the experiment. The animals were fed with standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India.

Treatment of animals

Acute toxicity studies of the methanolic extract of the *Vitis vinifera L.* fruit were carried out. Male albino rats weighing l50-200g were used for the study and were divided into six groups

each containing six animals. They received orally varying doses of the plant extract (l50, 250, 350,450 and 550mg/ kg b.w). Animals provided with normal pelleted diet and water served as control. During the dosing periods, all the animals were observed daily for clinical signs before dosing, immediately after dosing and up to 4 hr.

Serum Isolation and Hematology

At the end of experimental period, the animals were anathsized, blood samples collected through cardiac puncture and taken into heparinized tube for hematological studies and non-heparinized tube from which serum was isolated by centrifugation at 3000 rpm for 10 minutes and used for biochemical estimations. The liver and kidney were excised immediately and thoroughly washed in ice cold saline and weights were recorded

Hematological parameters namely, White blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb), MCV, MCH, Platelets, neutrophils and lymphocytes were analyzed by collecting blood from sino-orbital puncture and centrifuged for 30 min. at 2000 rpm to separate serum for testing the haematological parameters on the 30^{th} and 90^{th} day.

RESULTS AND DISCUSSION

The present study entiled subacute toxicity studies of methanolic fruit extract of *vitis vinifera L.* in albino rats was discussed with the following results.

Table 1: Phytochemical *analysis of Vitis vinifera* L. (Fruit).

S.No	Test	Water	Petrolium Ether	Methanol	Ethanol	Chloroform
1	ALKALOIDES	-	-	++	+	-
2	FLAVONOIDS	+++	-	++	+	-
3	TANNINS	++	-	++	++	-
4	SAPONINS	-	++	-	++	+
5	STEROIDES	+++	-	+++	++	+
6	TERPINOIDS	++	+	++	+	+
7	RESINS	-	-	-	-	-
8	GLYCOSIDES	+++	-	++	+	-
9	PHENOL	-	-	-	-	-

The phytochemical screening with water, ethanol, methanol, petroleum ether and chloroform of *Vitis vinifera L* (fruit) possess secondary metabolite which are shown in table 1.

Qualitative phytochemical analysis of the methanol fruit extract and it was found in the presence of maximum phytochemicals. The phytochemical study with methanolic fruit extract of selected medicinal plant extract show the presence of alkaloids, flavonoids, tannins,

steroids, terpenoids and glycosides. So the methanolic fruit extract of selected medicinal plant was used for the further study.

Mean body weight of rats treated with methanolic fruit extract of *Vitis vinifera L* for 90days were recorded in table 2 the control also gain body weight throughout the duration of treatment. There was no statistically significant weight gain or loss in experimental rats when compare to control rats for the doses tested.

Table 2: Body weight of experimental rats in the final systemic toxicity studies due to the methanolic fruit extract of *Vitis vinifera L*.

Dose mg/kg b.w	0^{th} DAY	30^{th} DAY	90^{th} DAY
Control (Group I)	166.86±0.07	171.39±0.06	175.12±0.07
150 (Group II)	$168.56±0.16a^{ns}$	$173.26±O.O9a^{ns}$	$177.2±0.13a^{ns}$
250 (Group III)	$168.59±0.13b^{ns}$	$173.53±0.16b^{ns}$	$177.58±0.13b^{ns}$
350 (Group IV)	$167.72±0.13c^{ns}$	$172.69±0.19c^{ns}$	$176.47±0.23c^{ns}$
450 (Group V)	$167.67±0.19d^{ns}$	$172.44±0.22d^{ns}$	$175.39±0.17d^{ns}$
550 (Group VI)	$166.60±0.21e^{ns}$	$172.47±0.27e^{ns}$	$175.38±0.19e^{ns}$

Values are expressed as mean ± S.D of six animals

Statistical comparisons

a- Group II is compared with group I b- Group III is compared with group I

c. Group IV is compared with group I d- Group V is compared with group I

e- Group VI is compared with group I ns- non significant at 5% level *-p<0.05

Table 3: Haematological values of the rats treated with Vitis *vinifera L* fruit extract for 30 days.

Haematological parameters	Dose (mg/kg b.w.)/ Groups					
	Control Group I	150 mg/ kg b.w Group II	250 mg/ kg b.w Group III	350 mg/ kg b.w Group IV	450 mg/ kg b.w Group V	550 mg/ kg b.w Group VI
RBC(10^{12}/L)	9.56±0.10	$8.55±0.15a^{ns}$	$9.66±0.15b^{ns}$	$9.73±0.16c^{ns}$	$9.78±0.16d^{ns}$	$9.76±0.15e^{ns}$
Hb(g/dl)	14.60±0.10	14.65±0.12 a^{ns}	15.67±1.47 b^{ns}	16.40±10 c^{ns}	15.62±0.16d^{ns}	15.65±0.19 e^{ns}
WBC(10^9/L)	6.69±0.14	6.74±0.16 a^{ns}	6.51±0.12 b^{ns}	6.66±0.14 c^{ns}	6.68±0.16d^{ns}	6.70±0.17 e^{ns}
MCV(µM^6)	79.55±0.13	79.56±0.11 a^{ns}	76.59±0.13 b^{ns}	77.49±0.15 c^{ns}	77.45±0.13d^{ns}	79.53±0.11 e^{ns}
MCH(pg)	27.34±0.12	28.59±0.16 a^{ns}	29.95±0.15 b^{ns}	25.53±0.13 c^{ns}	27.43±0.11d^{ns}	25.49±0.10 e^{ns}
MCHC(g/dl)	33.56±0.12	35.53±0.11 a^{ns}	36.52±0.10 b^{ns}	39.52±0.12 c^{ns}	34.60±0.11d^{ns}	33.89±0.13 e^{ns}
PCV(%)	44.89±0.06	45.56±0.23 a^{ns}	46.76±0.25 b^{ns}	45.53±0.21 c^{ns}	44.48±0.43d^{ns}	46.77±0.25 e^{ns}
Platelet(10^9)	873.28±0.25	888.59±0.17a^{ns}	877.47±0.24b^{ns}	859.57±0.14c^{ns}	834.79±0.12d^{ns}	885.67±0.20 e^{ns}
Neutrophil (%)	46.37±0.27	46.61±0.20 a^{ns}	46.60±0.19 b^{ns}	46.41±0.16 c^{ns}	46.44±0.26d^{ns}	46.78±0.13 e^{ns}
Lymphocyte (%)	81.72±0.12	81.62±0.18 a^{ns}	81.48±0.21 b^{ns}	81.68±0.16 c^{ns}	82.43±0.19d^{ns}	82.60±0.12 e^{ns}

Values are expressed as mean ± S.D of six animals

Statistical comparisons

a-Group II is compared with group I b- Group III is compared with group I

c-Group IV is compared with group I d- Group V is compared with group I

e- Group VI is compared with group I ns- non significant at 5% level *-p<0.05

Table 4: Haematological values of the rats treated with Vitis *vinifera L.* fruit extract for 90 days.

Haematological parameters	Dose (mg/kg b.w.)/ Groups					
	Control Group I	150 mg/ kg b.w Group II	250 mg/ kg b.w Group III	350 mg/ kg b.w Group IV	450 mg/ kg b.w Group V	550 mg/ kg b.w Group VI
RBC(10^{12}/L)	7.65±0.17	7.66±0.14 a^{ns}	7.59±0.13 b^{ns}	7.45±0.13 c^{ns}	7.47±0.13 d^{ns}	7.95±0.11 e^{ns}
Hb(g/dl)	14.67±0.13	14.65±0.11 a^{ns}	14.49±0.12 b^{ns}	14.38±0.14 c^{ns}	14.59±0.15 d^{ns}	14.63±0.14 e^{ns}
WBC(10^{9}/L)	5.72±0.12	5.63±0.13 a^{ns}	5.59±0.10 b^{ns}	5.49±0.20 c^{ns}	5.65±0.14 d^{ns}	5.62±0.13 e^{ns}
MCV(µM^{6})	78.24±0.16	78.24±0.23 a^{ns}	78.22±0.12 b^{ns}	78.33±0.22 c^{ns}	78.39±0.17 d^{ns}	78.22±0.17 e^{ns}
MCH(pg)	26.34±0.17	26.43±0.24 a^{ns}	26.39±0.13 b^{ns}	27.16±0.23 c^{ns}	26.33±0.18 d^{ns}	27.54±0.12 e^{ns}
MCHC(g/dl)	35.57±0.22	35.59±0.10 a^{ns}	35.46±0.13 b^{ns}	34.71±0.19 c^{ns}	33.73±0.14 d^{ns}	33.65±0.15 e^{ns}
PCV (%)	45.36±0.19	47.41±0.17 a^{ns}	45.45±0.13 b^{ns}	46.68±0.18 c^{ns}	46.59±0.24 d^{ns}	45.55±0.19 e^{ns}
Platelet(10^{9})	881.67±0.18	885.53±0.17 a^{ns}	857.98±0.11 b^{ns}	863.69±0.19 c^{ns}	879.47±0.1 d^{ns}	854.25±0.13 e^{ns}
Neutrophil (%)	52.64±0.18	52.67±0.17 a^{ns}	52.73±0.14 b^{ns}	52.43±0.17 c^{ns}	52.52±0.19 d^{ns}	52.67±0.10 e^{ns}
Lymphocyte (%)	82.56±0.22	82.59±0.20 a^{ns}	82.56±0.08 b^{ns}	82.81±0.12 c^{ns}	83.64±0.24 d^{ns}	83.76±0.11 e^{ns}

Values are expressed as mean ± S.D of six animals

Statistical comparisons

a-Group II is compared with group I b- Group III is compared with group I

c-Group IV is compared with group I d- Group V is compared with group I

e- Group VI is compared with group I ns- non significant at 5% level *-p<0.05

The haematological values of rats treated with *Vitis vinifera L* were given in table 3 and 4. Assessment of haematological parameters can be used to explain blood relating function of plant extract. The non-significant effect of the extract at various doses on the RBC and indicates relating to it throughout the experimental period is an indication that there was no destruction of matured RBCs. The non-significant effect on RBC and Hb also implies that there was no change in the oxygen carrying capacity of the blood and the amount of oxygen delivered to the tissue following the extract administration, since RBC and Hb are very important in transferring respiratory gases.

The calculated blood indicates MCV, MCH and MCHC have particular importance in anaemia diagnosis in most animals. This implies that the methanolic fruit extract of *Vitis vinifera L.* does not possess any adverse effects throughout 90 days period of administration.

The non-significant effect on calculated blood indicates, WBC, platelets, neutrophils and lymphocytes suggest that there was no infections and leukaemia. The decrease of such

indicates makes the rats more susceptible to various physiological stresses resulting in disease, poor growth and greater mortality.

The effect of administration of *Vitis vinifera L* fruit extract at different concentration on haematological parameters at the end of 30 and 90 days treatments are shown in tables 4 & 5 respectively. Administration of the fruit extract at all the selected concentrations did not produce any significant change ($p<0.05$) in all the haematological parameters tested.

Table 6: Liver marker enzymes and biochemical parameters in serum of the rats treated with *Vitis vinifera L* for 90 days.

Biochemical Parameters	Control Group I	150 mg/ kg b.w Group II	250 mg/ kg b.w Group III	350 mg/ kg b.w Group IV	450 mg/ kg b.w Group V	550 mg/ kg b.w Group VI
AST	40.25±0.15	40.65±0.13a^{ns}	40.64±0.12b^{ns}	40.53±0.11c^{ns}	40.76±0.20d^{ns}	41.76±0.12e^{ns}
ALT	37.62±0.16	37.53±0.23a^{ns}	37.54±0.14b^{ns}	36.52±0.17c^{ns}	36.49±0.17d^{ns}	35.50±0.15e^{ns}
ACP	58.43±0.21	58.60±0.16a^{ns}	58.61±0.11b^{ns}	58.64±0.11c^{ns}	58.77±0.12d^{ns}	59.53±0.14e^{ns}
ALP	56.80±0.16	56.52±0.17a^{ns}	56.74±0.17b^{ns}	56.42±0.10c^{ns}	56.68±0.25d^{ns}	56.59±0.19e^{ns}
LDH	111.45±0.13	111.52±0.18a^{ns}	98.93±0.18b^{ns}	111.53±0.13c^{ns}	111.45±0.13d^{ns}	110.89±0.16e^{ns}
PROTIEN	6.89±0.19	6.93±0.19a^{ns}	6.69±0.10b^{ns}	6.95±0.11c^{ns}	6.85±0.11d^{ns}	6.79±0.12e^{ns}
UREA	14.84±0.16	14.92±0.10a^{ns}	14.76±0.11b^{ns}	14.54±0.16c^{ns}	14.61±0.13d^{ns}	14.57±4.0.9e^{ns}
ALBUMIN	2.51±0.20	2.62±0.05a^{ns}	2.62±0.12b^{ns}	2.65±0.07c^{ns}	2.75±0.14d^{ns}	2.69±0.12e^{ns}
URIC ACID	5.36±0.21	5.42±0.18a^{ns}	5.35±0.20b^{ns}	5.53±0.19c^{ns}	5.34±0.20d^{ns}	5.28±0.13e^{ns}

Values are expressed as mean ±S.D of six animals

Statistical comparisons

a- Group II is compared with group I b-group III is compared with group I

c- Group IV is compared with group 1 d-Group V is compared with group I

e - Group VI compared with group I ns – non significant at 5% level * -$p<0.05$

Liver marker enzymes and biochemical parameters in serum of experimental rats treated *Vitis vinifera L* extract for 90 days were given in the table 6.

As far as the liver marker enzymes and biochemical parameters concerned, no significant alterations in relation to control group appeared on 90 day treatment. Toxicity studies indicated that the methanolic fruit extract of *Vitis vinifera L.* was found to be nontoxic at all the selected dose levels since the serum biochemical parameters are within, its normal levels.

CONCLUSION

The result of this study had demonstrated that the methanolic fruit extract of *Vitis vinifera L* fruit contains significant active metabolites when compared with other extracts. In toxicity

studies the methanolic fruit extract had administrated orally to rats at a highest dose of 2000mg/kg b.w. It did not produce any significant change in autonomic and behavioural response during the observation. Our result also show that there is no significant difference in the body weight, Haematological and biochemical parameters gain in acute toxicity studies in varying doses of administration. It was considered as nontoxic, safe and will be used for future studies.

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