Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

ACUTE ORAL TOXICITY STUDIES OF *VITIS VINIFERA* (GRAPES) PRODUCED BY MICROBIAL FERTIGATION AND FOLIAR SPRAY OF PANCHAGAVYA

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DOI: 10.7897/2277-4572.033147
Published by Moksha Publishing House. Website www.mokshaph.com

Received on: 05/05/14 Revised on: 02/06/14 Accepted on: 16/06/14

ABSTRACT
Acute oral toxicity of organic grapes produced by microbial fertigation and foliar spray of panchagavya was evaluated in albino rats. In the present study, albino wistar rats were administered *Vitis vinifera* fruit extract orally with dosages of 200, 400 and 800 mg Kg^{-1} body weight. Mortality, body weight and haematological parameters were observed for 7 days after treatment. Effect of methanolic extract of *Vitis vinifera* on pylorus ligated rats and HCl – Ethanol induced gastric lesion in rats was studied simultaneously. The methanol extract of *Vitis vinifera* was found to be non toxic and safe as oral intake. The methanol extract of organic grapes was found to possess a protective effect against gastric ulcer and acidity.
Keywords: *Vitis vinifera,* Acuteoral toxicity, organic grapes, microbial fertigation, panchagavya, antiulcer.

INTRODUCTION

Grape (*Vitis vinifera*) belonging to Family Vitaceae is a commercially important fruit crop which has got adapted to sub-tropical climate of peninsular India. Continuous use of pesticide in large quantity has led to accumulation of it in the agricultural crops[1,2]. The problem of pesticide residue (PR) accumulation needs more attention in vegetables and fruits because mostly these are consumed either raw or without much storage time. Due to carcinogenic effect of these pesticides, ethical committee has tightened the rules and regulation for import of grapes. The high use of chemical pesticides, current spray practice and the spray equipment used have led to chemical residues on the fruit, poor control of pests, diseases, increased pressure on the environment and a reluctance of consumers to purchase grapes[3]. In the present global scenario, the view of scientists are directed to use bio-control in the form of fungicides or mineral fertilizers that can be beneficial to control soil borne plant pathogens and produce the natural clear fruits free from mineral residues[4]. To grow grapes economically and to get a high yield of grapes with a good quality, farmers are using lots of (toxic and nontoxic) pesticides. Chronic toxicity studies of Mancozeb usage in viticulture showed impaired thyroid function[5] and birth defects and cancer in experimental animals (U.S. Environmental Protection Agency, 1987). Cypermethrin is a synthetic pyrethroid acting as a neurotoxic agent causing anaphylactoid reactions including bronchospasm, oropharyngeal oedemaand shock[6]. Metalxyl, is a sytemicphenlyamide fungicide that has a tendency to migrate to deeper soil horizons with a potential to contaminate ground water, particularly in soils with low organic matter and clay content[7]. GC MS Studies of grapes has revealed the presence of about 27 pesticides out of 171 pesticides which indicates that the stability of these pesticides is very high or they retain in the grape fruit for a long time after use of them[8]. It is necessary to ensure that such residues was not be found in food or feed at levels presenting an unacceptable risk to humans. Investigation was carried out to evaluate the acute toxicological effect of organic grapes produced by microbial fertigation and foliar spray of Panchagavya. The purpose of the study was to determine safety of organic grapes with antiulcer activity for consumption.

MATERIALS AND METHODS

Plant material

Fresh organic grapes were harvested, rinsed and mixed with dry ice to form frozen grapes. The frozen grapes were ground to grape mixture which was stored in moisture proof container at < 32º F. The above prepared mixture was used for the animal toxicology studies.

Experimental animals

Swiss albino rats (Wistar strain) weighing 120-200 g were procured from King's Institute, Guindy, Chennai, India. All the rats were housed in standard plastic cages with stainless steel overlids and wheat straw as bedding material. Animals were given a week's time for acclimatizing lab conditions before starting the experiment. Rats were fed with standard rat feed from Hindustan lever Ltd, India. The study protocol was approved by the CPCSEA ethical committee.

Experimental grouping of rats and administration of the standardised methanolic extract of *Vitis vinifera*

To study toxicological effect on changes in rats total body weight, four groups of six animals were used. Group I served as the control group and the other groups II, III and IV were maintained as the test groups (200, 400 and 800 mg Kg^{-1} body weight respectively). All the animals were fasted overnight prior to dosing. Before commencing the experiment, the body weight of rats was recorded. The standardised methanolic extract of *Vitis vinifera* were administered as a single dose of 200, 400 and 800 mg Kg^{-1} body weight. The control animals received only vehicle. The organs such as brain, lungs, heart, liver, spleen and kidney were isolated and the weights of the organs were measured compared with the control.

Determination of haematological parameters

To study the toxicological effect on blood parameters, four groups of six animals were used. Group I served as the control group and the other groups II, III and IV were maintained as the test groups (200, 400 and 800 mg Kg^{-1} body weight respectively). On the 8th day, the animals were made to fast overnight and the blood was collected. The haematological parameters (RBC count, Haemoglobin, Haematocrit value and PCV) were determined using an auto analyser.

Effect of methanolic extract of *Vitis vinifera* (grapes) on aspirin plus pylorus ligated rats

The methanolic extract of *Vitis vinifera (*grapes) was pre treated orally to all animals in group III and IV for 5 days. On the day 6, rats were weighed and fasted for 24-36 hours and pylorically ligated. Under anaesthesia, the pyloric end of the stomach was ligated, replaced carefully and the abdominal wall was closed by interrupted sutures. After 4 hours of pyloric ligation, animals were sacrificed and dissection was carried out. The gastric contents were drained into calibrated centrifugal tubes. The parameters such as volume and pH of gastric content, free acidity, total acidity, Ulcer score and ulcer Inhibition (%) were evaluated using the gastric content and exposed stomach[9].

Effect of methanolic extract of *Vitis vinifera* fruits against HCl – Ethanol induced gastric lesion in rats

The methanolic extract of *Vitis vinifera* were pre treated orally to all animals in group III and IV for 5 days. On the day 6, rats were weighed and allowed to starve for 24 hours with free access to drinking water. The test drug (HCl/ethanol mixture (1.5 mL of 0.15 N HCl in 70 % ethanol) was administered through oral route 1 hour before the necrotizing agents (1 ml of absolute alcohol). The absolute alcohol was administrated through oral route. After 1 hour of alcohol administration, animals were sacrificed and the isolated stomach was observed for total number of lesions and % of Ulcer inhibition.

Statistical analysis

All values were expressed as mean ± SEM of six animals. The statistical comparison was determined by means of one way ANOVA test. Values were considered statistically significant at $p < 0.05$[10].

Table 1: Organ weight (g) of rats under the doses of the standardized Methanolic extract of *Vitis vinifera*

Organs(g)	Control	Methanolic extract of *Vitis vinifera* whole fruit (mg/Kg BW)		
		200	400	800
Brain	2.12 ± 1.01	1.98 ± 2.01*	1.92± 1.27	2.29 ±2.11
Lungs	2.13 ± 1.09	2.09 ± 1.01	2.16 ± 2.09	2.82 ± 2.21
Heart	1.61 ±1.02	1.64 ± 2.06	1.28 ± 2.04	1.77± 1.24
Liver	14.12 ± 1.15	13.11 ± 2.45	15.32 ± 3.13	15.31 ± 2.08*
Spleen	1.07 ± 1.01	0.92 ± 1.02	1.02 ± 2.11	1.43 ± 2.12*
Kidney	1.41 ± 1.02	1.28± 1.0*	1.28 ± 2.24	1.58 ± 1.24

Values are expressed as mean ± SEM. n =6. * Significantly different from control, $p < 0.05$

Table 2: Hematological parameters under the doses of the standardised methanolic extract of *Vitis vinifera*

		Hematological parameters			
		RBC (mL/cu.mm.)	Hemoglobin (g/dl)	HCT (%)	PCV (%)
Control	0.5%CMC (200 mg/kg)	6.19 ± 0.37	22.23 ± 0.45	36.23 ± 2.10	43.42 ± 1.55
EVV	P.O/day (200 mg/kg)	21.46 ± 1.01	32.75 ± 2.09	666.50 ± 42.01	9.69 ± 0.82
EVV	P.O/day (400 mg/kg)	20.18 ± 0.79	40.71 ± 1.01	721.50 ± 50.31	11.33 ± 0.97
EVV	P.O/day (800 mg/kg)	20.31 ± 0.80	36.44 ± 1.42	701.40 ± 61.44	11.31 ± 1.14

Data are expressed as Mean ± SEM, n = 6, EVV = Methanolic extract *Vitis vinifera*: RBC: Red blood cell, PCV: Packed cell volume, HCT: Haematocrit value

Table 3: Clinical blood chemistry values of rats in the subchronic toxicity test of standardised methanolic extract of *Vitis vinifera*

Parameters	Control	Standardized methanolic extract of grapes (mg/kg)			
		200	400	800	2000
Glucose (mg/dl)	121.32 ± 1.31	123.01 ± 2.23	126.25 ± 2.20	126.63 ± 2.32	130.12 ± 2.66
Urea (mg/dl)	4.31 ± 0.68	4.21 ±0.65	5.06 ± 0.47	5.03 ± 0.86	5.05 ± 0.78
Creatinine (mg/dl)	0.42 ± 0.05	0.41 ± 0.10	0.41 ± 0.06	0.42 ± 0.05	0.41 ± 0.42
Total protein (g/dl)	5.65 0.21	5.42 ± 0.13	5.67 0.87	5.64 ± 0.36	6.16 ± 0.11
Albumin (g/dl)	2.99 ± 0.16	3.53 ± 0.06	3.43 ± 0.08	3.56 ± 0.10	4.54 ± 0.03
Total bilirubin (mg/dl)	0.14 ± 0.00	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.03	0.12 ± 0.02
Direct bilirubin (mg/dl)	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	0.031 ± 0.01
SGOT/AST (U/l)	62.71 ± 2.51	62.47 ± 4.24	65.53 ± 3.47	63.00 ± 3.23	63.73 ± 4.72
SGPT/ALT (U/l)	39.78 ± 1.54	38.61 ± 2.24	39.46 ± 2.32	40.12 ± 3.24	43.32 ± 2.36
ALP (U/l)	41.20 ± 1.76	40.94 ± 1.72	42.56 ± 1.61	40.58 ± 1.71	43.21 ± 1.63
Cholesterol (mmol/L)	0.86 ± 0.02	1.02 ± 0.23	1.01 ± 0.31	1.07 ± 0.36	1.12 ± 0.62

Values are expressed as mean ± SEM., n= 6

Table 4: Effect of methanol extract of *Vitis vinifera* against HCl – Ethanol induced gastric lesion in mice (n = 6)

Treatment	Dose (mg/kg b.wt)	Gastric lesion	Ulcer inhibition (%)
Control	1 % SCMC	21.55 ± 2.809	
Sucralfate	100	1.017 ± 0.512	91.85
EVV (100 mg/kg)	100	1.8 ± 4.46	82.71
EVV (200 mg/kg)	200	1.5 ± 3.54	86.56

Table 5: Effect of methanolic extract of *Vitis vinifera* grapes on gastric secretion, acidity, pH and ulcer score in pylorus ligated rats

Treatment	Volume of gastric acid secretion (ml/100 g)	Free acidity (mEq/l/100 g)	Total acidity (mEq/l/100 g)	pH	Ulcer score	Ulcer inhibition (%)
Vehicle control	2.633 ± 0.642	225.00 ± 6.124	555.00 ± 7.55	2.200 ± 0.163	3.600 ± 0.200	
EVV 100 mg/kg	0.953 ± 0.073	134.00 ± 10.882	542.50 ± 9.714	2.13 ± 0.210	1.567 ± 0.207**	51.61
EVV 200 mg/kg	1.003 ± 0.083	137.00 ± 10.782	522.50 ± 11.124	2.43 ± 0.250	1.267 ± 0.301	57.69

RESULTS AND DISCUSSION

In acute toxicity study, *Vitis vinifera* fruits produced by means of organic farming, at a single dose of 2000 mg/Kg did not show any toxicity signs which suggests that *Vitis vinifera* was practically nontoxic after an acute exposure in rats. Administration of methanolic extract did not show detectable abnormalities in organ weight, haematological parameters, clinical parameters.

Organ weight

Organ weight is an important index of physiological and pathological status in man and animals. The heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicants[11]. The weight of lungs, Heart and Kidney was almost same in control and treated groups. The liver weight is slightly increased in treated groups as compared to control. The organ weight of other organs like brain and spleen showed a similar trend like that of liver. Administration of methanolic extract of *Vitis vinifera* did not show any effect on organ weight of all important organs (Table 1). In addition, gross examination of internal organs of all rats revealed no detectable abnormalities. Thus, it can be suggested that the methanolic extract of *Vitis vinifera* is virtually nontoxic.

Haematological parameters

Blood parameters analysis is relevant to risk evaluation as the haematological system has a higher predictive value for toxicity in humans (91 %) when assay involve rodents non-rodents[12]. Blood is an important index of physiological and pathological status in man and animals and parameters usually index of physiological and pathological status in man and animals and the parameters usually measured are haemoglobin, total red blood cell (RBC), leukocyte (WBC), PCV, MCV, MCH, and MCHC[13]. The normal range of these parameters can be altered by the intake of some toxic plants[14]. The RBC level and haematocrit value of treated groups was markedly increased when compared with control. The haemoglobin level was found to be increased than the control. The PCV % recorded a decrease in values when compared with control. However, these increased or decreased levels were statistically non significant (Table 2). All the parameters of haematological analysis were statistically not significant when compared with the control group, discarding the possibility of anemia or disturbances in the erythrocytes or haemoglobin production.

Blood clinical parameters

The blood clinical data showed a slight increase in blood glucose level which was statistically insignificant and other blood clinical parameters also did not show any significant changes in their values (Table 3). Haemoglobin showed Positive increase corresponding to the increase of dosage.

Antiulcer activity

The methanol extract of *Vitis vinifera* (EVV) recorded higher ulcer inhibition rate against HCl- Ethanol induced gastric lesion when compared with control. It had been reported that ulceration via increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa caused by pylorus ligation[15]. Similar results showed consistence with increased gastric acidity and ulcer[16]. The results of the present investigation showed that the test extracts (EVV) have an effective anti secretory and antiulcer activity against pyloric ligation induced gastric ulcer in rats (Table 4). Ulcer induced by aspirin plus ligated rats, pre treated with EVV showed reduction in gastricacid secretion, free acidity, total acidity, ulcer score and increase in pH and ulcer inhibition rate when compared with control.

CONCLUSION

The methanol extract of *Vitis vinifera* was found to be nontoxic, when oral acute toxicity study in rats was performed. The findings are in agreement with the previous reports[17]. The results of the present study showed that the test extracts showed an effective anti secretory and antiulcer activity against pyloric ligation-induced gastric ulcer in rat. There was no significant change of clinical blood values which falls within the normal range[18]. Haemoglobin increase due to intake of organic grapes is noteworthy. The present study emphasizes that organic grapes produced by microbial fertigation are non-toxic and safe to consume and also they have a protective effect against gastric ulcer, anemia and acidity.

REFERENCES

1. Andersen JH. Results from the monitoring of pesticide residues in fruit and vegetables in the Danish market (1998-99). Food Addit. Contam 2001; 18: 906-931. http://dx.doi.org/10.1080/02652030110054759
2. Dogehim SM. Monitoring pesticide residues in Egyptian fruits and vegetables during 1996. Journal of AOAC Int. (Eds. Gad-Alla SA and Ei Marsafy AM) 2001; 84(2): 519.
3. Khan SA, Mulvaney RL, Ellsworth TR, Boast CW. The myth of nitrogen fertilization for soil carbon sequestration. Journal of Environmental Quality 2007; 36: 1821-1832. http://dx. doi.org/10.2134/jeq2007.0099
4. Russo RO, Berlyn GP. The use of organic bio stimulants to help low input sustainable agriculture. J. Sustainable Agric 1990; 1(2): 19-42. http://dx.doi.org/10.1300/J064v01n02_04
5. Edwards IR, Ferry DG, Temple WA. Fungicides and related compounds. Handbook of Pesticide Toxicology. Hayes WJ and Laws ER, Eds. Academic Press, New York; 1991. p. 4-2.
6. Wax PM, Hoffman RS. Fatality associated with inhalation of a pyrethrin shampoo. ClinTox 1994; 32(4): 457-460.
7. Sukul P, Spiteller M. Metalaxyl: persistence, degradation, metabolism and analytical methods. Rev. Environ. Contam. Toxicol 2000; 164: 1-26.
8. Mukesh Kumar Raikwar, Vikas Bhardvaj, Urmila Sawant, Virendra Vora. Study of Contamination Level of Pesticide Residues in Grapes (*Vitis vinifera*) in Maharashtra. The Green Pages; 2011.
9. Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 1945; 5: 43–61.
10. Anadan R, Reckha RD, Saravanan N, Devaki T. Protective effects of *Picrorrhiza lauroa* against HCl/ethanol-induced ulceration in rats. Fitoterapia 1998; 70(5): 498–501. http://dx.doi.org/10.1016/S0367-326X(99)00081-7
11. Dybing E, Doe J, Kleiner J, O Brien J. Hazard characterization of chemicals in food and diet: dose response, mechanism, extrapolation issues. Food Chem. Toxicol 2002; 42: 237-282. http://dx. doi.org/10.1016/S0278-6915(01)00115-6
12. Olson H, Betton G, Robison D, Thomas K, Monro A. Concordance of toxicity of pharmaceuticals in humans and in animals. Regul. Toxicol. Pharmacol 2000; 32: 56-67. http://dx.doi.org/10.1006/rtph.2000.1399

13. Schalm OW, Jain NC, Carrol EJ. Veterinary Haematology. 3rd ed. Lea and Febiger Publication, Philadelphia; 1975. p. 807-809.
14. Ajagbonna OP, Onifade KI, Suleiman U. Haematological and biochemical changes in rats given extract of *Calotropis procera*. Sokoto J Vet Sciences 1999; 1: 36–42.
15. Jainu M, Devi CS. Anti ulcerogenic and ulcer healing effects of *Solanum nigrum* (L) on experimental ulcer models- Possible mechanism for the inhibition of acid formation. J. Ethnopharmacol 2006; 104(1-2): 156-163. http://dx.doi.org/10.1016/j.jep.2005.08.064
16. Sood S, Muthuraman A. Activity of tacrolimus: An immunosuppressant, in pyloric ligation-induced peptic ulcer in the rat. Yakugaku Zasshi 2009; 129(12): 1523-1528. http://dx.doi.org/10.1248/yakushi.129.1523
17. Kim DC, Kim SH, Choi BH, Baek NI, Kim D, Kim MJ, Kim KT. *Curcuma longa* extract protects against gastric ulcers by blocking H2 histamine receptors. Biol. Pharm. Bull 2005; 28(12): 2220-2224. http://dx.doi.org/10.1248/bpb.28.2220
18. Angkhasirisap W, Inala P, Sirimontaporn A, Inpunkaew R, Rungrojejinda K, Kengkoom K, Ratanasak W, Buripadi Lawson D. Blood chemistry profiles of outbred Sprague-Dawley rat in the Facility of National Laboratory Animal Centre. 28th Congress on Science and Technology of Thailand; 2002.

Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
S. Geetha and Aruna Devaraj. Acute oral toxicity studies of *Vitis vinifera* (Grapes) produced by microbial fertigation and foliar spray of Panchagavya. J Pharm Sci Innov. 2014;3(3):245-248 http://dx.doi.org/10.7897/2277-4572.033147