



## Research Paper

# ***In vivo* antioxidant activities of green and black grape (*Vitis vinifera* L.) berries against DMBA/Croton oil induced oxidative stress in mice**

Accepted 21<sup>st</sup> November, 2021

### ABSTRACT

Grape's antioxidants are associated with anti-inflammatory activity which is apparently related with chemo preventive actions. Since grapes come in various colours and varieties, it becomes essential to know which one is more beneficial and what kind of benefits it accounts for. Current research was carried out for evaluating *in vivo* antioxidant activities of green and black grape berries (*Vitis vinifera* L.) against DMBA/Croton oil induced oxidative stress. The effect of methanolic green and black grapes separately and in combination was evaluated to modulate oxidative stress in swiss albino mice through lipid peroxidase test (LPO), superoxide dismutase (SOD) method and reduced glutathione (GSH) estimation. Swiss albino mice were divided into five experimental groups according to their treatment procedures. According to the result, black grape methanolic extract was potent in preventing the damage caused due to oxidative stress followed by green + black grape methanolic extract and green grape methanolic extract as compared to control group.

Meenal Sharma<sup>1\*</sup> and Ritu Thakur Bais<sup>2</sup>

<sup>1</sup>Research Scholar, Department of Microbiology, Barkatullah University, Bhopal (M.P.)-462026, India

<sup>2</sup>Professor, Department of Botany, Govt. MLB Girls PG (Auto) college, Bhopal (M.P.)-462010, India

\*Corresponding author. E-mail: msmeenalsharma88@gmail.com

**Key words:** *Vitis vinifera* L., chemoprevention, *in vivo* test, antioxidant activity, swiss albino mice, DMBA/Croton oil, oxidative stress, green grapes, black grapes, methanolic extract, LPO, SOD, GSH.

**Abbreviations:** DMBA, 7,12-Dimethylbenz[a]anthracene; LPO, Lipid Peroxidase test; SOD, Superoxide Dismutase; GSH, Reduced Glutathione Estimation; CPCSEA, Control and Supervision of Experiments on Animals; GGME, Green Grape Methanolic Extract; BGME, Black Grape Methanolic Extract.

## INTRODUCTION

Researchers and scientists have acknowledged various natural antioxidative compounds capable to shield cellular components from oxidative damage and avoid diseases (Gülçin, 2010). It has been proved that berry, leaves, and roots of several medicinal and aromatic plants biosynthesize natural phytochemicals comprising antioxidant properties (Yu et al., 2005). *Vitis vinifera* L. is a member of family Vitaceae, innate to Mediterranean region, central Europe and south western Asia. It is consumed raw or processed to make wine or juice and dried to produce raisins. Its fruit is classified as 'berry', since it stays connected with stem. Grape berry is amongst chief edible fruits having numerous nutritional and medicinal

properties which can be used for the screening of novel medicines and various industrial products. *Vitis vinifera* L. skins and seeds enfold abundant quantity of polyphenolic compounds, mainly monomeric catechin, epicatechin, gallic acid, polymeric and oligomeric procyanidins (Monagas et al., 2003). Grapes are mainly rich in polyphenols which includes simple phenolics, flavonoids, anthocyanins, stilbenes, pro anthocyanins, and vitamin. Flavonoids and stilbenes, well known for their antioxidative effects are produced in large amounts in grape berry (Ali et al., 2010). It has been proven in previous studies that grape seed procyanidins possess potent free radical scavenger ability (Da Silva et al., 1991).

Grape seed extract is documented to possess *in vivo* antioxidant property and it is as efficient as vitamin E in preventing diseases caused by oxidative stress by inhibiting the production of free radicals (Bouhamidi, 1998). Oxidative stress is created by reactive oxygen species that are continuously formed in human body. Sometimes, natural (endogenous) antioxidant defence system is not able to completely neutralize the adverse effects of oxidative stress which may lead to oxidative damage of DNA, lipids and proteins. Cancer and various neurodegenerative diseases are a resultant of such oxidative damages. So, consumption of antioxidant rich diet can be an important step in protection against such chronic diseases (Halliwell, 1996). In recent years, there has been a marked increase in consumption of natural products among large amount of population which can be attributed to their lesser side effects. Although various studies have been conducted for evaluating *in vitro* and *in vivo* antioxidant properties of *Vitis vinifera* L., but literature lacks comparative and combinational studies of its different coloured varieties. So, present study evaluates the effect of methanolic green and black grapes singly and in combination against DMBA/Croton oil induced oxidative stress in swiss albino mice through lipid peroxidase test (LPO), superoxide dismutase (SOD) method and reduced glutathione (GSH) estimation.

## MATERIALS AND METHODS

### Collection of sample

Fresh green and black grape berries (samples) were collected from Ganesh grape garden, Nashik, Maharashtra (India). Collected samples were taken to laboratory for further processing. Samples were thoroughly washed with double distilled sterile water. Extraction was done by using maceration process (Das K, 2010).

### Extraction of sample through maceration process

500 grams of undamaged and disease-free samples were grounded and dipped in 50% methanol. The mixture was left for four days with occasional shaking or stirring. The extract was taken out and allowed to dry in oven. This process was repeated with the left-over grounded residue of berries until the solvent runs clear.

### Experimental animals

Female Swiss albino mice within the age group of 5-6 weeks, weighing 20-25 gm were grouped and kept in plastic cages with stainless steel wire lid and there were 6

mice per cage. Temperature of the room was maintained up to 30°C. Experiments were conducted in accordance with the guidelines set by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The experimental protocols were approved by the Institutional Animal Ethics Committee of Pinnacle Biomedical Research Institute (PBRI), Bhopal, India (Reg. No. 1824/PO/Ere/S/15/CPCSEA).

### Treatment groups

Female mice were divided into five different groups with six mice in each group as following:

**Group 1:** Mice belonging to group 1 were orally administered methanolic extracts of green grapes daily from third week up to the end of study that is, sixteen week post topical treatment with DMBA and dose of mice was recalculated every week after observing their weight.

**Group 2:** Mice belonging to group 2 were orally administered methanolic extracts of black grapes daily from third week up to the end of study that is, sixteen week post topical treatment with DMBA and dose of mice was recalculated every week after observing their weight.

**Group 3:** Mice belonging to group 3 were orally administered with methanolic extracts of both green grapes and black grapes combined in 50/50 ratio daily from third week up to the end of study that is, sixteen week post topical treatment with DMBA and dose of mice was recalculated every week after observing their weight.

**Group 4 (Positive control):** Mice of this group were administered with normal saline daily from third week post topical treatment with DMBA.

**Group 5 (Negative control):** Mice of this group were administered with double distilled water with normal feed daily till the end of study.

### *In Vivo* biochemical tests for oxidative stress

Biochemical tests of the affected skin were carried out before their histological analysis to determine oxidative stress. Mice were sacrificed group wise by cervical dislocation. The dorsal skin affected by DMBA/Croton oil was quickly excised and washed thoroughly with chilled saline. It was then weighed and blotted dry. After that following biochemical tests were carried out:

### *Lipid peroxidase method (lpo)*

Lipid peroxidation can be defined as oxidative degradation

of lipids. In this process free radicals take electrons from lipids in cell membrane resulting in damage. Lipid peroxidase was determined using the method of Okhawa (1979). 10 % w/v tissue homogenate in 0.15 M Tris HCl buffer (pH 7.4) was prepared. Tissue sample of skin obtained from DMBA/Croton oil induced oxidative stress was weighed and added with 10% (w/v) of 0.15 M tris HCl buffer. Each sample mixture was homogenized under chilled condition using a motor -driven Glass-Col® homogenizer and the supernatant tissue homogenate was collected. 0.2 ml tissue homogenate + 0.2 ml 8.1 % SDS + 1.5 ml 20 % acetic acid + 1.5 ml 8 % TBA was taken and with 4 ml double distilled sterile water volume was make up. Mixture was heated on water bath (95°C) for 60 min using glass ball as condenser. The mixture was cool and with 5 ml of water volume was make up. 5 ml of butanol: pyridine (15:1) was added to the mixture. The mixture was vortexed for 2 min and centrifuged at 3000 rpm for 10 min. Upper organic layer was separated and its Optical density at 532 nm was measured. Butanol: pyridine (15:1) was used as blank. Interpretation was based on standard curve of MDA.

#### **Superoxide dismutase method (SOD)**

One unit of SOD activity is defined as that amount of enzyme required to inhibit the reduction of nitro blue tetrazolium (NBT) by 50% under the specified conditions. The percent inhibition of NBT reduction can be used to quantify superoxide-scavenging. Superoxide dismutase was determined using the method of Sapakal et al. (2008). 10% w/v tissue homogenate in 0.15 M Tris HCl or 0.1 M phosphate buffer was prepared in which tissue samples of skin obtained from DMBA/Croton oil induced oxidative stress was weighed and added with 10% (w/v) of 0.15 M tris HCl buffer. Each sample mixture was homogenized under chilled conditions using a motor-driven Glass-Col® homogenizer and the supernatant tissue homogenate was collected. The homogenates were centrifuged at 15000 rpm for 15 min at 4°C. 0.1 ml of supernatant (sample) was taken and mixed with 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.052 M) + 0.1 ml phenazine methosulphate (186 µM) + 0.3 ml of 300 µM Nitroblutetrazolium + 0.2 ml NADH (750 µM). 0.1 ml of the mixture was taken and incubated at 30°C for 90 sec. 0.1 ml glacial acetic acid was added to the mixture. The mixture was stirred with 4.0 ml n-butanol and allowed to stand for 10 min. The mixture was centrifuged and butanol layer was separated. Optical density was measured spectrophotometrically at 560 nm. Butanol was taken as blank. Absorbance values were compared with a standard curve generated from known SOD. Interpretation was based on % Inhibition.

$$\% \text{ Inhibition} = \frac{(\Delta \text{ Absorbance control} - \Delta \text{ Absorbance sample})}{\Delta \text{ Absorbance control}} \times 100$$

#### **Reduced glutathione estimation (GSH)**

GSH is an intra-cellular reductant and plays major role in catalysis, metabolism and transport. Reduced Glutathione was determined by using the method described by Ellman (1959). 10% w/v tissue homogenate (0.1 M phosphate buffer, 7.4 pH) was prepared in which tissue sample of skin obtained from DMBA/Croton oil induced oxidative stress was weighed and added with 10% (w/v) of 0.1 M phosphate buffer (pH 7.4). Each sample mixture was homogenized under chilled condition using a motor-driven Glass-Col® homogenizer. Tissue homogenate was collected. 20 % TCA and 1 mM EDTA was added to 0.2 ml of homogenate. The homogenate was set aside for 5 min. Then the homogenate was centrifuged for 10 min at 2000 rpm. 200 µl supernatant was taken and transferred to a new tube. 1.8 ml of Ellman's reagent (5,5'- dithio bis-2-nitrobenzoic acid (0.1 mM) prepared in 0.3 M phosphate buffer, pH 7 with 1 % sodium citrate solution) was added to the supernatant. 2 ml of distilled water was used to make up volume. Optical density was measured at 412 nm and water was taken as blank.

#### **Statistical analysis**

The experiments were repeated three times and the average values were recorded with standard deviation.

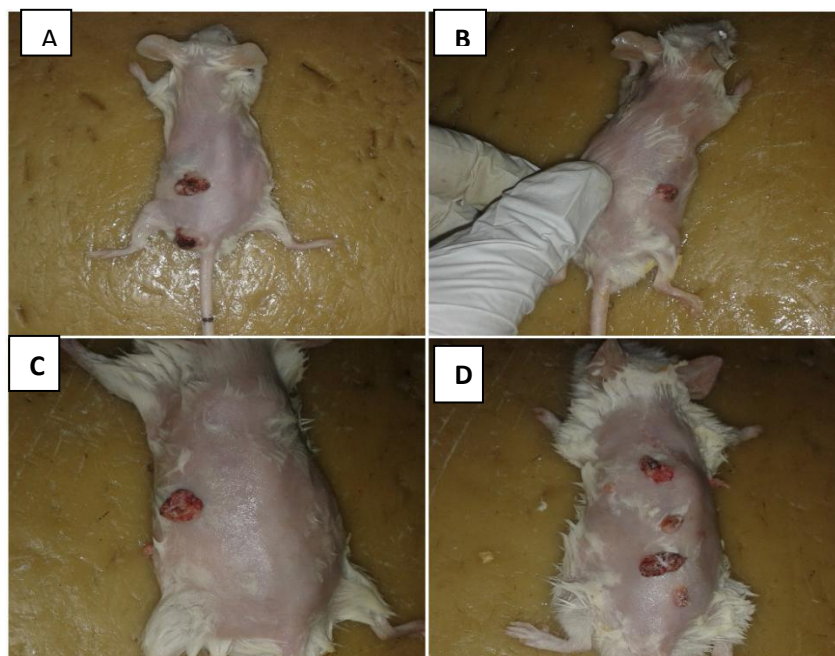
#### **RESULT AND DISCUSSION**

The result of oxidative stress is summarised in Table 1. LPO of green grape methanolic extract, black grape methanolic extract and green + black grape methanolic extracts was recorded as  $56.8 \pm 2.5$  nM MDA/gm wet tissue,  $36.1 \pm 2.8$  nM MDA/gm wet tissue and  $48.25 \pm 4.2$  nM MDA/gm wet tissue respectively. Similarly, SOD of green grape methanolic extract, black grape methanolic extract and green + black grape methanolic extracts was observed as  $94.7 \pm 12.1$  U/gm wet tissue,  $165.2 \pm 12.4$  U/gm wet tissue and  $122.6 \pm 10.1$  U/gm wet tissue and GSH of green grape methanolic extract, black grape methanolic extract and green + black grape methanolic extracts was found to be  $0.6 \pm 0.01$  nmol/gm wet tissue,  $0.9 \pm 0.04$  nmol/gm wet tissue and  $0.7 \pm 0.03$  nmol/gm wet tissue respectively. Whereas LPO, SOD and GSH of positive control group was recorded as  $74.4 \pm 3.9$  nM MDA/gm wet tissue,  $54.1 \pm 6.5$  U/gm wet tissue and  $0.4 \pm 0.02$  nmol/gm respectively. The obtained data shows that grapes produced protection by decreasing the activity of lipid peroxidation and at the same time increase the levels of GSH and SOD. Result of present study can be correlated with the studies of BVS et al. (2014) and Almajwal and Elsadek (2015). Black grapes are known to improve damages caused by oxidative stress.

**Table 1:** Effect of green and black grapes (methanolic extracts) oral supplementation on *in vivo* oxidative stress in skin tissues of mice [GGME: Green grape methanolic extract & BGME: Black grape methanolic extract].

GROUPS	LPO (nM MDA/gm wet tissue)	SOD (U/gm wet tissue)	GSH (nmol/gm wet tissue)
GGME (I)	56.8 ± 2.5	94.7 ± 12.1	0.6 ± 0.01
BGME (II)	36.1 ± 2.8	165.2 ± 12.4	0.9 ± 0.04
GGME+BGME (III)	48.25 ± 4.2	122.6 ± 10.1	0.7 ± 0.03
CONTROL (IV)	74.4 ± 3.9	54.1 ± 6.5	0.4 ± 0.02

Data represents mean ± standard deviation (S.D.)



**Figure 1:** Pictures of mice from different groups where A represent group I, B represents group II, C represents group III & D represents group IV

In the present work, administration of black grape extract in mice, lowered down lipid peroxidation, increased antioxidative defence and levels of SOD and GSH. Presence of flavonoids, tannins and saponins in grapes leads to inhibition of lipid absorption. Dietary intake of grape seed is documented to be effective against alcohol-induced liver injury and oxidative stress. Thus, it helps in bringing MDA levels back to normal. In the present work antioxidant properties of green grape methanolic extract (GGME), black grape methanolic extract (BGME) and green + black grape methanolic extract (GGME + BGME) against oxidative stress in swiss albino mice was evaluated. The result suggests that black grape methanolic extract is potent in preventing the damage caused due to oxidative stress followed by green + black grape methanolic extract and green grape methanolic extract as compared to control group. The combination of

green grape and black grape methanolic extract was found more effective in inhibiting oxidative stress as compared to green grape methanolic extract alone which reveals that mixture of green and black grape methanolic extract synergistically enhance *in vivo* antioxidant activities. In control group, increased level of lipid peroxidation and decreased levels of GSH and SOD resulted from an elevation of reactive oxygen species caused due to stress condition in the mice with DMBA/Croton oil intoxication. Figure 1 shows the pictures of mice from different groups.

## CONCLUSION

It can be concluded that the antioxidant properties of both black grapes and green grapes suppress superoxide anion

and lipid peroxidation. The mixture of green and black grape methanolic extract synergistically enhances *in vivo* antioxidant activities. Both green and black grapes have been reported having amplified antioxidant properties. They help in reducing aging, recover skin and have huge amounts of fibre for digestion and vitamins to aid with immunity and strength. More studies involving the combination of various grape species can be helpful in finding out their effect in combating various types of cancer and other dreadful diseases.

## ACKNOWLEDGEMENTS

We are grateful to Dr. Amit Nayak, Pinnacle Biomedical Research Institute, Bhopal-462001 for his valuable instruction while performing animal studies. Part of this study involving animal studies was performed at Pinnacle Biomedical Research Institute, Bhopal approved by Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

## REFERENCES

- Ali K, Maltese F, Choi Y, Verpoorte R (2010). Metabolic constituents of grape vine and grape-derived products. *Phytochem. Rev.* 9(3): 357–378.
- Almajwal AM, Elsadek MF (2015). Lipid-lowering and hepatoprotective effects of *Vitisvinifera* dried seeds on paracetamol-induced hepatotoxicity in rats. *Nutr. Res. Pract.* 9(1): 37-42.
- Bouhamidi R, Prévost V, Nouvelot A (1998). High protection by grape seed proanthocyanidins (GSPC) of polyunsaturated fatty acids against UV-C induced peroxidation. *C R Acad. Sci.* 321(1): 31–38.
- Das K, Tiwari RKS, Shrivastava DK (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *J. Med. Plants Res.* 4 (2): 104-111.
- DaSilva JMR, Darmon N, Fernandez Y, Mitjavila S (1991). Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *J. Agric. Food Chem.* 39(9): 1549–1552.
- Ellman GL (1959). Tissue Sulfhydryl Groups. *Arch. Biochem. Biophys.* 82(1): 70-77.
- Gülçinİ (2010). Antioxidant properties of resveratrol: a structure– activity insight. *Innov. Food Sci. Emerg. Technol.* 11(1): 210–218.
- Halliwell B (1996). Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radic. Res.* 25(1): 57–74.
- Lakshmi BVS, Sudhakar M, Aparna M (2014). Attenuating effect of black grapes on cadmiuminduced nephrotoxicity in rats-A preliminary study. *Int. J. Adv. Pharm. Med.Bio. Sci.* 2(1): 25-29.
- Monagas M, Cordovés GC, Bartolomé B, Laureano O, Ricardo da Silva JM (2003). Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* 51(22): 6475–6481.
- Ohkawa H, Onishi N, Yagi K,(1979). Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Anal.Biochem.* 95(2): 351–358.
- Sapakal VD, Shikalgar TS, Ghadge RV, Adnaik RS, Naikwade NS, Magdum CS (2008). *In Vivo* Screening of Antioxidant Profile: A Review. *J. Herb. Med. Toxic.* 2(2): 1-8.
- Yu LL, ZhouK K, Parry J (2005). Antioxidant properties of cold pressed black caraway, carrot, cranberry, and hemp seed oils. *Food Chem.* 91(4):723–729.

### Cite this article as:

Sharma M, Bais RT (2021). *In vivo* antioxidant activities of green and black grape (*Vitis vinifera* L.) berries against DMBA/Croton oil induced oxidative stress in mice. *Acad. J. Med. Plants.* 9(12): 180-184.

### Submit your manuscript at:

<https://www.academiapublishing.org/journals/ajmp/>