

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.510.072>

Effect of Aqueous Extract of *Annona muricata* (Graviola Tea) on the Oxidative Stress produced by the Artificial UV Source *in Vivo* and *in Vitro*

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ABSTRACT

Keywords

Blood samples, graviola tea, oxidative stress, rats, UV exposure.

Article Info

Accepted:
20 September 2016
Available Online:
10 October 2016

The prime purpose of the study was to investigate the effects of Graviola tea on the oxidative stress induced by the ultraviolet light. In the *in vivo* part, the rats were divided into the three groups of six animals each. The 1st group in which healthy animals (Control), 2nd group in which only UV treatment was given to the animals (Non-treated), 3th group in which Graviola tea was given after UV exposure to the animals (Treated) and for *in vitro* part, blood samples were collected group wise i.e. i) Control which contains blood sample of healthy individuals, ii) Non treated group in which blood samples were exposed to UV radiation for different time intervals and in the iii) Treated group blood sample were exposed to UV radiation and subsequently treated with graviola tea for different time intervals under aseptic conditions. Then the biochemical assays like Lipid Peroxidation, Protein Carbonyl Oxidation, Superoxide Dismutase, Catalase, Glutathione Peroxidase were performed and positive results were obtained.

Introduction

The primary source of the ultraviolet radiation is the solar radiation for all the living system. UV rays have the range of 100-400 nm. UV radiation can be divided into three categories based on the wavelength I) UV A: 315-400 nm, II) UV B: 280- 315 nm, III) UV C: 100-280 nm as said by International Commission on Illumination (Duthie *et al.*, 1999; Clydesdale *et al.*, 2001). Ultraviolet radiation has a few helpful health effects like use in the treatment of vitiligo along with the combination of drugs and helps in vitamin D₃ formation. Ultraviolet radiation

plays a vital role in the initiation of some pathological conditions in skin. Ultraviolet radiation is considered the major cause of erythema, pigmentation, immuno-suppression and delayed effects are skin aging, DNA damage and skin cancer (Kligman, 1989; Matsui and Leo, 1991; Hussein, 2005). Ozone layer absorbs UV radiation up to 310 nm. Thus it absorbs all UV C and most of the UV B but does not absorb UV A (Gruijl and Van der Leun, 2000). In the skin because of the reaction of UV photons with endogenous photosensitizers more of Reactive Oxygen

Species (ROS) is produced which may disturb the antioxidant protective mechanisms ending in the antioxidant/ pro-oxidant disequilibrium known as oxidative stress (Heck *et al.*, 2004).

ROS are inseparable and natural component of metabolism. Fibroblasts and keratinocytes constantly generate ROS in the skin and swiftly removed by enzymic antioxidant like glutathione reductase, superoxide dismutase, glutathione Peroxidase, catalase and non enzymic antioxidants like glutathione, tocopherol, ascorbic acid and ubiquinol that sustain the anti-oxidant/ pro-oxidant balance, thus resulting in tissue and cell stabilization (Sander *et al.*, 2004; Inal *et al.*, 2001). As UV A is having long wavelength compared to UV B and UV C, UV A can penetrate much deep in the skin. In the humans UV A is more vital to cause skin inflammation than UV B (Nishigori *et al.*, 2004). Oxidative damage to the DNA can be increased as inflammatory cells generate ROS (Halliday, 2005). UV A has a bigger influence on oxidative stress than UV B in the skin as UV A can induce RNS/ROS which can damage lipids, proteins and DNA and also lead to depletion of NADH and hence loss of energy from the cell (Halliday, 2005).

Annona muricata Linn is commonly known as soursop or graviola, belongs to the family of Annonaceae. It is a tropical tree and native of Central America. The leaves are dark green in color and glossy that had been conventionally used to treat asthma, headaches, cough, hypertension, and used as sedative, antispasmodic and anti-fungal activity (Taylor, 2002). The phytochemicals found in *Annona muricata* are flavonoids, alkaloids, tannins, saponins, terpenoids, phytosterol, cardiac glycosides, proteins and carbohydrates and annonaceous acetogenins.

Materials and Methods

The study was divided into two parts of *in vivo* and *in vitro*.

In vivo part

The study was aimed to check the effect of Graviola tea on the oxidative stress induced rats.

Animal care and Monitoring

The present study was carried out on healthy adult male and female rats of Albino Wistar strains weighing of approximately 150-200gm obtained from inbred colony maintain in animal house of Sardar Patel University where rats were housed 1 per cage in polypropylene cages that were fitted with stainless steel wire mesh bottom under standard laboratory condition $25\pm 5^{\circ}\text{C}$ temperature, $50\pm 15\%$ relative humidity and 12 ± 3 hr dark and light photoperiod. The animals were given standard food pellets and water ad libitum throughout the period of experiment. The animals used in present study were maintained and handled according to the University and Institutional Legislation, regulated by the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics committee (IAEC) of Sardar Patel University, V.V.Nagar, Anand, Guajrat. The experimental protocol was approved by the IAEC.

Animals were randomized into four groups of six rats each:

Group I: No treatment given. Healthy (Control)

Group II: Animals were given UV exposure for 21 days. (Non-treated)

Group III: Animals were given UV exposure for 21 days and then given Graviola extract of 3ml for 15 days. (Three times in a day) (Treated)

Irradiation treatment

Rats were shaved in a 6 x 8 cm² of dorsal area. Any damage to the animal skin was carefully avoided. The UV exposure was given for 1 hour. The ultraviolet light was used with a wave length starting from 290 to 315 nm, at a distance of 5 cm from the skin.

Equipment

Ultraviolet lamps from Blackwood present a 220 – 230 V connection. UV lamps were fitted into the spot light dome and the according to that stands were made. The cages were made from stainless steel grill.

Sample preparation for *in vivo* study

The skin tissues were minced with the help of a scissors and homogenized in ice cold 50mM phosphate buffers (pH-7.4) by potter and elvejem type homogenizer equipped with a Teflon pestle (Remi motor, Bombay) to obtain homogenate. The debris was removed by centrifuge at 10,000rpm for 15minutes at 4°C in cooling centrifuge (Hettich Zentrifuge, Germany). The clear supernatant was used for all the biochemical parameters of oxidative stress.

***In Vitro* part**

5ml of blood from the normal healthy males aged between 25-30 years was collected and then various biochemical test were carried out and those who have normal range of oxidation only those blood samples were

considered as samples. A total of 30 blood samples were considered. From those samples 500µl of blood was distributed evenly in small petri plates. Two sets were made named treated and untreated. The UV radiation was given to the timing of 3 minutes, 6 minutes, 9 minutes and 12 minutes in duplicates under aseptic conditions. The 125 W black bulb was used as the radiation source at the distance of 5 cm. The luminance flux measured using the Lux meter was 205±3 Lux or Lumen/m². After UV exposure treated set was cultured with 8ml of RPMI- 1640 media and 1 ml of graviola tea. The untreated set was cultured with 8ml of RPMI- 1640 media only. For control, no irradiation was applied to the blood sample and was directly added to the culture media.

Cell Culture

All the samples were cultured for 48 hours in the incubator. . The medium has PHA-M, streptomycin and penicillin. Culture conditions were maintained at 37°C using incubator.

Sample Preparation for *in vitro* study

After 48 hours of culturing, the cells were harvested by centrifuging the culture tubes at 2000 rpm for 20 minutes. The supernatant was discarded and same volume of hypotonic solution of KCl (0.5% KCl) was added and gently mixed with the pellet of cells followed by incubation of 20 minutes at 37°C. The cell lysate thus obtained was used as sample for all the biochemical assays performed. All the assays were performed in triplicates.

Collection of the plant samples

Plant sample (*Annona muricata*) was collected from a nursery in Bangalore,

Karnataka. The leaves and stems were washed with distilled water. The leaf and small pieces of stems were dried in the shade.

Graviola tea procedure

In the glass bowl take 1 liter of distilled water and then add 30 graviola dried leaves and add 4 small pieces of stem and start boiling the water. Then boil water until 900ml of water remains in the bowl. Cool it.

Biochemical assays

In this study, the following biochemical tests were performed as per standard operating procedure.

Lipid Peroxidation

The Thiobarbituric Acid Reactive Substances (TBARS) as a product of unsaturated fatty acid in the tissue was measured according to method described by (Ohkawa *et al.*, 1979).

Glutathione Peroxidase

The glutathione peroxidase activity was checked by the method of (Rotruck *et al.*, 1973)

Protein Oxidation

Determination from protein oxidation is chemically stable and serves as markers of oxidation. Estimation of protein carbonyl (PCO) content: Protein carbonyl (PCO) was

measured by using the method of (Reznick and Packer *et al.*, 1994).

Superoxide dismutase

SOD activity was measured by quantifying the inhibition of NBT transformation to formazan, applying the process of (Kakker *et al.*, 1984).

Catalase

The catalase activity was measured by the method of (Sinha, 1972).

Statistics

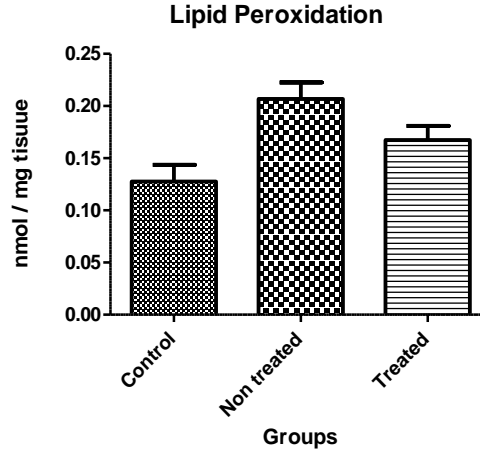
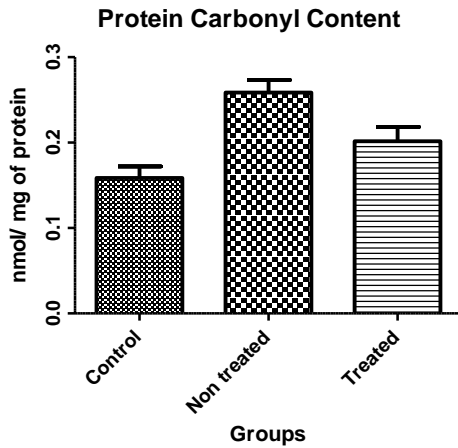
The entire experiment was performed in triplicates and the data were described as mean \pm standard deviation (SD). Statistical study was carried out by one –way ANOVA. Level of signification was determined by Graphpad Prism 5.0 (USA) and was found to be $P<0.05$.

Results and Discussion

***In vivo* part**

Protein carbonyl content and Lipid peroxidation

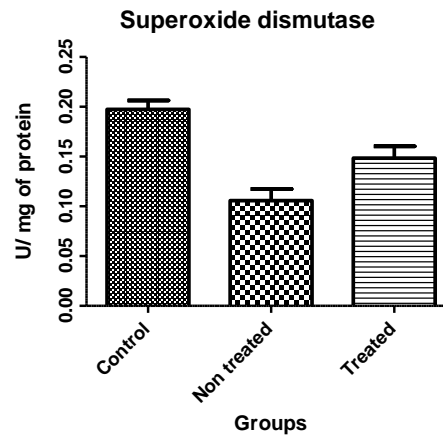
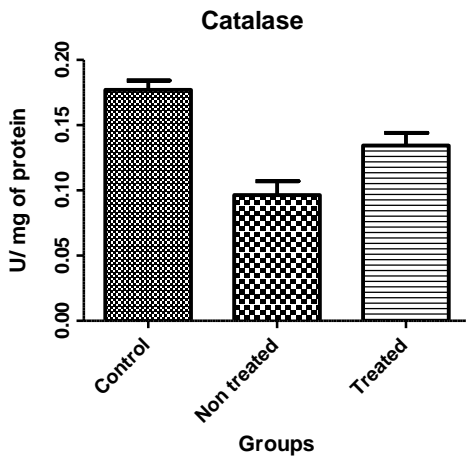
In the protein oxidation there was 66% increase from control in the non treated group where as 34% increase from control in treated group. In the lipid peroxidation there was 58% increase compare to control in the non treated group and 25% increase from control in the treated group.



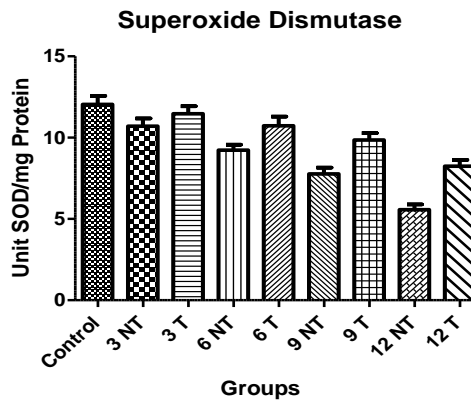
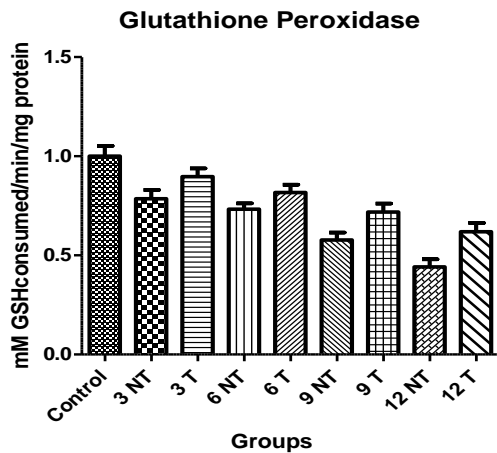
Superoxide dismutase and Catalase

In superoxide dismutase and catalase there was 47% and 42% decrease from control in

the untreated group respectively where as 23% and 26% decrease compare to control in treated group in superoxide dismutase and catalase respectively.



In vitro part

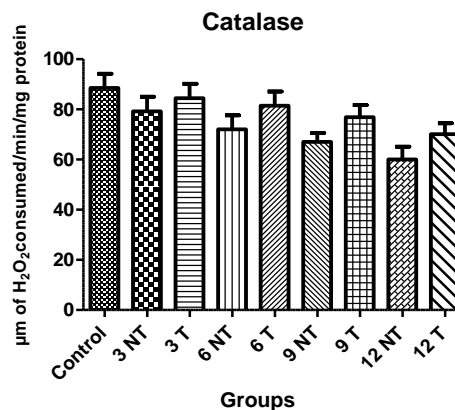
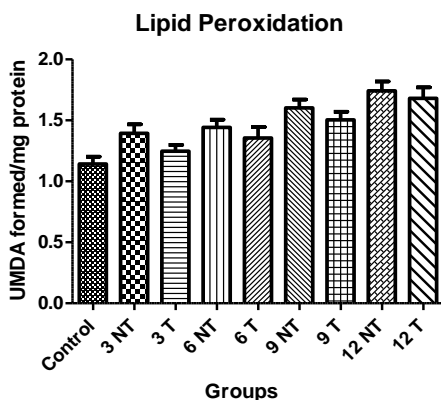


3NT (Non Treated) - Only 3minutes of UV radiation and 3T (Treated) - 3minutes of UV radiation and later treated with graviola extract; Same applies to the rest.

Glutathione Peroxidase: There was 21% decrease in 3NT where only 11% decrease in 3T and 27% decrease in 6NT and 19% decrease in 6T furthermore 43% decrease in

9NT and 30% decrease in 9T moreover 56% decrease in 12NT and 39% decrease in 12T.

Superoxide dismutase: It was 11%, 23%, 35%, 55% decrease in 3NT, 6NT, 9NT and 12NT respectively where as 5%, 11% , 18% ,32% decrease in 12T respectively compare to control.



Lipid peroxidation: it was observed 18% increase in 3NT and 10% increase in 3T compare to control along with 27% increase in 6NT, 17% increase in 6T futher more 45% increase in 9NT and 36% increase in 9T moreover, 54% increase in 12NT and 45% increase in 12T.

Catalase: There was 10 %, 18%, 24%, 31% decrease in 3NT, 6NT, 9NT and 12NT correspondingly where as 4.5%, 8%, 14%, 21% decrease in 3T, 6T, 9T and 12T respectively compare to control.

From the centuries, plants are investigating for their diverse therapeutic properties that might be successful in management, prevention, and cure of various diseases. Naturally occurring compounds are proving a better choice than synthetic compounds as naturally occurring compounds have lesser side effects.

Phytochemicals that have a definite physiological function are important factors

to decide the medicinal value of a plant. The most significant phytochemicals are phenolic compounds, tannins, flavonoids, alkaloids (Hill, 1952). Flavonoids work as antioxidants and are known to boost the Vitamin C's effects. They are biologically active against microbes, viruses, tumors, liver toxins (Korkina *et al.*, 1997). Tannins possess anti-oxidants activities (Tebib *et al.*, 1997). Tannins and flavonoids belong to the phenolic compounds and plant phenolic compounds are a chief group that acts as free radical scavengers or prime antioxidants (Polterait, 1997). The amounts of total phenolics in the extract was quantified and found to be significantly higher. In the study by (Vijayameena *et al.*, 2013) had proved that aqueous extract of *A.muricata* shows non enzymatic antioxidants like Vit.C and Vit E and enzymatic antioxidants like SOD and Catalase. The prior study by (Baskar *et al.*, 2007) had shown that leaf ethanolic extract of graviola exhibits considerable antioxidant property so *A.muricata* possess anti oxidative activity may be credited to

annonaceous acetogenins. The aqueous extract of *Annona muricata* (Graviola tea) has shown considerable antioxidant potentials in this experiment. However, further investigation should be carried out to make sure it's safe usage.

Conflict of Interest: The authors declare that they have no conflict of interest.

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How to cite this article:

Bhaumik D. Vaghela, Devarshee Bhavsar and Nayan K. Jain. 2016. Effect of Aqueous Extract of *Annona muricata* (Graviola Tea) on the Oxidative Stress produced by the Artificial UV Source *in Vivo and in Vitro*. *Int.J.Curr.Microbiol.App.Sci*. 5(10): 648-655.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.510.072>