Antioxidative Effect of Graviola Aqueous Extract on the Blood of the Patients from Diabetes

INTRODUCTION

Diabetes is a condition in which blood sugar level is relatively high in the body. There are three types of diabetes i) type 1 diabetes, ii) type 2 diabetes, iii) gestational diabetes. Diabetes causes reactive oxygen species (ROS) and that damages cells of the body. Damaged cells lead to secondary complications in diabetes[1,2]. There are many toxic repercussions of ROS on catalase, superoxide dismutase, protein, lipid, glutathione metabolism, and plasma's antioxidative capacity. In resistance to insulin and its succession to glucose intolerance oxidative stress works like arbitrator[3].

Annona muricata Linn is generally famous as graviola or soursop which is from Annonaceae family. The leaves of the plant are by tradition used for various purposes like to treat hypertension, cough, headaches, asthma, anti-spasmodic, anti-bacterial and anti-fungal activity[4]. There are many phytochemicals present in graviola which are phytosterol, alkaloids, flavonoids, saponins, tannins, Terpenoids, carbohydrates, proteins, cardiac glycosides and also annonaceous acetogenins.

MATERIALS AND METHODS

Graviola extract

The leaves and stems of graviola were collected and washed with the distilled water. After that they were dried in a cool place away from sunlight. The 30 leaves and 5 small stems were crushed into fine powder then allowed to dissolve in 100 ml of double distilled water. Then the mixture was filtered with the filter paper grade 4.

Sample collection

All the blood samples were collected from the Civil Hospital, Ahmedabad, Gujarat. The ethical committee approval of the hospital was taken prior. The blood sample of 5 ml was collected from the 30 diabetic male patients aged between 45–50 years. After that sample was divided into equal half in the laminar airflow under aseptic conditions. From the first half of blood sample blood was cultured with 8ml of RPMI-1640 media along with 1 ml of the graviola aqueous extract that was termed as treated group, and the second half was cultured only with RPMI-1640 media and it was known as diabetic group. Blood of 5 ml from the age and gender matched healthy individuals was collected.

ABSTRACT

This study was aimed to look into the antioxidative effect of the graviola aqueous extract on the blood of the patients from diabetes. 5 ml Blood was collected from the patients of 45-50 years of age group and then equally divided into two groups non-treated (Diabetic) and treated. Oxidative tests were carried out directly from the diabetic group whereas; in the treated group 1 ml of the graviola aqueous extract was added. Various biochemical tests for example, Lipid Peroxidation (LPO), Catalase (Cat), Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD) were carried out. Results were positive showing antioxidative effect of the graviola aqueous extract.

KEYWORDS diabetes, graviola aqueous extract, oxidative stress
collected for control and cultured only with the media. After 48 hours of culturing the biochemical tests were performed.

**Cell culture**

Blood samples of all the groups were in incubator for 48 hours. The medium has penicillin, streptomycin and PHA-M. The temperature was constantly maintained at 37°C during the incubation time.

**Sample preparation**

Cells were harvested using centrifuge at 2000 rpm for 20 minutes after 48 hours of culturing.

Then supernatant was removed. Hypotonic solution of KCl that is 0.5% KCl of equal volume was added and mixed gently along with the pellet of cells. Then there was 20 minutes of incubation at 37°C. Thus the cell lysate obtained was utilized as sample in all biochemical tests. All the tests were carried out in triplicates.

**Biochemical tests**

All the tests like Lipid peroxidation by Ohkawa; Glutathione peroxidase by Rotruck, Catalase by Sinha, Superoxide dismutase by Kakker were performed as per standard operating procedure.

**Statistics**

The data represent as a mean±standard deviation (SD). Statistics analysis was performed by one way ANOVA and level of signification was calculated in Graphpad prism 5.0 software. It was $P < 0.05$.

**RESULTS AND DISCUSSIONS**

Graph 1 depicts that in the diabetic group Lipid peroxidation level was more than double compare to control and after the treatment from the graviola aqueous extract the level reduced in the treated group significantly where as in the Graph 2 the concentration of Glutathione peroxidase was almost half compare to control and later on after the exposure of the extract the level was increased considerably.

**Lipid peroxidation and glutathione peroxidase**

It is evident that in diabetic group lipid peroxidation was quite high compare to control and with use of graviola extract it was lowered in the treated group. Glutathione peroxidase level low compare to control whereas after the treatment from graviola extract level was increased in the treated group. Peroxyl radicals can take out the hydrogen from the lipids and thus generating hydroperoxides which can further proliferate pathway of free radical (V. Lobo et al., 2010). Oxidized lipids can make malondialdehyde (MDA) which can damage the cell membranes. In oxidative stress and free radical intervened lipid damage, MDA can be recognised as a major biomarker. In diabetic patients, increased level of MDA in serum and plasma has been observed.

In serum and in red blood cells, considerably increased level of thiobarbituric acid-reactive substances (TBARS) have been found in diabetic condition. It is reported that in diabetes patients decreased level of glutathione and reduced glutathione level can be one of the reason for the oxidative DNA damage in diabetes type 2 (Figs. 1 and 2).

Graph 3 illustrates that the level of Superoxide dismutase in diabetic group was lower than control group but in the treated group the level of superoxide dismutase was higher than diabetic group and less than control group and same applies to the Catalase enzyme essay of Graph 4.

**Superoxide dismutase and Catalase**

Superoxide dismutase and catalase level was low compare to control in diabetic group and was raised in treated group because of the effect of the graviola
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Extract. Superoxide dismutase plays an extremely vital role in protection against histological and cellular damages, which are generated by reactive oxygen species. Reduced levels of superoxide dismutase have been observed in blood and in diabetes tissue\textsuperscript{16}. Catalase is present in most of the living organisms and very important in oxidative stress in diabetes\textsuperscript{17}. People with decreased level of catalase are at enhanced risk of diabetes\textsuperscript{18} (Figs. 3 and 4).

In the study by Baskar\textsuperscript{19} published that alcoholic leaf extract of \textit{A. muricata} shown considerable anti oxidative activity. Other research works published by Bhauumik D. Vaghela\textsuperscript{20} and Vijayameena\textsuperscript{21} also demonstrated that \textit{A. muricata} contains anti oxidative property.

**CONCLUSION**

In this experiment graviola extract has exhibited tremendous antioxidative activity but still further investigation should be conducted for its more safe usage.

**SOURCES OF SUPPORT**

Gujarat University.

**REFERENCES**