

Testicular Antioxidants and Serum Lipid Effects of the Fruit of *Solanum Melongena* on Wistar Rats

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Abstract

Solanum Melongena is a highly nutritive medicinal plant which is cultivated in many countries including Nigeria where it is mainly grown in the northern part of the country. The plant is also applied in folklore remedies in the treatment of different ailments. The present study was carried out to investigate the testicular antioxidant and lipid effects of the fruit of *Solanum Melongena* on male wistar rats. The rats were divided into three (3) groups of six (6) rats each. Group one (1) served as control and received distilled water. Group two (2) and group three (3) were treated with 200mg/kg bw and 400mg/kg bw of the hydromethanol (20:80) extract of *Solanum Melongena* respectively. Extract was administered orally as single daily dose for a period of 30 days. The results obtained showed that the extract caused significant increase in the superoxide dismutase and catalase enzyme activities and also significantly reduced malondialdehyde level. The serum concentrations of total cholesterol and low density lipoprotein cholesterol levels were significantly reduced but the serum concentrations of the high density lipoprotein cholesterol was increased. This study have shown that extract of *Solanum Melongena* inhibit lipid peroxidation and may reduce the risk of coronary artery disease in male wistar rats.

Keywords: *Solanum Melongena*, antioxidant, lipids, hydromethanol, male wistar rats.

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INTRODUCTION

Solanum Melongena Linn is a herbaceous plant with coarse leaves, and white to purple flowers that give rise to a fleshy berry like fruit containing seeds with large endosperm [1]. The fruits are of varying shapes and colours. The shapes range from oblong, ovoid, obovoid or cylindrical while the commoner colours of the fruit range from purple, white, yellow, green, with or without stripes of white/light green and combination of more than two colours [2, 3]. There are over 1000 species worldwide but in Nigeria, only about 25 species have been identified and the plant is commonly cultivated in the northern parts of the country [4-6].

The plant is economically viable. The fruits are usually purchased in the market or from hawkers in the streets and consumed raw or in cooked form where it adds more spice and flavor to soups, stews and other food preparations. The fruits are also offered to guests in social and traditional gatherings in Southern Nigeria. The plant is highly valued for its nutritional benefits and medicinal properties. According to reports, *Solanum Melongena* is a good source of vitamins and

minerals, consisting of significant amount of vitamins B₁, B₃, B₆ and K, as well as high values of calcium and magnesium [2]. In traditional medicine, *Solanum Melongena* is applied in folklore remedies in the treatment of illnesses such as, asthma, allergic rhinitis [7], diabetes and bronchitis [7, 8]. Some documented pharmacological effects of the extracts of *Solanum Melongena*, includes; anti-inflammatory [9], hepatoprotective [10], hypotensive [11], and anticancer [12] activities as well as, anti-asthmatic [13] and intra ocular pressure lowering effects [1]. Usually, observed actions are due to the total amount and nature of bioactive substances present in the extract. The extract of *Solanum Melongena* possesses several biologically active substances such as alkaloids, tannins, saponins [14] and flavonoids [15]. Furthermore, solanoflavone- a biflavonoid glycoside was found in the fruits of *Solanum Melongena* [16]. The findings in a study [14] showed that *Solanum Melongena* may also be beneficial to some categories of individuals such as those watching their weight and those with ischemic heart diseases. However, in view of apparent dearth of scientific reports on the effects of this plant on the lipid profile and oxidative stress, the present study was

carried out with the objective to explore the testicular antioxidant and possible lipid lowering effects of the extract of *Solanum Melongena*.

MATERIALS AND METHODS

Plant material

Fresh fruits of *Solanum Melongena* were purchased from local farmers in Lere Local Government Area of Kaduna state, in northern Nigeria and identified at the herbarium, Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The fruits were washed to remove dirt and then chopped to small pieces and air-dried. The dried forms were blended to fine powder using a Manual blender. The powdered samples were kept in polythene bags and preserved at room temperature.

Preparation of plant extracts

A total of 500g of powdered sample was soaked in 2 liters of solvent (Hydromethanol, 20:80) and left to stand for 24 hours to allow for extraction at room temperature. Thereafter, the solution was filtered with a Whatman's filter paper and the filtrate concentrated under reduced pressure using a rotary evaporator. The yield was stored air tight in a refrigerator until required for use.

Animal models

Adult male wistar rats weighing 150 to 200g were used for the study. Animal care and handling conformed to standard guidelines on the use of experimental animal in research [17]. Institutional ethical approval was obtained before commencement of study. The wistar rats were divided into three (3) groups of six (6) rats each. Group one (1) served as control and received distilled water. Group two (2) and group three (3) were treated with 200mg/kg bw and 400mg/kg bw of the hydromethanol extract respectively. The extract was administered orally, once per day for a period of 30 days. The rats were sacrificed under chloroform anaesthesia on day 31 after 24hours of last administered dose.

Determination of antioxidant activity

The rat's testis was removed following an incision at the inguinal area. The organ was blotted with tissue paper then cut very thinly with sterile scapel blade and homogenized in ice-cold 0.25M (mol/L) sucrose solution (mass-to-volume ratio of 1:5). The homogenates was centrifuged for 10 minutes at 4000r/minute at 4°C to obtain a clear supernatant. The supernatant was then carefully aspirated with pasteur pipette into sample bottle and stored frozen at -20°C until used for biochemical assays.

The methods adopted in determination of some enzymatic and non-enzymatic antioxidant activity in

this study have been previously documented. Briefly, the principle involved is such that, the malondialdehyde (MDA) produced in a mixture of the supernatant and the chromogenic reagent, 2-thiobarbituric acid (TBA) causes a coloured reaction. Using excitation wavelengths of 525 nm and 547nm for emission, the absorbance was measured [18].

The principle involved in the determination of catalase activity is based upon the ability of catalase to cause the breakdown of hydrogen peroxide (H₂O₂) in a sample preparation which is then measured spectrophotometrically at 240nm. The amount of protein that converts a unit (μmol) H₂O₂ utilized per minute is equivalent to one unit of catalase enzyme activity [19]. The reduction of nitrobluetetrazolium (NBT) to blue formazan by the superoxide anion was determined spectrophotometrically at 560nm. The ability of superoxide enzyme to cause a 50% inhibition in NBT reduction refers to one unit of enzyme activity [20]. Gluthathione (GSH) reacts with 5,5'-dithio-bis (2-nitrobenzoic acid) (DNTB, Ellman's reagent) to produce the conjugate GS-TNB as well as the yellow TNB (5'-thio-2-nitrobenzoic acid) detected at 412nm. The rate of TNB production was proportional to the GSH concentration in the extract [21, 22].

Collection of blood

Blood samples were collected through cardiac puncture into dry sample tubes and allowed to stand for about 15-20 minutes to clot. It was further centrifuged at 3000 rev/min for 15 minutes using a table centrifuge machine. The serum was separated using a pasteur pipette into sterile sample tubes and stored at -4°C until used.

The biochemical analysis of serum was carried out for estimation of Total cholesterol (TC), High density lipoprotein cholesterol (HDL-c), Triglycerides (TG) and Low density lipoprotein cholesterol (LDL-c). The TC and the different cholesterol fractions were determined using commercially available diagnostic kits based on standard techniques in accordance to manufacturer's recommendations. The concentration of LDL-c was determined using the Friedewald equation [23].

STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS) version 20.0 software tool was used for the statistical data processing. Values were expressed as mean±SEM. The differences in the mean values between groups were analyzed using the analysis of variance with post hoc least significant difference, and considered statistically significant at p<0.05.

RESULT

Result presentation

The results are presented in tables 1 to 4.

Table-1: Level of antioxidant activity

| Groups/Dose (mg/kg) | Testicular antioxidants | | | |
|---------------------|---|---------------------|-----------------------|-----------------------------|
| | Gluthathione ($\mu\text{g}/\text{min}/\text{mg.protein}$) | SOD (Ug/mg.protein) | MDA (Umol/mg.protein) | Catalase (Units/mg.protein) |
| Control | 0.12 \pm 0.01 | 0.46 \pm 0.05 | 1.44 \pm 0.13 | 23.03 \pm 0.88 |
| 200 | 0.13 \pm 0.01 | 0.52 \pm 0.07 | 1.13 \pm 0.14 | 26.22 \pm 0.69* |
| 400 | 0.14 \pm 0.01 | 0.78 \pm 0.06* | 0.77 \pm 0.09* | 28.58 \pm 1.41* |

Values expressed as Mean \pm SEM. n=6. Significant at [$*(P<0.05)$] when compared to control group

Table-2: Predictor ratio of association of oxidative stress markers

| Dose(mg/kg) body weight | Antioxidant ratio | | | | | | |
|-------------------------|-------------------|---|--------|---|---------|---|----------|
| Control | GSH[1] | : | SOD[4] | : | MDA[12] | : | CAT[191] |
| 200 | GSH[1] | : | SOD[4] | : | MDA[8] | : | CAT[201] |
| 400 | GSH[1] | : | SOD[6] | : | MDA[5] | : | CAT[204] |

Table-3: Effect of extract on serum lipid profile

| Groups/Dose (mg/kg) | Serum lipids (mg/dl) | | | |
|---------------------|----------------------|-----------------|------------------|------------------|
| | Total cholesterol | Triglyceride | LDL-c | HDL-c |
| Control | 3.00 \pm 0.35 | 2.09 \pm 0.26 | 4.40 \pm 0.42 | 3.67 \pm 0.40 |
| 200 | 2.22 \pm 0.14* | 1.66 \pm 0.14 | 3.55 \pm 0.41 | 5.20 \pm 0.53 |
| 400 | 2.10 \pm 0.12* | 1.60 \pm 0.12 | 2.36 \pm 0.30* | 5.62 \pm 0.60* |

Values expressed as Mean \pm SEM. n=6. Significant at [$*(P<0.05)$] when compared to control group

Table-4: Mean values of the lipid ratio

| Groups/Dose (mg/kg) | Lipid ratio (mg/dl) | | | | | |
|---------------------|---------------------|------------------|------------------|-----------------|------------------|------------------|
| | TG/HDL-c | TC/HDL-c | LDL-c/HDL-c | LDL-c/TC | LDL-c/TG | HDL-c/LDL-c |
| Control | 0.62 \pm 0.11 | 0.88 \pm 0.16 | 1.26 \pm 0.16 | 1.54 \pm 0.20 | 2.23 \pm 0.29 | 0.85 \pm 0.09 |
| 200 | 0.34 \pm 0.04 | 0.45 \pm 0.06* | 0.73 \pm 0.11* | 1.67 \pm 0.28 | 2.16 \pm 0.20 | 1.58 \pm 0.26 |
| 400 | 0.29 \pm 0.02* | 0.39 \pm 0.04* | 0.43 \pm 0.06* | 1.12 \pm 0.14 | 1.46 \pm 0.13* | 2.53 \pm 0.35* |

Values expressed as Mean \pm SEM. n=6. Significant at [$*(P<0.05)$] when compared to control group

RESULT ANALYSIS

Hydromethanol extracts of the fruit of *Solanum melongena* were administered for a period of 30 days for testicular antioxidant and lipid studies, as low dose of 200mg/kg bw (group 2) and higher dose of 400 mg/kg bw (group 3). Table 1 showed that catalase level increased significantly at $p<0.05$ in a dose dependent manner while the higher dose significantly increased superoxide dismutase activity and reduced malondialdehyde levels when compared to control. Table 2 highlighted the predictor ratio of association of the various markers of oxidative stress.

The ratio of superoxide dismutase and catalase were increased while the malondialdehyde ratio was decreased in the test groups relative to control. In table 3, there was a significant ($p<0.05$) decrease in total cholesterol with both doses of extract and a decrease LDL-c level as well as, an increased HDL-c level with the higher dose. Table 4 show the mean values for various lipid ratios and statistically significant differences are highlighted.

DISCUSSION

The testicular antioxidant and serum lipid effects of *Solanum Melongena* were assessed in the present study. This assessment was based on the effects of the extracts on some antioxidant enzyme system, lipid peroxidation and general lipid metabolism. There was a significant ($p<0.05$) increase in the activity of superoxide dismutase and catalase (Table 1) in the treated groups but the glutathione reductase level was not significantly affected, although its level were marginally higher in the tests groups. These observations were made when the value of enzyme activities obtained in the test groups were compared to control. The increase in superoxide and catalase enzyme activities implies that extracts of *Solanum Melongena* are capable of boosting the antioxidant production and defense against the negative effects of oxidative stress in the experimental animal models. Both catalase and superoxide dismutase work closely to prevent free radical damage to the body. The superoxide dismutase converts the dangerous superoxide radical to hydrogen peroxide which is then

converted to harmless water and oxygen by catalase [24]. GSH act as electron donor in various reductive processes essential for synthesis and degradation of proteins, formation of deoxy-ribonucleotides and reduction of H₂O₂ and it's a critical molecule that functions in resisting oxidative stress and maintains the reducing environment of the cell [25]. When cells are exposed to oxidative stress, glutathione disulphide levels increase significantly with a concomitant decrease in glutathione [26]. Glutathione reductase catalyzes the reduction of glutathione disulfide to the sulfhydryl form, glutathione. Furthermore, the level of malondialdehyde was significantly ($p < 0.05$) reduced in the extract treated groups. The malondialdehyde has been reported as a byproduct of lipid peroxidation. Reactive oxygen species generated spontaneously in cells during metabolism causes degradation of polyunsaturated lipids leading to the production of malondialdehyde [27]; which serves as a biomarker to measure the level of oxidative stress in an organism [28, 29]. In addition, the predictor ratio of association of oxidative stress markers show an increased ratio of catalase and superoxide dismutase to a decreased ratio of malondialdehyde in the *Solanum melongena* treated groups. The antioxidant action of *Solanum Melongena* may be due to its ability to inhibit lipid peroxidation by the removal of free radical intermediates. In this study, the change in these oxidative stress markers suggest that the extract of *Solanum Melongena* may play important roles in preventing toxic stress in testicular cells.

The medicinal properties of the plant are derived from its phytochemical constituents. Several bioactive compounds such as tannins, saponins and flavonoids [14, 15] are present in the fruit of *Solanum Melongena*. The plants antioxidant property may be due to the flavonoids content. Flavonoids are reportedly good antioxidants [30], which may possess the capacity to control or prevent oxidative stress and associated disorders. Although animals exposed to reproductive toxicants may suffer varying degrees of reproductive failure, the antioxidants which have positive influence on testicular function may protect spermatogenesis in this animals [31]. Hypothetically, lipid peroxidation is considered a key element in the causal pathway of atherogenesis.

The results of the lipid profile showed that extracts of *Solanum Melongena* caused a significant ($p < 0.05$) decrease in the serum concentrations of total cholesterol and LDL-c, as well as a significant ($p < 0.05$) increase in the serum concentration of HDL-c but did not significantly alter the concentration of triglycerides. As an initial broad medical screening tool for lipid abnormalities, the lipid profile can identify certain genetic diseases and determine the approximate risks for cardiovascular disease, certain forms of pancreatitis and other diseases. It is often recommended in the evaluation of dyslipidemia, although, emphasis is

placed on LDL-c which is regarded as "bad lipoprotein [32]". In view of this, existing evidence show that an elevated LDL-c concentration is atherogenic, but a high HDL-c is cardioprotective [33, 34]. In addition, oxidatively modified LDL-c contributes to the pathogenesis of atherosclerosis [35] and also effect some morphologic changes on the vascular endothelium [36]. *Solanum Melongena* may prevent these changes occurring due to an abnormal lipid profile.

It has been established from reports in various studies, that even when the conventional lipid profile test appear supposedly normal, lipid ratios such as the Castelli's risk index-I (CRI-I), Castelli's risk index-II (CRI-II), and other ratios may be used as an alternative diagnostic tool in predicting the risk of developing cardiovascular disease [37-39], especially in individuals with intermediate risk. Hence, evaluation of the lipid ratios may be useful indicator of an existing risk as well as early identification and diagnosis of dyslipidemia at its earliest stage [40]. The findings in this study showed a significant ($p < 0.05$) reduction in CRI-I and CRI-II (Table 4), for the group treated with higher dose (400mg/kg) extract of *Solanum Melongena* when compared to control. There is an association between a high CRI-I and CRI-II with higher risk of ischaemic heart disease. In some reports, abnormally high CRI-I and LDL-c/HDL-c ratio (CRI-II) was associated with a higher risk of coronary plaques formation [41, 42]. In the present study, these ratios (CRI-I and CRI-II) were reduced with the extract of *Solanum Melongena*.

CONCLUSION

The extracts of *Solanum Melongena* possess antioxidant potential with the capacity to prevent cell death due to lipid peroxidation by inhibiting the lipid peroxidation process. The extract also reduced serum concentrations of LDL-c and elevated serum concentrations of anti atherogenic HDL-c concentration as well as reduced critical lipid ratios associating lipids levels with risk of cardiovascular events.

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