

A Biochemical Study on the Effects of Fullerene C₆₀ and Fruit Extract of *Balanites aegyptiaca* Plant in Ameliorating the Toxicity Induced by Doxorubicin in Streptozotocin Model of Diabetic Male Albino Rats

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Abstract

There were relationship between hyperglycaemic and pharmacokinetic of toxicity of doxorubicin that lead to increase oxidative stress on cells. This study was aimed to detect the curative effect of some antioxidants drugs (fullerene C₆₀ and mesocarp fruit extract of *Balanites aegyptiaca*) on the haematological and biochemical of kidney function parameters after induced by diabetes and toxicity with doxorubicin on the rats. Nine groups of adult male albino rats were established (n=8). The first group (Group 1) was served as normal group. Group 2 was injected intraperitoneal (i.p) with streptozotocin at single dose (45 mg/kg body weight). Diabetic rats (Group 3) were injected intraperitoneal (i.p) with doxorubicin at dose (5 mg/kg body weight) for three days. Diabetic rats (Group 4) were given orally fullerene C₆₀ daily at dose (4 mg/kg body weight). Diabetic rats (Group 5) treated orally by mesocarp fruit extract of *Balanites aegyptiaca* at dose (1.5 ml/kg body weight). (Group 6) treated orally with fullerene C₆₀ plus mesocarp fruit extract of *Balanites aegyptiaca*. Diabetic rats (Group 7) intoxicated DOX treated orally with fullerene C₆₀. Diabetic rats (Group 8) intoxicated DOX treated orally with mesocarp fruit extract of *Balanites aegyptiaca*. Diabetic rats (Group 9) diabetic rats treated orally with fullerene C₆₀ plus mesocarp fruit extract of *Balanites aegyptiaca*. Whole blood and serum were collected for haematological and biochemical of kidney functions examinations, respectively. Diabetes induce, hematotoxicity was determined by a highly significant decrease in Red Blood Cells (RBCs), White Blood Cells (WBCs), Blood Platelets (PLTs), Haemoglobin (Hb) and PCV value, as well as nephropathy was determined by a highly significant increase in creatinine, urea and uric acid. Diabetic rats intoxicated DOX showed reduction in haematological parameters, also, there were highly significant increases in kidney function parameters. Fullerene C₆₀ and mesocarp fruit extract of *Balanites aegyptiaca* ameliorated haematological and kidney functions indices. It could be concluded that Fullerene C₆₀ and *Balanites aegyptiaca*, clarified a modulatory role against the cellular damage produced by oxidative stress.

Keywords: Diabetic rats; Doxorubicin; Fullerene; *Balanites aegyptiaca*

Introduction

Diabetes mellitus is a major endocrine and a metabolic disorder with multiple etiologies, characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin function or both [1]. Nephropathy is one of the major complications of both type 1 and types 2 diabetes mellitus, and the morbidity and mortality due to Diabetic Nephropathy (DN) continue to increase in industrialized nations [2].

STZ-induced diabetes cause abnormality of hemorheological indices in form of increasing RBCs count [3], Hb concentration and PCV while decrease WBCs count and platelets but STZ- induced diabetes cause anemia in form of decreasing RBCs count, Hb concentration and PCV while increase WBCs [4]. It was reported that diabetes can change hematological parameters and the immune system in diabetes mellitus [5,6].

Doxorubicin (DOX) is an anthracycline antibiotic, although, a total chemical synthesis is now possible. DOX has demonstrated high antitumor efficacy. However, the use of DOX has been limited largely

due to possible diverse cardiac, renal, hematological and testicular toxicities [7].

The use of DOX has been limited largely due to possible diverse cardiac, renal, haematological and testicular toxicities [7]. DOX induces rat nephropathy, which is characterized by massive proteinuria, hypoalbuminemia and dyslipidemia [7].

Nanoscience is one of the most important research and development frontiers in modern science. Nanotechnology is now widely used throughout the pharmaceutical industry, medicine, electronics, robotics, and tissue engineering. The use of nanoparticle (NP) materials offers many advantages due to their unique size and physical properties [8].

Fullerene (C₆₀), the third carbon allotrope, is similar in structure to graphene but rolled up to form hollow spheres with closed structure [9]. Fullerene autonomous cytostatic, immunosuppressant and antioxidant properties [10]. Fullerene C₆₀ is known to be able to inactivate hydroxyl radicals by attaching to double bonds [11]. However, this mechanism cannot explain sufficient (nearly two times) increase in lifespan of rats. Such kind of antioxidative activity is also attributed to natural phenolic antioxidants that do not possess high senescence retarding activity [12].

The study on the medicinal plants is essential to promote the proper use of herbal medicine in order to determine their potential as a source

for the new drugs [13]. *Balanites aegyptiaca* is commonly known as nanjunda. It has been used in a variety of folk medicine in India and Asia. Various parts of the plant are used in Ayurvedic and other folk medicine for the treatment of various ailments such as syphilis, jaundice, liver and spleen problem, epilepsy and yellow fever and the plant also has insecticidal, anthelmintic, antifeedant, molluscicidal and contraceptive activities [14].

Mesocarp fruit extract of *Balanites aegyptiaca* can be used as complementary natural ant diabetic agent to prevent diabetic complications. Mesocarp fruit extract of *Balanites aegyptiaca* also has beneficial effects on other target tissues as kidney, and shows beneficial effects of mediators of large vessel damage, this concept appears attractive for the prevention or delay of diabetic nephropathy [15].

Materials and Methods

Experimental animals

White albino rats (*Rattus norvegicus*) from order Rodentia and family Muridae were used in the present study. Experiments were carried out on 72 male albino rats at age (10-12 weeks) and weight about (250-280 g), which was obtained from the animal house of the Egyptian Organization for Biological Products and Vaccines (VACSERA), Helwan, Cairo, Egypt. The animals were housed in the animal house of the Faculty of Science, South Valley University, Qena, Egypt; rats were divided into nine groups (8 rats/group).

Adult rats were kept under observation for 2 weeks before experimentation to exclude any intercurrent infection and to acclimatize the animals to the new conditions. The selected animals were marked, housed in controlled suitable cages with the separate bottom and kept at room temperature (23 ± 2°C), and 12 h light/dark period, and fed on a balanced stable commercial diet. For drinking tap water was provided ad libitum.

Streptozotocin

Streptozotocin (STZ) was purchased from Sigma-Aldrich Chemie GmbH, Germany. It induces diabetes in experimental animals. It injected intraperitoneally (i.p) at a dose of 45 mg/kg by weight (single dose) [16].

Doxorubicin

Doxorubicin (DOX) is anticancer drug and was purchased from Ebewe Pharma Co. Austria (Doxorubicin -injection 15 mg) according to [17,18].

Fullerene

C₆₀ (purity 99.9%) was obtained from Lydow Group Limited Research Corporation (China) and used without further purification.

Virgin olive oil

It is obtained from a Colavita Extra Virgin Olive Oil Company which extracted from Olives harvested and pressed in Italy.

C₆₀-olive oil solution preparation

After sourcing the high purity C₆₀, we prepared C₆₀-olive oil solution according to [19]. Fifty mg of C₆₀ were dissolved in 10 ml of

olive oil by stirring for 2 weeks at ambient temperature in the dark. The resulting mixture was centrifuged at 5.000 g for 1 h and the supernatant was filtered through a Millipore filter with 0.25 mm porosity.

Balanites aegyptiaca

Fruits of *Balanites aegyptiaca* were collected from South Valley University, Qena, Egyptian February 2017. They were taxonomically identified by the Department of Botany, Faculty of Science, South Valley University of Qena, Egypt.

Preparation of *Balanites aegyptiaca* extract

Collected fruitsof *Balanites aegyptiaca* were properly washed in water, rinsed using distilled water and the coat epicarp was gently removed by hand while a cleaned, dried knife was used to peel the mesocarp of the fruit. Cleaned mesocarp (fleshy outer part) was separated from the hard inner shell containing the seed. Mesocarp was air dried at room temperature in the laboratory and was powdered using coffee mill. This was thereafter placed in a dry plastic container and later put inside the refrigerator until required for use. 100 g powdered was extracted using 200ml distilled water; it was stirred continuously for 10 mins and filtered. Filtrate was concentrated at 60°C to obtain thick brown viscous semi-solid [20]. It administrated orally fruit mesocarp extract of *Balanites aegyptiaca* daily 1.5 g/kg by weight for 45 days [21].

Experimental design

The animals were randomly assigned into 9 groups (8 rats for each group). The first eight rats were separated for group1, which were injected i.p with 0.9% isotonic saline solution at a dose (10 ml/kg by weight) and used as normal group. All remaining animals were injected with single dose of streptozotocin (45 mg/kg by weight) and divided into eight groups (groups 2-8). Group 2, diabetic rats were injected intraperitoneal (i.p) with Virgin olive oil at a dose (10 ml/kg by weight) and used as diabetic group. Group 3, diabetic rats were injected intraperitoneal (i.p) with doxorubicin at a dose of (5 mg/kg by weight.) for three days. Group 4, diabetic rats were orally administered with fullerene C₆₀ (4 mg/kg by weight.). Group 5, diabetic rats were orally administered with mesocarp fruit extract of *Balanites aegyptiaca* (1.5 g/kg by weight). Group 6, diabetic rats were orally administered with fullerene C₆₀ and mesocarp fruit extract of *Balanites aegyptiaca*. Group 7, diabetic rats intoxicated with DOX were orally administered with fullerene C₆₀ (4 mg/kg by weight.). Group 8, diabetic rats intoxicated with DOX were orally administered with mesocarp fruit extract of *Balanites aegyptiaca* (1.5 g/kg by weight). Group 9, diabetic rats intoxicated with DOX were orally administered with fullerene C₆₀ and mesocarp fruit extract of *Balanites aegyptiaca*. Groups 3-8 were treated with drugs after induced diabetes by STZ with 10 days, daily and the experiment were continuous for 60 days. All animals were sacrificed at the end of the experiment.

Blood collection

The blood were collected from all animals and divided into two portions, one portion was taken in EDTA containing tubes and used for haematological examination. The other portion of blood was left in clean tubes at room temperature to clot, after an hour, then serum was separated by centrifugation for 30 min at 3000 rpm. The sera were collected in labelled eppendorf tubes and stored at -20°C until used for

biochemical analysis. A part of right lobe of liver was dissected and washed with physiological saline solution, dried, weighed and homogenized in phosphate buffer (pH 7.4) and kept frozen until used for biochemical assays.

Haematological analysis

The haematological evaluation consisted of erythrocytes (RBCs), White Blood Cells (WBCs), Platelets (PLT) counts and Haemoglobin content, determination by Automated Haematology Analyser (Diff3) Mek6410/Mek-6420.

Biochemical analysis

Urea was determined by enzymatic colorimetric method which described by [22]. Creatinine was determined by kinetic method which described by [23]. Uric Acid was determined by enzymatic colorimetric method which described by [23].

Statistical analysis

The results are expressed as mean $\pm$ S.E. The means comparisons were made by using one-way Analysis of Variance (ANOVA) using Graph Pad Prism 03n software. Statistical significance was set at $p < 0.05$.

Results

Haematological indices

Rats treated with STZ resulted in a highly significant decrease in RBCs count, WBCs count, platelets count, Hb concentration and PCV value at ($p < 0.01$) when compared with the normal rats. Diabetic rats intoxicated with DOX showed a highly significant decrease in RBCs count, WBCs count, platelets count, Hb concentration and PCV value at ($p < 0.01$) when compared with the diabetic rats and normal rats. These results were recorded in Figure 1.

Diabetic rats treated with fullerene C₆₀, mesocarp fruit extract of *Balanites aegyptiaca* and (fullerene C₆₀ + mesocarp fruit extract of *Balanites aegyptiaca*) there were improvement ($p < 0.01$) in RBCs count, WBCs count, platelets count, Hb concentration and PCV value when compared with diabetic rats (group 2) but it was not reach to normal animals (group 1). Diabetic rats intoxicated with DOX treated with fullerene C₆₀, mesocarp fruit extract of *Balanites aegyptiaca* and (fullerene C₆₀ + mesocarp fruit extract of *Balanites aegyptiaca*) for 60 days show enhancement at ($p < 0.01$) in RBCs count, WBCs count, platelets count, Hb concentration and PCV value when compared with diabetic (group 2) and diabetic rats intoxicated with DOX (group 3).

Effect on biochemical parameters

Kidney function

As shown in Figure 2 creatinine, urea and uric acid in serum of rats treated with STZ (group 2) resulted in a highly significant increase at ($p < 0.01$) when compared with the normal rats. Diabetic rats intoxicated with DOX (group 3) showed a highly significant increase at ($p < 0.01$) in comparison with diabetic rats. Diabetic rats treated with fullerene C₆₀, mesocarp fruit extract of *Balanites aegyptiaca* and (fullerene C₆₀+mesocarp fruit extract of *Balanites aegyptiaca*) indicated revealed a highly significant decrease at ($p < 0.01$) in serum

creatinine, urea and uric acid levels comparing to diabetic rats (group 2).

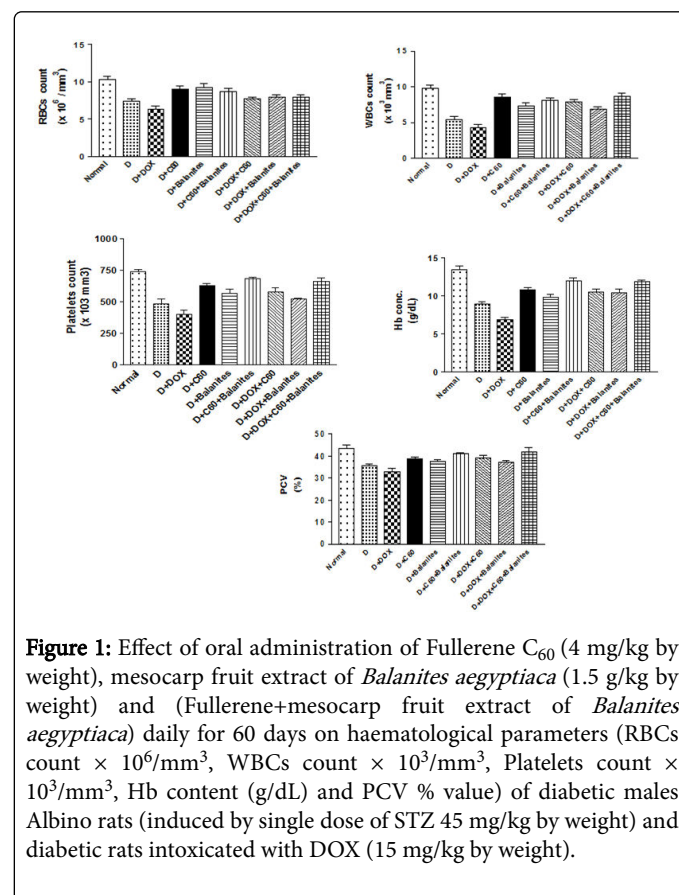


Figure 1: Effect of oral administration of Fullerene C₆₀ (4 mg/kg by weight), mesocarp fruit extract of *Balanites aegyptiaca* (1.5 g/kg by weight) and (Fullerene+mesocarp fruit extract of *Balanites aegyptiaca*) daily for 60 days on haematological parameters (RBCs count $\times 10^6/\text{mm}^3$, WBCs count $\times 10^3/\text{mm}^3$, Platelets count $\times 10^3/\text{mm}^3$, Hb content (g/dL) and PCV % value) of diabetic males Albino rats (induced by single dose of STZ 45 mg/kg by weight) and diabetic rats intoxicated with DOX (15 mg/kg by weight).

As well as, diabetic rats intoxicated with DOX (group 3) treated with fullerene C₆₀, mesocarp fruit extract of *Balanites aegyptiaca* and (fullerene C₆₀+mesocarp fruit extract of *Balanites aegyptiaca*) revealed a highly significant decrease at ($p < 0.01$) in serum creatinine, urea and uric acid levels comparing with diabetic rats (group 2) and diabetic rats intoxicated with DOX (group 3).

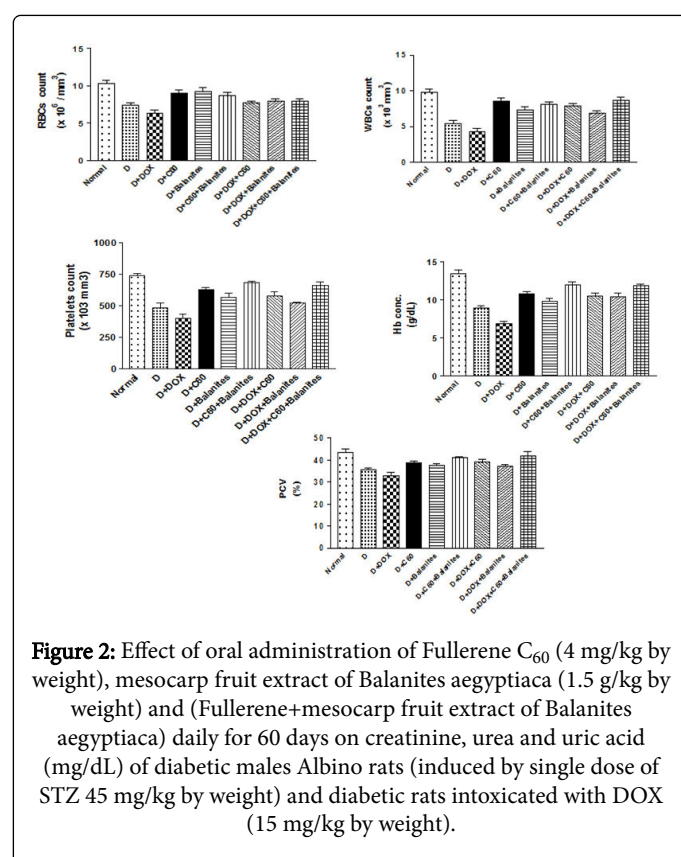
Discussion

Chronic hyperglycaemia is associated with long term damage, dysfunction and failure of various body organs by involvement of micro and macro-vasculature [24]. Hyperglycaemia generates more Reactive Oxygen Species (ROS) and promotes oxidative stress, which is known to play crucial role in the pathogenesis of DM [25].

Data of the present study have indicated that diabetic rats resulted in disorders in the haematological constituents as manifested by a highly significant decrease in the number of RBCs, WBCs, platelets, remarkable fall in haemoglobin content (Hb) and highly significant drop in PCV value, which may be due to alteration in bone marrow as well as haemopoietic system of the animals. Similar observations were obtained by [26] who reported that the decreased levels of WBC and platelet in diabetic rats indicate a suppression of the immune system. The decreased immunity can contribute to the various complications associated with diabetes. Also, high levels of free radicals, during diabetes, cause damage to cellular proteins, membrane lipids and nucleic acids, and cell death. It has been indicated that anaemia has

failed erythropoietin production that fails in the kidneys and raises non-enzymatic glycosylation of membrane proteins of red blood cells. Based on the findings, changes in RBC, Hb content and PCV levels in diabetic rats are the cause of anaemia [6,27].

Besides that, the present recorded results in this study showing a decrease in RBCs, WBCs and PLTs value in diabetic rats intoxicated with DOX. DOX induced reduction in RBCs, WBCs, platelets, haemoglobin content (Hb) and PCV value [28]. It is noticeable doxorubicin in the rats induced a marked leukopenia and thrombocytopenia may be attributed to the destructive effects of DOX on peripheral blood cells and bone marrow [29,30]. DOX can induce platelet lysis directly through the sequential mechanisms of oxidative stress [31]. The developed anaemia in DOX- treated rats could be explained by haematopoiesis and haemoglobin synthesis reduction and probably due to a severe haemolysis in response to antioxidant system imbalance. It was proven that free radicals interact with cell membranes and thereby induce cell leakage and lyses [32].



The present study demonstrated that, the nanosphere of fullerene C₆₀ administrated to diabetic rats and diabetic rats toxicated with DOX, caused haematological changes in blood of rats, which characterized by a pronounced improvement of total number of RBC, WBCs and platelets, remarkable improvement in haemoglobin contents (Hb) and significant increase in PCV value. This improvement may be attributed to the fullerene C₆₀ reacting with both; the drug and the membrane proteins of the erythrocytes. Therefore it may be diminish the complication of diabetes and the side effects of DOX [33]. The antioxidant activity of fullerene C₆₀ may be due to inhibition of lipid peroxidation by scavenging free radicals where haematological parameters were alleviated.

Moreover, it is clear that there is an increase in the RBCs, WBCs, Hb content and PCV value count after mesocarp fruit extract of *Balanites aegyptiaca* treatment at the end of the experimental period. Improvement of WBCs and platelets due to its antioxidant activity was observed [20]. The antioxidant activity of mesocarp fruit extract of *Balanites aegyptiaca* may be due to inhibition of lipid peroxidation by scavenging free radicals and stabilization of red blood cell membranes where RBCs and related parameters were alleviated. In another view, flavonoids of mesocarp fruit extract of *Balanites aegyptiaca* can stimulate the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce red blood cells [34].

It is worthily mention that diabetic nephropathy is one of the most complications of diabetes. It include hyper filtration and renal hypertrophy [35,36]. The levels of non-protein nitrogenous substances are always used as significant markers for the assessment renal dysfunction characterized by proteinuria. The results showed an increase in urea, creatinine and uric acid due to STZ-induced metabolic disturbances as well as renal dysfunction. Another possibility, the increase of these parameters may be attributed to protein catabolism and glomerular injury [36,37].

Also, DOX-produced renal injury was evidenced by an elevation in serum urea and creatinine. DOX induced nephrotoxicity due to free radical formation and generation of oxidative stress. These results might be attributed to the induction of oxidative stress and inflammation cascade resulting in an increase in glomerular filtration rate, structural integrity derangement in the renal cells and kidney dysfunction [38,39].

The recorded results when comparing the levels of urea, creatinine and uric acid in the groups treated with fullerene C₆₀ with their diabetic rats and diabetic rats intoxicated DOX. Additionally, the fullerene C₆₀ has the ability to aggregate in serum and it is mainly distributed to liver and kidney [40] and they act as the free radical scavenger and protect the cells from damage which lead to return kidney function parameter to the normal value.

In regard to mesocarp fruit extract of *Balanites aegyptiaca* treatment animals, when compared with diabetic rats and diabetic rats intoxicated with DOX the enhancement take place. The preventive of mesocarp fruit extract of *Balanites aegyptiaca* effects on diabetic rats and diabetic rats intoxicated with DOX, side effects have been attributed to the ability of mesocarp fruit extract of *Balanites aegyptiaca* preventing the decline of the renal antioxidant status or due to have antioxidant and radical scavenging- activity of mesocarp fruit extract of *Balanites aegyptiaca* has beneficial effects of mediators of large vessel damage so it has ability to the prevention or delay of diabetic nephropathy [15]. *Balanites aegyptiaca* fruits as herbal tea showed enhanced the renal function where it induced a reduction in the creatinine and urea [41].

Conclusion

It could be concluded that, mesocarp fruit extract of *Balanites aegyptiaca* and fullerene C₆₀ could be used as a powerful antioxidant against diabetes related complication and the side effects of doxorubicin.

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