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Role of *Annona muricata* (L.) in Oxidative Stress and Metabolic Variations in Diabetic and Gamma-irradiated Rats

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A. MURICATA L., commonly known as graviola, is a plant widely distributed throughout the world rich in phytochemicals and minerals. The objective of this study is to investigate the influence of *A.muricata* on oxidative stress in the liver, kidney and pancreas along with variations in glucose, insulin, lipid profile, liver and kidney functions in diabetic (DM) and γ-irradiated rats (IRR).Diabetes was induced by a single intraperitoneal injection of streptozotocin (65 mg/Kg body weight). Irradiation was performed as a whole body γ-irradiation (5 Gy) administered in a single acute dose.*A.muricata* leaves aqueous extract (100 mg/Kg body weight) was administered via gavages during 2 weeks to diabetic rats or during 2 weeks before γ-irradiation. Diabetic and irradiated rats received *A.muricata* during 2weeks before irradiation. Animals were sacrificed 24 hours post irradiation and/or *A.muricata* trearment. *A.muricata* treatment has significantly attenuated hyperglycemia, hypoinsulinemia and dyslipidemia, and reduced the increase of serum alanine and aspartate amino transferase activities and serum urea and creatinine levels. The amelioration in metabolic variations was associated to significant improvement of oxidative stress in tissues notified by a higher superoxide dismutase (SOD) activity and glutathione (GSH) content and a lower malondialdehyde (MDA) content.It could be suggested that the synergistic relationship between the different elements found in the leaf of *A.muricata* could be beneficial in ameliorating liver and kidney functions and correcting metabolic variations associated with oxidative stress in the liver, kidney and pancreas.

Keywords: *Diabetes, streptozotocin,γ-irradiation, A.muricata, oxidative stress, metabolic variations.*

Introduction

In recent years, an interest in the phytochemistry of plant has been sparked. The genus *Annona* belonging to the Custard Apple family, Annonaceae, is widespread in the tropical regions of the world and includes the species *Annona muricata* (Linn). The phytochemical analysis conducted on *A.muricata* dried and powdered leaves revealed the presence of flavonoids, alkaloids, tannins, saponins and reducing sugars. Mineral analysis showed the presence of potassium (363.05 mg/kg), calcium (11,183.50 mg/kg), sodium (694.86 mg/kg), magnesium (9,619 mg/kg), iron (139.50 mg/kg), zinc (8.34 mg/kg), manganese (8.25 mg/kg), chromium (3.75 mg/kg), copper (14.25 mg/kg) and cadmium (5.49 mg/kg) (Usunomena and Paulinus, 2015).

Attention to the role of *A. muricata* in human health has increased following the discovery that it possesses anticancer (Asare et al.,2015; Coria-Tellez et al., 2016),antidiabetic(Ahalya et al., 2014), anti-inflammatory (Isholaet *al*., 2014), hepatoprotective(Art hur et al., 2012), antioxidantandfree radical scavenging activities (George et al., 2015; Coria-Tellez et al., 2016). The plant A. m*uricata* was even described as a "Miracle Fruit" (Patel and Patel, 2016).

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia due to defects in insulin production and/or action (American Diabetes Association, 2009). Along with hyperglycemia,diabetes is associated with degenerative complications in many organs including the pancreas, liver, kidney, heart, muscles, and eyes.Oxidative stress was reported to have a central role in the onset of DM and its complications (Wang *et al*., 2015).

On the other hand, exposure to ionizing radiation has become inevitable due to the increase in the development of nuclear

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technology.Experimental studies demonstrated that receiving an acute dose of ionizing radiation induces oxidative stress associated with metabolic alterations (Saada *et al*., 2016).Efficient defense and repair mechanisms exist in living cells to protect against oxidant species. Superoxide dismutase (SOD) catalyzes the reduction of superoxide anion to hydrogen peroxide (H_2O_2), which is broken down by catalase and glutathione peroxidase (GSH-Px) (Sun et al., 1998). However under abnormal conditions, the antioxidant system may not be adequate to protect from oxidative stress and metabolic alterations.

The objective of this study is to investigate if *A. Muricata* leaves aqueous extract would alleviate oxidative stress associated with some metabolic alteration in diabetic, γ-irradiated and diabetic- γ-irradiated rats.

Materials and Methods

Animals

Healthy male adult albino rats *Sprague-Dawley* (10 ± 2 weeks old; 120 ± 20 g) were obtained from the Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt.Animals were housed under standard laboratory conditions of ventilation, temperature and humidity in specially-designed plastic cages along the experiment period. The rats were fed with commercially available rat pelleted diet, containing all the required nutritive elements. Water and food were provided *ad libitum* throughout the study.Experimental analyses were performed in the morning at 11:00 ± 1.00 hour.All animal procedures were approved by the Ethics Committee of the National Research Center conformed to the "Guide for the care and use of Laboratory Animals" published by the National Institutes of Health (NIH publication No. 85–23, revised 1996).

Induction of Diabetes

Streptozotocin (STZ), purchased from Sigma Chemical Company, St. Louis Missouri, USA, in the form of 1 g vial was administered to rats in a single intraperitoneal (i.p.) injection at a dose of 65 mg/Kg body weight, dissolved in freshly prepared 0.1 M cold sodium citrate buffer (pH 4.5) (Erejuwa et al., 2011). Owing to the high destructive power of STZ on pancreatic β-cells and massive release of insulin, 10% sucrose solution was allowed to rats for the next 24hours to avoid hypoglycemic shock (Gandhi and Sasikumar, 2012). Monitoring of blood glucose levels was performed 72 hours after STZ administration, using an Accu-check blood glucose meter (Roche Diagnostics, Basel, Switzerland) in tail vein blood. Rats with blood glucose levels ≥ 250 mg/dL were considered diabetics and selected for this study.

Radiation Treatment

A whole-body γ -irradiation of rats with 5 Gy, applied as a single acute dose, ata dose rate of 0.5 Gy/minute was carried out at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The source of radiation was a Canadian Gamma cell-40 (Cesium-137), which ensured a homogeneous dose distribution all over the irradiation tray.

Annona muricataTreatment

Annona muricata leaf aqueous extract was purchased from USA, under the trade name of Graviola 750 in the form of capsules. The content was dissolved in saline and administered to rats daily via gavages at doses of 100 mg/Kg body weight/day (in 1m L saline) during 14 consecutive days according to Florence *et al*. (2014).

Experimental Design

A total of 80 rats were divided into 8 groups (10 rats per group):1- Control group: Rats given 1 mL saline during 14 days via gavages. 2- *A. muricata* group: Rats daily supplemented with *Annona muricata* (100 mg/Kg body weight/day) during 14 days via gavages.3- DM group: Diabetic rats given 1mL saline daily during 14 days via gavages.4- DM + *A. muricata* group: Diabetic rats given *A. muricata* (100 mg/Kg body weight/day) daily during14 days via gavages.5- IRR group: Rats given 1 mL saline during 14 days via gavages then exposed to a whole body γ-irradiationat a dose rateof 5Gy.6- *A. muricata*+ IRR group: Rats given *A. muricata* (100 mg/Kg body weight/day) daily during 14 days via gavages before γ-irradiation (a whole body exposure at 5 Gy).7- DM + IRR group: Diabetic rats given saline via gavages during 14 days then whole body γ-irradiated with 5 Gy.8- DM + *A. muricata*+ IRR group: Diabetic rats given *A. muricata* via gavages during 14 days then whole body γ-irradiated with 5 Gy.

Collection and Processing of Blood and Tissue Samples:

The animals were sacrificed 24 hr. post-irradiation or *A. muricata* treatment after a fasting period of 12 hr. Blood samples were obtained via heart puncture by sterilized syringe and the serum obtained after centrifugation at 3000 rpm for 15 min

(Cent rifuge, PLC-036, Taiwan). The liver, kidney and pancreas were quickly excised washed in ice-cold saline. A homogenate was prepared in 0.9 % saline (20% W/V) using digital homogenizer (WiseTis HG-15D, Germany) and the homogenates were stored at –20º C until a further biochemical analysis.

Biochemical Analysis

Chemicals and reagents were purchased from Sigma-Aldrich, St Louis, MO, USA otherwise mentioned. Measurement of absorbance was performed using a T60 UV/VIS spectrophotometer, PG instruments, London, UK. Tissue homogenates were obtained using digital homogenizer, WiseTis HG-15D, Germany. Centrifugation was carried out using cooling centrifuge, Hettich, MIKRO 22R, Germany.

Estimation of Metabolic Variations

Glucose was determined using diagnostic kit purchased from Spectrum Egypt according to the method described by Trinder (1969). *Insulin* was determined using enzyme-linked immunosorbent assay (ELISA) according to Clark and Hales (1994).The variation of serum *lipid profile* was carried out using Spectrum Egypt diagnostic kit for the estimation of serum triglycerides (TG) (Fossati and Prencipe, 1982), total cholesterol (Richmond,1973) and high-density lipoprotein-cholesterol (HDL-C) (Friedewald et al.,1972). Low-density lipoprotein-cholesterol (LDL-C) was calculated using the formulaof Friedewald et al. (1972):LDL-C level = Total Cholesterol - (TG/5) – HDL-C.The *atherogenic index* (AI); a logarithmic ratio between the concentrations of TG to HDL-C [Log (TG/HDL-C)] used for the diagnosis and prognosis of cardiovascular disease (CVD) (Dobiasova, 2006)was calculated using the online Calculator of atherogenic risk.

AI <0.11 was considered a low risk for CVD; AI (0.11 to 0.21): was considered a medium risk for CVD; AI >0.21 was considered a high risk for CVD.

Assessment of Liver and Kidney Functions

Liver function was assayed by the measurement of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities according to Reitman and Frankel (1957). *Kidney function* was evaluated by measuring serum creatinine and urea levels according to Henry et al. (1974) and Patton and Crouch(1977), respectively.

Assessment of Oxidative Stress

Lipid peroxidation was determined as described by Yoshioka et al. (1979) based on the determination of malondialdehyde (MDA), an end product of lipid peroxidation, which can react with thiobarbituric acid in acidic medium to yield a pink colored trimethine complex which wasmeasured at absorbance of 532 nm.*Superoxide dismutase activity (SOD)* was determined according to the method of Kakkar *et al.*(1984). *Glutathione (GSH)*content was determined according to the method of Beutler et al. (1963).

Statistical Analysis

All values are represented as Mean ± Standard deviation. All groups were compared by one-way analysis of variance (ANOVA) and post hoc multiple comparisons were done with LSD test in SPSS/PC software program (version 20.0; SPSS Inc., Chicago, IL, USA) to determine the differences between the studied groups. Differences were considered statistically significant at $p \leq 0.05$ and highly significant at $P \leq 0.01$.

Results

Supplementation of rats with the aqueous extract of *A. muricat a*leaves (100 mg/Kg b.wt/ day) daily via gavages during 14 days has not induced significant changes in SOD, GSH and MDA levels in the liver, kidney and pancreas, compared to control(Tables 1&2). SOD activity and GSH content were lower and MDA level was higher in the liver, kidney and pancreas of diabetic (DM),γ-irradiated (IRR) and diabetic-γ-irradiated (DM+IRR) rats compared to contro l.*A. muricata* treatment has significantly ameliorated oxidative stress by increasing SOD and GSH and decreasing MDA as recorded in DM+ *A.muricata*, *A.muricata*+ IRR and DM+ *A.muricata*+ IRR groups (Tables 1&2).

The supplementation of rats with the aqueous extract of *A. muricata* leaves(100 mg/Kg b.wt/ day) daily via gavages during 14 days had no significant effect on glucose, insulin and lipid profile (Table 3).The level of glucose was significantly higher and insulin was significantly lower in the diabetic (DM), γ-irradiated (IRR) and diabetic-γ-irradiated (DM+ IRR) groups, compared to control. *A. muricata* treatment restored glucose and insulin to the normal level (Table 3).The levels of triglycerides, cholesterol and LDL-C were significantly higher and HDL-C

was significantly lower in the diabetic (DM), γ-irradiated (IRR) and diabetic-γ-irradiated (DM+ IRR) groups, compared to control (Table 3).*A. muricata* treatment has significantly ameliorated the variations in lipid profile by reducing triglycerides, cholesterol and LDL-C and increasing HDL-C (Table 2). The atherogenic index (AI) in diabetic rats was 0.113 indicating a low risk of CVD while in diabetic irradiated rats AI was 0.229 indicatinga high risk. *A. muricata* treatment has significantly improved the AI in diabetic and diabetic irradiated rats (Table 3).

The supplementation of rats with the aqueous extract of *A. muricata* leaves(100mg/Kg b.wt/day) daily via gavages during 14 days had no effect on AST and ALT activities,urea and creatinine levels, compared to control (Table 4). AST and ALT activities,urea and creatinine levels were significantly elevated in the diabetic (DM),γ-irradiated (IRR) and diabetic-γ-irradiated (DM+IRR) rats, compared to control. *A. muricata* treatment has significantly lowered ALT and AST activities as well as urea and creatinine levels (Table 4).

TABLE 1. Influence of Annona muricata (AM) on antioxidants in different tissues of diabetic (DM), γ-irradiated (IRR) and diabetic-γ-irradiated (DM+IRR) rats.

Rat Groups	Control	AM	DM	DM +AM	IRR	AM +IRR	DM +IRR	DM +AM +IRR
Superoxide dismutase (U/g tissue)								
Liver	36.33 ±0.52	36.58 ±0.49	20.26 ±2.76 (-44%) a**	30.08 ±0.92 (-17%) b**	28.17 ±1.17 (-23%) a**	30.60 ±4.73 (-16%) b**	28.47 ±0.93 (-22%) a**	31.79 ±0.34 (-13%) b**
Kidney	32.00 ±3.43	30.92 ±0.49	23.50 ±1.38 (-27%) a**	30.08 ±1.11 (-6%) b**	29.00 ±0.89 (-9%) a*	31.58 ±1.36 (-1%) b*	25.83 ±0.41 (-19%) a**	29.10 ±0.86 (-9%) b*
Pancreas	7.35 ±0.07	7.14 ±0.21	6.13 ±0.68 (-17%) a**	6.96 ±0.28 (-5%) b*	5.87 ±0.80 (-20%) a**	7.1 ±0.26 (-3%) b**	6.16 ±0.96 (-16%) a**	7.26 ±0.12 (-1%) b*
Glutathione (mg/g tissue)								
Liver	20.73 ±3.04	19.52 ±1.05	6.46 ±0.96 (-69%) a**	19.28 ±1.07 (-7%) b**	7.46 ±0.61 (-64%) a**	21.35 ±0.63 (-3%) b**	7.55 ±1.13 (-64%) a**	11.80 ±1.41 (-43%) b**
Kidney	25.02 ±2.26	23.67 ±4.50	11.8 ±0.94 (-53%) a**	24.04 ±2.15 (-4%) b**	10.71 ±0.95 (-57%) a**	24.0 ±2.94 (-4%) b**	9.86 ±0.80 (-61%) a**	21.35 ±1.70 (-15%) b**
Pancreas	7.48 ±0.68	7.72 ±1.48	5.59 ±0.22 (-25%) a**	7.60 ±0.70 (+2%) b**	6.56 ±0.20 (-12%) a*	7.58 ±0.43 (+1%) b*	6.08 ±0.63 (-19%) a*	7.70 ±0.31 (+3%) b**

Data are expressed as Mean ± Standard Deviation (n=10). Numbers between brackets show the percentage of change from the respective control value. a: significance vs control. b: significance vs respective DM, IRR and DM+IRR groups not treated with *A muricata*. *: significant at P≤0.05; **: highly significant at P≤0.01

TABLE 2. Influence of Annona muricata (AM) on lipid peroxidation marker Malondialdehyde (nmol/g tissue) in different tissues of diabetic (DM), γ-irradiated (IRR) and diabetic-γ-irradiated (DM+IRR) rats.

Rat Groups	Control	AM	DM	DM +AM	IRR	AM +IRR	DM +IRR	DM +AM +IRR
Liver	139.0 ±01.90	134.8 ±09.52	247.8 ±14.33 (78%) a**	160.5 ±14.40 (16%) b**	243.0 ±11.54 (75%) a**	167.2 ±12.40 (20%) b**	345.7 ±11.06 (149%) a**	260.2 ±15.29 (87%) b**
Kidney	170.7 ±17.26	174.2 ±10.76	610.2 ±18.83 (258%) a**	419.3 ±21.40 (146%) b**	498.5 ±10.31 (192%) a**	327.2 ±11.70 (92%) b**	974.2 ±37.86 (471%) a**	569.3 ±17.51 (234%) b**
Pancreas	187.3 ±9.75	195.7 ±6.92	299.5 ±13.90 (+60%) a**	189.2 ±9.17 (+1%) b**	231.7 ±13.44 (+24%) a**	183.7 ±11.83 (-2%) b**	337.2 ±7.52 (+80%) a**	257.8 ±10.03 (+38%) b**

Data are expressed as Mean ± Standard Deviation (n=10). Numbers between brackets showthe percentage of change from the respective control value. a:significance vs control. b:significance vs respective DM, IRR and DM+IRR groups not treated with *Amuricata*. *: significant at P≤0.05; **: highly significant at P≤0.01

TABLE 3. Influence of Annona muricata(AM) on some metabolic variations in the serum of diabetic (DM), γ-irradiated (IRR) and diabetic-γ-irradiated (DM+IRR) rats.

Rat Groups	C	AM	DM	DM +AM	IRR	AM +IRR	DM +IRR	DM +AM +IRR
Glucose mg/dL	80.29 ±5.91	81.33 ±6.03	281.4 ±26.79 (250%) a**	78.13 ±11.18 (-3%) b**	131.10 ±7.5 (63%) a**	86.43 ±12.78 (7%) b**	145.17 ±21.2 (80%) a**	85.33 ±6.32 (6%) b**
Insulin µIU/dL	5.72 ±0.09	5.88 ±0.95	4.12 ±0.36 (-28%) a**	5.38 ±0.20 (-6%) b**	4.82 ±0.55 (-16%) a*	5.38 ±0.23 (-6%) b*	4.80 ±0.20 (-16%) a*	5.32 ±0.36 (-7%) b*
Trigly-cerides mg/dL	71.50 ±4.76	72.33 ±4.36	150.6 ±4.86 (111%) a**	88.67 ±9.11 (24%) b**	94.14 ±4.17 (31%) a**	83.16 ±7.44 (16%) b*	170.5 ±8.76 (138%) a**	102.3 ±4.1 (43%) b**
Choles-terol mg/dL	135.7 ±14.39	136.6 ±12.89	382.8 ±16.03 (182%) a**	182.0 ±26.87 (34%) b**	325.3 ±14.12 (140%) a**	165.7 ±11.60 (22%) b**	391.6 ±16.32 (188%) a**	190.8 ±25.38 (41%) b**
HDL-c mg/dL	85.71 ±8.75	87.00 ±9.44	50.67 ±6.71 (-41%) a**	64.00 ±2.76 (-25%) b*	64.10 ±7.68 (-25%) a**	75.63 ±3.91 (-12%) b*	44.17 ±6.11 (-48%) a**	65.17 ±2.78 (-24%) b**
LDL-c mg/dL	35.66 ±6.44	35.16 ±6.96	301.8 ±9.49 (757%) a**	93.16 ±13.63 (166%) b**	242.5 ±8.19 (592%) a**	73.33 ±7.00 (109%) b**	313.5 ±10.84 (796%) a**	112.3 ±13.41 (220%) b**
AI	-0.439	-0.440	0.113	-0.218	-0.193	-0.319	0.229	-0.165

Data are expressed as Mean ± Standard Deviation (n=10). Numbers between brackets show percentage change from the respective control value. a:significance vs control. b:significance vs respective DM, IRR and DM+IRR groups not given AM. *:significant at P≤0.05; **:highly significant at P≤0.01.
Atherogenic index (AI) < 0.11: low risk of cardiovascular disease (CVD); AI (0.11 to 0.21): medium risk of CVD; AI > 0.21 high risk of CVD.

TABLE 4. Influence of Annona muricata(AM) on liver injury markers (ALT and AST activities) and kidney injury markers (urea and creatinine levels) in the serum of diabetic (DM), γ-irradiated (IRR) and diabetic-γ-irradiated (DM+IRR) rats.

Rat Groups	Control	AM	DM	DM +AM	IRR	AM +IRR	DM +IRR	DM +AM +IRR
ALT U/L	21.92 ±1.16	21.52 ±1.22	51.42 ±1.50 (134%) a**	22.36 ±0.70 (2%) b**	25.85 ±2.12 (18%) a**	23.36 ±1.75 (6.6%) b*	30.50 ±1.41 (39%) a**	22.66 ±1.96 (3.4%) b**
AST U/L	30.83 ±3.18	31.29 ±4.37	148.00 ±15.31 (380%) a**	69.25 ±7.47 (124%) b**	76.25 ±5.60 (147%) a**	50.50 ±3.52 (64%) b**	73.09 ±1.81 (137%) a**	49.80 ±6.88 (61%) b**
Urea mg/dL	45.46 ±2.58	45.17 ±2.34	115.50 ±1.83 (154%) a**	52.21 ±6.75 (15%) b**	63.83 ±5.31 (40%) a**	44.64 ±2.08 (-2%) b**	100.20 ±3.76 (120%) a**	71.83 ±7.93 (58%) b**
Creatinine mg/dL	0.94 ±0.07	0.91 ±0.09	1.58 ±0.14 (68%) a**	1.03 ±0.14 (9%) b*	1.09 ±0.06 (16%) a*	1.01 ±0.16 (7%) b*	1.65 ±0.15 (75%) a**	1.20 ±0.16 (27%) b**

Data are expressed as Mean ± Standard Deviation (n=10). Numbers between brackets showpercentage change from the respective control value. a:significance vs control. b:significance vs respective DM, IRR and DM+IRR groups not given AM. *: Significant at P≤0.05; **: Highly significant at P≤0.01

Discussion

Annona muricata L. is a popular fruit tree that has long been used in traditional medicine (Bidlaet al., 2004). *In vivo* and *in vitro* studies revealed that the leaves of Annona muricata possess anti-inflammatory (Ishola et al., 2014), hepatoprotective (Arthur et al., 2012),antioxidant (George et al., 2015; Coria-Tellez et al., 2016) and antidiabetic activities (Florence et al., 2014; Rahmi et al., 2016).

In the current study, the administration of the aqueous extract of *A. muricata* leaves to normal rats at a dose of 100mg/Kg body weight for two weeks had no significant effect on the oxidative stress parameters of the liver, kidney and pancreas tissues. No significant changes were observed in serum glucose, insulin, lipid profile as well as liver and kidney functions. The results are in harmony with previous findings indicating that the oral administration of the aqueous extract of *A. muricata* leaves (100mg/kg bwt) during four weeks had no significant effect on SOD, catalase and MDA (Florence *et al.,* 2014), glucose, lipid profile, ALT and AST activities and urea and creatinine levels (Arthur *et al*., 2011; Florence et al., 2014). The results support the suggestion that *A. muricata* leaves are practically non-toxic (Utomo *et al.,*2015).

In diabetic, γ-irradiated and diabetic-γ-irradiated rats, *A. muricata* treatment has significantly alleviated oxidative stress verified by a higher level of the antioxidant SOD activity and GSH content associated witha lower level of the lipid peroxidation end-product MDA inthe liver, kidney and pancreas compared to their relative levels in rats not treated with *A. muricata*. Experimental studies revealed that the decrease of antioxidants is caused by their increased utilization to neutralize free radicals together with a decreased synthesis (Yoshida et al., 2008; Matsunami et al., 2010) while lipid peroxidation arises by the interaction of $^{\bullet}OH$ radicals with unsaturated fatty acids (Bartsch and Nair, 2002; Spitz et al., 2004).The protective capacity of *A. muricata* is attributed to its role against $^{\bullet}OH$ radicals (Baskar et al., 2007) and H_2O_2 (Muthu and Durairaj, 2015; George et al., 2015). The antioxidant potential of *A. muricata* might be ascribed to the presence of phytochemicals (Muthu and Durairaj, 2015) including luteolin, quercetin, epicatechingallate and emodin(George et al., 2015),nonenzymatic antioxidants such asVitaminE (Muthu and Durairaj, 2015), VitaminC and carotenoids (Usunomena and Paulinus, 2015), andenzymatic antioxidants including catalase, glutathione reductase and SOD (Muthu and Durairaj, 2015). The presence of ascorbic acid suppresses

peroxidation in both aqueous and lipid region of cells (Dadheech et al., 2006). It traps peroxyl radicals before they can initiate lipid peroxidation and helps in the regeneration of Vitamin E (Chatterjee and Nandhini, 1991).The results are in harmony with previous findings indicating that the aqueous extractof *A. muricata*leaves protects against oxidative stress (Adewole and Ojewole, 2009; Olakunle et al., 2014) and enhancesSOD andcatalase activities, increases GSH content and reduces MDA (Moghadamtousi et al., 2015).

In diabetic, γ-irradiated and diabetic-γ-irradiated rats *A. muricata* treatment has significantly alleviated hyperglycemia, hypoinsulinemia and dyslipidemia. Hyperglycemia is generally the consequence of insulin deficiency (Akbarzadeh et al., 2007) due to oxidative stress and degeneration of pancreatic β-cells (Szkudelski, 2012), in addition to oxidative damage of DNA (Nieman and Schalinske, 2011**)** causing depression of insulin synthesis(Kaneto et al.,1999). Dyslipidemia might be attributed to oxidative stress in liver tissues and alteration ofcholesterol, triglycerides, and lipoproteins synthesis (Zakim and Thomas, 2002). Moreover, oxidative stress induces damage to the receptors on the surface of many cells in the body which prevents the ingestion of LDL-C by endocytosis and might contribute to the increase of total cholesterol (Gent and Braakman, 2004). *A muricata* treatment has significantly reduced the levels of glucose,triglycerides and LDL-Csupporting the anti-hyperglycemic and anti-hyperlipidemic properties of *A.muricata* (Adeyemi*et al.,* 2008a).

The results corroborate that the oral administration of the aqueous extract of *A. muricata* leaves reduced glucose level (Florence et al., 2014). The modulatory role of *A. muricata* on hyperglycemia and hypoinsulinemia might be attributed to its role in the protection of pancreatic β-cells against oxidative stress (Florence et al.,2014) and regeneration of β-cells (Adeyemi et al., 2008b). The antioxidant effect is probably mediated by the inactivation of NF-κB and the consequent decrease in formation of nitric oxide (NO),a mediator of islet beta cell damage (Wolff et al., 1991). Supporting this postulation, histological examination of pancreas tissues revealed that *A.muricata* protected and preserved pancreatic β-cell integrity in parallel to a significant decrease of blood NO (Adewole and Caxton-Martins, 2006).Furthermore, the leaves of *A.muricata* contain magnesium (Mg), Chromium (Cr) and zinc (Zn) (Usunomena and Paulinus, 2015). Magnesium helps insulin secretion from the beta cells (Gommers et al., 2016) and thusregulates insulin level.Chromiumis an essential mineral that is thought to be necessary for normal glucose and lipid homeostatsis(Cefalo and Hu, 2004). In this line, Zhang et al. (2014) suggested that chromium improves blood glucose in diabetic rats by activating insulin synthesis in islet.On the other hand (Emdin et al., 1980) found that zinc plays an important role in insulin production in the β-cell.

Also the hypoglycemic activity of *A.muricata* leaves might be attributed to the presence of certain flavonoids that have the ability to inhibit α-glucosidase (Rahmi*et al.,* 2016) thus decreasing carbohydrate metabolism and glucose absorption (Hardokoet al., 2015). Additionally, the glycemic index and the glycemic load of *A.muricata* were found to be low in *A. muricata*, which supports its hypoglycemic potential (Passos et al., 2015).

In the current study,the improvement of dyslipidemia in *A. muricata*-treated rats are in agreement with the findings of Ahalya *et al.* (2014) suggesting that *A. muricata* reduces cholesterol and triglyceride levels. The results are also in harmony with the findings of Adewole and Ojewole (2009) and Florence et al. (2014) who reported that the oral administration of the leaf aqueous extractof *A. muricata* (100 mg/kg/day) to diabetic rats during four consecutive weeks has significantly decreased the elevated total cholesterol, triglycerides and LDL-C. Moreover, *Annona muricata* leaves contain saponins, known to produce inhibitory effect on inflammation (Just et al., 1998). Saponins as a class of natural products are involved in complexation with cholesterol to form pores in cell membrane bilayers (Francis et al., 2002), and as such may be used as anti-cholesterol agents or cholesterol lowering agents.

In diabetic, γ-irradiated and diabetic-γ-irradiated rats,theelevationof serum AST and ALT activities indicate liver injury (Botros and Sikaris, 2013) while the increase of urea and creatinine indicates kidney damage (Siew et al., 2011). In the current study, liver and kidney injury appears to be the consequence of oxidative stress verified by the increase of MDA associated with a decrease of SOD activity and GSH content. Increased lipid peroxidation of cell membrane causes alteration of cell membrane permeability. *A. muricata* treatment has significantly alleviated liver and kidney dysfunction. The results corroborate the

hepatoprotective role of *A. muricata*(Adewole and Ojewole, 2009) and that *A.muricata* aqueous leaf extract could restore liver function toward normal levels (Arthur et al., 2012).

Experimental evidence suggests that pro-inflammatory cytokines such asInterleukin-1β (IL-1β) and Tumor necrosis factor-alpha (TNF-α) play an important role in liver (Christiansen et al., 2007) and kidney damage (Chan *et al.,* 2010; Hamid et al., 2012).Thus, in the current study, the amelioration of liver and kidney functions in *A. muricata*- treated rats might be attributed to its anti-inflammatory properties (Ishola et al., 2014) and attenuation of TNF-α and IL-1β protein expression (Chan et al., 2010; Hamid et al., 2012). Moreover, the amelioration of liver and kidney functions appears to be the consequence of the improvement of oxidative stress.

The present study demonstrates that *A. muricata* ameliorates oxidative stress in the liver, kidney and pancreas, associated with improvement of hyperglycemia, hypoinsulinemia, hyperlipidemia besides improvement of both liver and kidney functions which could be attributed to the synergistic relationship between the different elements found in the leaves of *A.muricata.* It could be concluded that supplementation of *A.muricata* could be beneficial in ameliorating liver and kidney functions and correcting metabolic variations associated with oxidative stress in the liver, kidney and pancreas.

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دور"أنونا ميوريكاتا" في الإجهاد التأكسدى و التغيرات الأيضية فى الجرذان المُصابة بالسكرى و المُعرضة لأشعة جاما

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يُعْرف نبات "أنونا ميوريكاتا" بـ "الجرافيولا" و هو نبات مُنتشر علي نطاق واسع فى جميع أنحاء العالم و يمتاز باحتواءه على الكثير من المواد الكيميائية النباتية و المعادن.تهدف هذه الدراسة إلي معرفة تأثير أوراق نبات "أنونا ميوريكاتا" على الكبد ،و الكُلى ،و البنكرياس المُصابة بالإجهاد التأكسدى فضلاً عن التغيرات بمستويات الجلوكوز ، الأنسولين ، الدهون, وظائف الكبد و الكُلى لدى جرذان التجارب المُصابة بداء السكرى و المشعة جاميًا. ولإستحداث مرض السكرى تمّ حقن جرذان التجارب بجرعة ٦٥ مجم/كجم من وزن الجسم على دفعة واحدة بمادة الستربتوزوتوسين عبر التجويف البريتونى.تمّ تشعيع مجموعة من الجرذان بأشعة جاما كجرعة حادة بمقدار ٥ جراى.و تمّ تجريع جرذان التجارب المُصابة بداء السكرى أوقبل تشعيعها جاميًا بالمستخلص المائى لأوراق "أنونا ميوريكاتا" بالفم بجرعة ١٠٠ مجم/كجم من وزن الجسم مرة واحدة يوميا لمدة ١٤ يومًا.

و قد تمّ تجريع جرذان التجارب المُصابة بداء السكرى بـ"أنونا ميوريكاتا" طيلة 14يومًاقبل تشعيعهم .وفي نهاية التجربة ، يتم ذبح الحيوانات بعد مرور 24 ساعة من الجرعة الإشعاعية أو جرعة "أنونا ميوريكاتا". وقد أبرزت النتائج أنه قد خفف العلاج بـ"أنونا ميوريكاتا" ارتفاع السكر، وانخفاض الأنسولين و تدهور مستويات الدهون بالدم بدرجة ملحوظة كما أنه عَمَلَ على خفض فرط نشاط إنزيمى الكبد إنزيم الانينأمينو ترانسفيريز(ALT)و أسبارتات أمينو ترانسفيريز(AST) إلى جانب خفض مستويات اليوريا و الكرياتينين بمصل الدم.

كانت التحسينات فى التغيرات الأيضية مصحوبة بتحسن ملحوظ في الإجهاد التأكسدى داخل الأنسجة و الملاحظ من خلال ارتفاعنشاط إنزيم سوبر أكسيد ديسميوتيز و المحتوى الجلوتاثيونى مع انخفاض نسب المالون داي ألدهيد.

ولقد أوضحت نتائج البحث أنه من المحتمل أن يكون هناك علاقة تآزر بين العناصر المختلفة الموجودةبأوراق نبات "أنونا ميوريكاتا" و التحسن الإيجابى الملاحظ بوظائف الكبد و الكلى فضلا عن تحسن التغيرات الأيضية المصاحبة للإجهاد التأكسدى بأعضاء الكبد ، والكلى ، و البنكرياس.