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Effects of some selected flavonoids on antioxidant status and kidney function parameters of streptozocin induced diabetic rats

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Abstract
Diabetes mellitus is one of such diseases whose onset and progression could be potentiated by reactive oxygen and nitrogen species (ROS/RNS). Diabetes mellitus if not properly managed could lead to several micro and macrovascular complications among which is diabetic nephropathy. The aim of the study is to determine the effects of some selected flavonoids on endogenous antioxidant systems of kidney and some kidney function parameters of streptozocin-induced Diabetic rats. Albino rats were acclimatized and divided into 14 groups of 6 animals each (84). Diabetes was induced in the rats using single intra peritoneal injection of streptozotocin 50 mg /kg body weight of the animals. Animals with fasting blood glucose (FBG) of 200 mg/dL or more after 48 to 78 hours of induction were considered diabetic. Three graded doses (100, 200 and 400 mg/kg body weight) per flavonoid samples were administered to the diabetic rats. Groups 1 - 3, 4 - 6 and 7-9 received graded doses of food grade quercetin, rutin and luteolin respectively in the order of 100, 200, and 400mg of flavonoid. Groups 10, 11, 12 were diabetic rats administered with vitamin C 1.5 mg, Vitamin E 2.31 IU and Glibenclamide 5 mg /kg body weight respectively, while groups 13 (Diabetic control) and 14 (Normal control) were administered with almond oil. After four (4) weeks of administration and sacrifice. Blood samples were collected and tissues (Kidney) were excised for the preparation of serum and homogenates. *In vivo* antioxidant activities of some endogenous kidney enzymatic antioxidant, glutathione and malondialdehyde were determined using the homogenate, while urea and creatinine were determined using serum. Data collected were analyzed using SPSS software 21 and Analysis of variance was performed with n=4 sample data. Among the flavonoids tested, rutin 100 mg / kg body weight improved the kidney antioxidant the most and also lowered the malondialdehyde level when compared with the diabetic control. However, rutin 400 treated group had the least serum creatinine level. Rutin and Luteolin improved kidney functionality at dose concentrations shown in serum parameters of kidney function test results. The combination of these antioxidants at the doses found effective may have synergistic action and may yield a more profound result.

Keywords: Kidney function, kidney antioxidant status, urea, creatinine, malondialdehyde.

Introduction

The increase in oxidative stress in diabetes mellitus could be attributed to elevated blood glucose levels, which upon auto-oxidation generates free radicals and damages the cell membrane through peroxidation of membrane lipids (Ito *et al*., 2019) and protein glycation (Negre- Salvayre *et al.,* 2009). Chronic hyperglycemia is positively correlated with long-term damage, dysfunction and failure of different organs (Lyra *et al*., 2006) especially the eye, nerve, heart, kidney and blood vessels. Chronic hyperglycemia is believed to play a major role in initiation of vascular complications through many metabolic and structural derangements which include advance glycation end product generation, abnormal activation of signaling cascade such as protein kinase C (PKC), elevated production of reactive oxygen species (which interact and damage biomolecules) and abnormal stimulation of hemodynamic regulation system such as renin-angiotensin system (Cade, 2008). Targeted glucose control can reduce vascular complications. For

every percentage decrease in hemoglobin AIc (HbAIc), there is 35% risk reduction of microvascular complication (UKPD, 1998). Among the diabetic micro vascular complications is nephropathy leading to renal failure; (ADA, 2014).

Flavonoids are secondary metabolites in plants that have some desirable characteristics (Ruiz-Cruz *et al*., 2017). The health benefiting effects of flavonoids is attributable to their antioxidant, antibacterial, antiviral and anti-inflammatory properties and also as a result of their ability to scavenge for free radicals and protect cells from oxidative damage activity as conferred on them by the presence of hydroxyl group (reducing power) (Ruiz-Cruz *et al*., 2017). In addition to the above, their anti-mutagenic, modulatory effects on key cellular enzyme function (Panche *et al*., 2016), hepatoprotective, coronary heart disease prevention and anticancer activities (Kumar and Pandey, 2013). This research studied the effects of flavonoids administration on the kidney endogenous antioxidant status and some kidney functions parameters of streptozocin induced diabetic rats. The results generated from this research will provide useful information for effective management and treatment of diabetes mellitus and its complications using the flavonoids. Treatment of individuals prior to disease manifestation may delay the onset of diabetes mellitus and its complication in individuals with predispositions to diabetes.

Materials and Methods

Source of food grade flavonoids used

Five hundred (500) g each of the food grades Quercetin and Rutin, with Cas number 117-39-5, 153-18-4 and product number CN Lab 161025, CN Lab 151022 respectively with percentage purity (98%), were purchased from a Food grade Chemical company, CN Lab Nutrition China. One hundred (100) g of the food grade, Luteolin, with product number HK 161125 with percentage purity 98% was purchased from a Food grade Chemical company Hunan Hua Kang Biotechnology, China.

Source of experimental animals

All the animals (albino rats) used for the study were purchased from National Veterinary Research Institute (NVRI), Vom, Plateau State and College of Health Sciences, Kogi State University, Anyingba, Kogi State. The rats were housed in well-ventilated clean aluminium cages in the department of Biochemistry animal house, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, and fed with vital feed and clean water for two weeks to acclimatize them prior to commencement of the study. The rats were maintained under standard laboratory conditions of temperature and humidity. After acclimatization, they were weighed and divided into 14 groups of 6 rats each.

Methods

Preparation of streptozotocin

Streptozotocin 20 mg/ml was prepared by dissolving streptozotocin in 0.1 M fresh cold citrate buffer, (pH 4.5).

Induction of diabetes mellitus

Diabetes mellitus was induced by single intraperitoneal injection of streptozocin 50 mg /kg body weight, into 8 hours-fasted rats (Burcelin *et al.*, 1995). Monitoring of the blood glucose level commenced 24 hours after induction. The blood sugar levels were determined with a glucometer (Accuchek) and any rat with fasting blood glucose level more than 200 mg/dL (11.1 mmol/L) was considered diabetic hence selected for the research.

Experimental design

Eighty four albino rats were placed in fourteen groups of six animals each. They were subsequently maintained with growers mash (vital feed) and clean water *ad libitum*.

Groups 1 - 3, 4 - 6 and 7-9 received graded doses of food grade Quercetin, Rutin and Luteolin respectively in the order of 100, 200, and 400mg /kg body weight. Groups 10, 11, 12 were administered with Vitamin C 1.5 mg, Vitamin E 2.31 IU and Glibenclamide 5 mg /kg body weights respectively, while groups 13 (Diabetic control) and 14 (Normal control) were administered with 0.5 ml of almond oil. The food grade flavonoids and vitamin E were separately dissolved in almond oil and administered orally to the rats daily for four weeks period using dosing needle. The volume administered to each rat was less than 2 ml. The fasting blood glucose was estimated on weekly basis (Ampa *et al*., 2017).

Animal sacrifice and serum sample collection

At the end of the fourth week, the animals were fasted overnight (8 hours) and blood was collected through ocular puncture using capillary tubes. Sera were harvested from the blood and used to assay for urea and creatinine respectively.

Collection and preparation of kidney tissue homogenate

Kidneys of the rats were quickly excised from the sacrificed animals and the tissues were washed in ice cold normal saline (0.9% NaCl) and weighed. Twenty five (25) % of the tissues respective homogenates were prepared by homogenizing the tissues using mortar and pestle in 10 mM phosphate buffer saline (PBS) of pH 7.4 (Brostrom and Jeffay, 1970; Priora *et al.,* 2010). The homogenates were centrifuged in a refrigerated centrifuge at 15,000 Xg for 15 min. The supernatants were collected and stored below -20 ^{o}C until used. Kidney supernatant was used for the determination of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA).

Preparation of serum for some biochemical parameters

Blood samples collected from the animals were centrifuged at 3000 Xg for 5 minutes in a refrigerated centrifuge. The supernatants (serum) were stored below -20 ^{o}C until used. Serum samples were used for the determination of urea, creatinine.

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) was assayed by colorimetric method of Misra and Fredovich, (1972).

Determination of catalase activity

Catalase activity was determined by colorimetric method of Sinha (1972).

Determination of glutathione-S-transferase (GST) activity

GST activity was determined spectrophotometrically at 37 ^{o}C as described by Habig *et al.*(1974).

Estimation of glutathione peroxidase (GPx)

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

Determination of reduced glutathione (GSH)

Reduced glutathione was determined by the method of Beutler *et al*. (1963).

Determination of malondialdehyde (MDA) level

MDA level was determined by the colorimetric method of Gutteridge and Wilkins (1982).

Determination of urea level

Blood urea was determined by the method described by Kassirer (1971).

Determination of creatinine level

Serum creatinine was estimated by Jaffe's method as described by Laron (1972).

Results

The results of the *in vivo* antioxidant parameters in Table 1 showed that there was a decrease in the activity of superoxide dismutase (SOD) of the albino rats as the concentration of quercetin was increased from 100 mg/kg body weight to 400 mg/kg body weight. The SOD activities in quercetin treated group (Q100) was also significantly higher ($p<0.05$) than the SOD activity observed in the normal control, vitamin E, glibenclamide, diabetic control. R100 had the highest SOD activity among the Rutin treated animal groups. The activity value (R100) was not significantly higher ($p>0.05$) than the normal control, diabetic control and standard controls (vitamin E and glibenclamide). The albino rats treated with luteolin 400 mg /kg body weight (L400) showed a higher SOD activity among the luteolin treated animal groups. Q100 treated groups had the highest SOD activity among all the food grade flavonoid treatment groups. The rat groups treated with quercetin 200 mg /kg, rutin 400 mg /kg, and luteolin 400 mg /kg body weight show a higher catalase (CAT) activity among the quercetin, rutin, and luteolin treated animal groups respectively. A significant increase ($p<0.05$) in catalase activity was observed in R400 treated group when compared with diabetic control, glibenclamide, vitamin C and vitamin E treated groupsrespectively. Catalase activity similarly increased as the concentration of luteolin administered to the rats increased from 100 to 400 mg/kg body weight. The catalase activity of L400 treated group was significantly higher ($p<0.05$) than all the test, standard and control groups. The rat groups treated with Quercetin 200 mg /kg, Rutin 400 mg /kg and luteolin 400 mg /kg body weight showed higher catalase (CAT) activity among the Quercetin, Rutin, and Luteolin treated groups, respectively. There was a dose dependent decrease in GSH level among the rutin treated groups. The GSH level in luteolin treated groups equally increased in a dose dependent manner. The level of GSH in L400 treated animal group was significantly higher ($p<0.05$) in the normal control, diabetic control, all the test groups and all standard groups (vitamin C, vitamin E and glibenclamide).

An increase was observed in glutathione peroxidase (GPx) activity in quercetin treated animal groups: Q100, Q200 and Q400, over the standard groups, control groups and flavonoid groups. This increase was significantly higher ($p<0.05$) in Q400 treated animal group when compared with the normal control, diabetic control, vitamin C, vitamin E and glibenclamide treated animal groups. The GPx activities in animals treated with rutin (R100, R200 and R400) were higher than the GPx activities of the diabetic control and glibenclamide treated animal groups. There was a dose dependent decrease in the GPx activity of the luteolin treated animal groups. The GPx activities in these groups (L100, L200 and L400) were higher when compared with the GPx activity in normal control, diabetic control, vitamin C, vitamin E and glibenclamide treated animal groups. The Q400 treated animal group showed the highest GPx activity of all the treatment groups. The glutathione -s- transferase (GST) activities of the animals in all the treatment groups were relatively constant and not affected by the treatments. There was dose dependent decrease in the lipid peroxidation product; malondialdehyde (MDA) in quercetin treated animal groups. The values were not significantly lower when compared with diabetic control group. The rutin treated animal group R200 showed increase in MDA level which was significantly higher than the normal control, vitamin E, vitamin C, and glibenclamide. The MDA values at L100 was lower than the value observed in diabetic control group. The overall lowest MDA level was observed in Q400 treated animal group with value of 4.68 ± 0.52.

Table 1: Effects of the food grade flavonoids on Kidney Antioxidant Enzymes

Treatments	SOD (U/g wet tissue)	CAT (U/g wet tissue)	GSH (mg/g wet tissue)	GPX (U/g wet tissue)	GST (U/g wet tissue)	MDA (nmol/g wet tissue)
Q100	94.37 ± 11.16^{cd}	112.02 ± 7.59^{ab}	1.29 ± 0.30^{ab}	4.60 ± 0.07^{a}	0.03 ± 0.01^{a}	9.08 ± 0.07^{bc}
Q200	88.08 ± 4.08^{bcd}	117.22 ± 13.26^{ab}	1.29 ± 0.21^{ab}	4.58 ± 0.03^{a}	0.03 ± 0.01^{a}	9.03 ± 0.07^{bc}
Q400	57.71 ± 5.66^{a}	104.90 ± 10.32^{a}	1.05 ± 0.18^{a}	5.64 ± 1.30^{b}	0.03 ± 0.01^{a}	7.77 ± 4.00^{ab}
R100	93.14 ± 5.83^{bcd}	116.36 ± 9.68^{ab}	1.35 ± 0.03^{ab}	4.46 ± 0.03^{a}	0.03 ± 0.01^{a}	8.71 ± 3.42^{abc}
R200	63.81 ± 15.71^{a}	120.27 ± 10.63^{ab}	1.30 ± 0.11^{ab}	4.46 ± 0.10^{a}	0.03 ± 0.01^{a}	11.30 ± 0.76^{c}
R400	80.19 ± 9.48^{b}	138.04 ± 11.50^{c}	1.18 ± 0.03^{a}	4.51 ± 0.01^{a}	0.02 ± 0.00^{a}	9.03 ± 2.12^{bc}
L100	80.89 ± 8.43^{b}	108.17 ± 4.62a	1.14 ± 0.12^{a}	4.58 ± 0.05^{a}	0.03 ± 0.01^{a}	8.53 ± 1.80^{abc}
L200	65.12 ± 3.51^{a}	113.51 ± 3.99^{ab}	1.41 ± 0.10^{ab}	4.56 ± 0.07^{a}	0.03 ± 0.01^{a}	9.19 ± 1.37^{bc}
L400	86.68 ± 2.58^{bcd}	161.97 ± 16.97^{d}	1.59 ± 0.09^{b}	4.54 ± 0.04^{a}	0.03 ± 0.01^{a}	10.02 ± 0.54^{bc}
Vit. C	99.06 ± 1.13^{d}	106.04 ± 2.17^{a}	1.28 ± 0.44^{ab}	4.56 ± 0.10^{a}	0.03 ± 0.01^{a}	7.34 ± 2.20^{ab}
Vit. E	85.86 ± 7.82^{ab}	120.20 ± 13.66^{ab}	1.26 ± 0.06^{ab}	4.48 ± 0.08^{a}	0.03 ± 0.01^{a}	6.97 ± 1.59^{ab}
Glib	82.51 ± 10.73^{ab}	114.69 ± 13.66^{ab}	1.16 ± 0.49^{a}	4.44 ± 0.09^{a}	0.03 ± 0.01^{a}	6.90 ± 1.79^{ab}
Dia. Ctrl	83.51 ± 0.03^{ab}	114.69 ± 13.66^{ab}	1.19 ± 0.13^{a}	4.38 ± 0.08^{a}	0.03 ± 0.01^{a}	9.40 ± 1.11^{bc}
Nor. Ctrl	86.31 ± 9.44^{bcd}	126.95 ± 3.98^{bc}	1.38 ± 0.12^{ab}	4.50 ± 0.13^{a}	0.03 ± 0.01^{a}	5.76 ± 0.20^{a}
Oneway F	8.061	7.827	1.480	3.038	0.530	2.292
Anova P	0.000	0.000	0.165	0.003	0.893	0.021

Results were expressed as mean ± standard deviation (n=4/ group). Means with different superscript letters are statistically significant down the column ($p<0.05$)

Key:

Q = Quercetin R = Rutin L = Luteolin

100, 200, 400 = 100, 200, 400 mg/kg body weights respectively

Vit. C = Vitamin C (Ascorbic acid) Vit. E = Vitamin E (α-Tocopherol)

Glib. = Glibenclamide Dia. Ctrl = Diabetic Control

Nor. Ctrl = Normal Control SOD = Supeoroxide Dismutase

CAT = Catalase GSH = Glutathione GPX = Glutathione Peroxidase

GST = Glutathione –S- transferase MDA =Malondialdehyd

From Table 2, the serum urea concentrations of quercetin (Q100) and rutin (R200) treated animal groups at quercetin 100 mg and rutin 200 mg /kg body weight, respectively were significantly lower ($p<0.05$) when compared with diabetic control animal group. The serum urea concentrations of luteolin (L200) treated group of 200 mg/kg body weight was significantly lower ($p<0.05$) when compared with vitamin E, glibenclamide, diabetic control animal groups. The least serum urea concentration was observed in L200 group in comparison with other flavonoid treated groups. There was a dose dependent decrease in serum creatinine concentration of the groups treated with rutin and luteolin. The creatinine concentrations of rutin treated animal groups at dose of 200 and 400 mg/ kg body weight were significantly lower when compared with vitamin C treated group. Dose concentration of Luteolin 400 mg /kg body weight the least serum creatinine concentration was observed in R400 group when compared with other flavonoid treated animal groups.

Table 2: Effects of quercetin, rutin, and luteolin on some serum urea and creatinine

Treatments	UREA (mmol/l)	CREATININE (µmol/l)
Q100	3.33 ± 0.29^{ab}	32.40 ± 1.50^{cd}
Q200	4.68 ± 0.32^{cde}	41.06 ± 0.31^{f}
Q400	5.71 ± 0.24^{e}	46.60 ± 1.93^{g}
R100	5.37 ± 0.20^{e}	30.96 ± 0.59^{cd}
R200	3.98 ± 0.73^{abcd}	28.86 ± 2.45^{bc}
R400	4.70 ± 0.74^{cde}	28.04 ± 0.20^{bc}
L100	3.85 ± 0.68^{abc}	47.03 ± 0.11^{g}
L200	3.06 ± 0.74^{a}	37.70 ± 0.71^{ef}
L400	4.02 ± 0.85^{abcd}	32.20 ± 1.11^{cd}
Vit. C	3.43 ± 1.00^{ab}	34.15 ± 0.49^{de}
Vit. E	4.91 ± 0.71^{de}	31.81 ± 0.59^{cd}
Glib	4.19 ± 0.84^{bcd}	25.03 ± 11.10^{b}
Dia. Ctrl	5.60 ± 0.31^{e}	32.77 ± 0.66^{cd}
Nor. Ctrl	3.81 ± 0.72^{abc}	19.44 ± 2.46^{a}
Oneway F	6.814	22.737
Anova P	0.000	0.000

Results were expressed as mean ± standard deviation (n=4/ group). Means with different superscript letters are statistically significant down the column ($p<0.05$)

Key:
Q = Quercetin
R = Rutin
L = Luteolin
100,200, 400 = 100,200, 400 mg/kg body weights respectively
Vit. C = Vitamin C (Ascorbic acid)
Vit. E = Vitamin E (α-Tocopherol)
Glib.= Glibenclamide
Dia. Ctrl = Diabetic Control
Nor. Ctrl = Normal Control

Discussion

From Table 1, the kidney of the animals responded differently in the expression of antioxidant markers upon flavonoids administration. Quercetin (Q100) treated animal group showed an improvement in the activity of SOD, CAT, GPx. In Rutin treated animal group (R100), the activity of SOD, and level of MDA and GSH mildly increased when compared to the diabetic group. The MDA levels in R100 and L100 groups were lower than the diabetic group. Gomathi *et al.* (2014), reported that *Evolvulus alsinoides* extracts effectively increased the antioxidant level and prevented oxidative stress in the kidney of STZ-induced diabetic rats. Increase in activity of enzymatic antioxidants and concentration of non-enzymic antioxidant indicates that the

flavonoid at that dose was stimulatory and induce the synthesis of these enzyme in response to oxidative stress as a result of diabetes mellitus and vice versa. The level of Malondialdehyde (MDA) showed the extent of oxidative damage. Hyperglycemia in diabetes mellitus triggers the onset of and progression of renal injury. Podocytes layer of the glomerular filtration barrier (GFR) is highly vulnerable to the reactive oxygen species attack. This is because this layer does not proliferate even when challenged by injury leading to early loss of the layer at the onset of diabetes mellitus with resultant excessive protein loss (Liu *et al*., 2013: Kumar *et al.,* (2014). Renal injury as a result of oxidative stress in diabetes mellitus could develop to Chronic Kidney Disease (CKD). ROS from NADPH oxidase and mitochondria electron transport chain (ETC) are the major source of ROS in the kidney of diabetic animal and this plays important role in promoting pathophysiological processes in kidney disease. Advanced glycation end product (AGEs), glucose autooxidation and uncoupled nitric oxide synthase (NOS) also contribute to ROS among others. NADPH oxidase (NOX4) is responsible for the generation of O_2^- in renal cells such as podocyte, mesangial and proximal tubules (Autor *et al*., 1976; Kimball *et al*., 1976; Stevens and Autor, 1977). Luteolin at 400 mg/kg body weight may not be protective to the kidney. Quercetin at 400 mg/kg body weight may be more nephroprotective as it reduced MDA level to 7.77 ± 4.00 in comparism with diabetic control 9.40 ± 1.11.

In Table 2, the serum levels of urea and creatinine are used as indices to measure the functionality of the kidney. Elevation in the serum levels of urea and creatinine could be as a result of reduced glomerular filtration rate. However, creatinine is a more specific parameter for this purpose. Quercetin at a low dose may confer little antioxidant protection to the glomerular filtration barrier (GFB). This is evident in the serum urea and creatinine concentrations of Q100 treated animal group which is slightly lower than the level of normal control and vitamin E animal groups as shown in Table 2, but as the concentration of quercetin increased, the level of both urea and creatinine increased. This progressive increase in the serum level of urea and creatinine is an indicator of progressive decrease in glomerular filtration rate (GFR) as the concentration of quercetin increased from Q100 to Q400. In Table 1, it is evident that there is decline in the activity of SOD, CAT and in the level of GSH as the concentration increased.

Quercetin may have pro-oxidant effect and could lead to initiation and progression of oxidation by reduction of metal ion and fenton reaction. Glomerular filtration barrier, podocytes, transporters involved in the secretion of creatinine and other vital parts of the kidney filtration unit nephron could be damaged by oxidants. Quercetin at high dose may not be beneficial for the treatment kidney related damage in diabetes mellitus. Pro-oxidant activities of flavonoids have been reported by Prochazkova *et al.,* (2011).

Administration of the flavonoid rutin and luteolin caused a decline in serum urea concentration from animals administered with 100 mg to 200 mg/kg body weight. However, they were effective in lowering the serum creatinine concentration in a dose dependent manner and could have protective effect on the glomerulus. Dose increase in rutin and luteolin above 400 mg/kg body weight may affect the serum creatinine positively but not urea.

Conclusion

Streptozotocin induced diabetes mellitus in albino rats were treated for four weeks (28) days with food grade flavonoid antioxidants (Quercetin, Rutin and Luteolin) were investigated for nephroprotective effect. Serum kidney function parameters indicated that Q100 may have better nephroprotective effect as the urea and creatinine values were lower than the diabetic control, while some kidney antioxidant parameters (e.g CAT) decreased with a corresponding decrease in MDA level at lowest dose (R100 and L100). However, similar observation was made with Quercetin (Q400), Rutin and Luteolin improved kidney functionality at dose concentrations (R400 and L400) as shown in serum parameters of kidney function test, R200 equally showed improved kidney function in the parameters analyzed in the serum. Based on serum kidney function parameters, it may be likely that quercetin at high dose may not be beneficial to the kidney. Luteolin and Rutin may possibly have a better kidney antioxidant protection at low dose concentration (R100). There is a possibility of having a synergistic response or a more enhanced/modified response on the animals if the effective doses of these flavonoids are combined and studied.

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