PAT December, 2020; 16 (2): 13-32 ISSN: 0794-5213

Online copy available at *www.patnsukjournal.net/currentissue*

Publication of Nasarawa State University, Keffi

Effects of Some Selected Flavonoids on Liver Function Parameters of Streptozocin Induced Diabetic Rats

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Abstract
The study evaluates how flavonoids administration in diabetic rats affects certain serum and liver function parameters. The aim of the study is to determine the effects of some selected flavonoids on liver function parameters of streptozocin-induced Diabetic rats. To achieve this aim, Albino rats were acclimatized and divided into 14 groups of 6 animals each (84). Diabetes was induced on thirteen groups of rats using single intra peritoneal injection of 50 mg streptozotocin /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 used for the study. 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 /kg body weight. Groups 10, 11, 12 were administered with 1.5 mg Vitamin C, 2.31 IU Vitamin E and 5 mg Glibenclamide/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 and liver tissues were excised and the effect of the administered flavonoids on liver function parameters was determined. Data collected were analyzed using SPSS software 21 and Analysis of variance was performed with n=4 sample data. Luteolin at dose, 400 mg, maintained normal liver enzyme (ALP, AST and ALT) and protein levels. However, the albumin level was significantly ($p<0.05$) higher than the normal control. The serum enzymes (ALP, AST and ALT), markers of liver damage in Q400 and L400 treated groups were significantly ($p<0.05$) lower than the diabetic control. The animal groups Q100, Q200, Q400 and R400 have a decreased atherogenic indices of 0.12, 0.28, 0.52 and 0.53 respectively. The serum albumin levels of R400 and L400 were 33.64 ± 0.53^{e} and 31.49 ± 0.26^{ed} and are significantly ($p<0.05$) higher than the serum albumin level of the diabetic control group (29.31 ± 0.61^{b}). while the serum protein levels of Q200 and L100 were 86.61 ± 0.32^{h} and 72.38 ± 0.62^{cde} and are significantly ($p<0.05$) higher than the serum albumin level of the normal control group (29.31 ± 0.61^{b}). The serum ALT and ALP levels of Q400 and L400 treated animals were significantly ($p<0.05$) lower than the diabetic control group while the serum AST level of R400 and L400 treated animals were significantly ($p<0.05$) lower than the normal control group. The serum total cholesterol and low density lipoprotein of L400 treated animal group decreased significantly ($p<0.05$) when compared with diabetic control group, the high density lipoprotein increased significantly ($p<0.05$) when compared with diabetic control group. The animal group L400, R400 and Q400 have atherogenic index of 0.08, 0.14 and 0.18 respectively.
Keywords: *Liver function parameters, diabetes mellitus,*

Introduction

Diabetes mellitus (DM) is a common metabolic disease characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action or both (Jayakar and Suresh, 2003; Bastaki, 2005).

Continuous high glucose levels after onset of either type 1 or type 2 diabetes has secondary adverse effects on the beta cell itself. The current knowledge is that oxidative stress plays a significant role in the development of diabetes and its complications (Dos-santos *et al*., 2019: Tabatabaei-Malazy *et al*., 2013). The major concern of the 2016 World Health Organization (WHO) day celebration was Diabetes mellitus (Takian and Kazempour-Ardebili, 2016). It was estimated that a population of 300 million people worldwide will suffer diabetes by the year 2025, with a prevalence rate of 7.7 % by 2030 (Kayar *et al*., 2017). Many more death at the rate of 3.96 % DM patient is expected to die annually and that 6.8 % cause of death will be attributable to DM (Kayar *et al*., 2017). However, the global burden on diabetes mellitus according to the 2016 global report on diabetes by WHO has now increased by several millions more than the 2025 projection. WHO in its report estimated that a total of 422million adult were living with DM in 2014 and that DM was the cause of 1.5 million death in 2012 (WHO, 2016). It was also estimated that 1.6 million deaths occurred in 2016 because of DM (WHO, 2020). According to the report from international diabetes federation the value has further increased to 451 million adult living with DM worldwide in the year 2017 and will further increase to 693 million by 2045 if no effective prevention method is adopted (Cho *et al.,* 2018).

Liver plays a central role in maintenance of glucose homeostasis. Derangement in liver function and particularly liver enzymes, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), Gammar Glutamyl Transpeptidase (GGT) widely co-exist in type 2 diabetes mellitus (Ni *et al.,* 2012: Shibabaw *et al.,* 2019). According to growing body of evidence, elevated liver enzymes are recently considered a risk factor for development of diabetes in conjunction with other risk factors (Abbassi *et al,* 2012: Kunutsor *et al.,* 2013). Bora *et* al. (2016) reported abnormalities in at least one liver function parameter in 71.25 % of patients sampled. They further stated that deranged liver enzymes (ALT and ALP) were most common in male and female and mostly associated with glycemic status. The pathophysiological mechanisms of diabetes mellitus induced abnormalities in liver biomarkers are still unclear however, there are speculations that DM may be responsible for deposition of fats in the liver and inflammation which alters the function of the liver and changes its biomarkers (Hanley *et al*., 2005).

Increase in liver enzyme and hepartomegaly because of glycogenic hepartopathy has been observed in type 1 diabetic patients. This is caused by excessive and irreversible accumulation of glycogen in the hepartocytes (Ucler *et al.,* 2015).

Leeds *et al*., (2009) reported that an elevated ALT was associated with glycemic control and that this elevation is not uncommon in type 1 diabetes. Hepartic glycogenosis associated with poor metabolic control was observed in an 18-year-old female type 1 diabetic patient. This condition was accompanied by elevated liver function test in. Liver enzymes declined after achievement of glycemic control (Yener *et al.,* 2009). Similar observation was made in a 19-year-old male whose hepartomegaly and elevated liver enzymes were resolved when put on glycemic control (Ucler *et al.,* 2015). The prevalence of abnormal liver function parameters among diabetic patients is till controversial (Getnet *et al.*, 2019). The presence study was aimed to substantiate this claim and to see if there will be improved liver function at the end of the experimental period. The effect of flavonoids on the liver function of streptozocin induced diabetic rats was studied. The results generated from this research will provide useful information for effective management and treatment of diabetes mellitus and its complications using the flavonoids. Also, treatment of individual prior to disease manifestation may delay the onset of diabetes mellitus in individuals who are predisposed to diabetes mellitus.

Flavonoids are group of natural substances with variable phenolic structure found in all the parts of plant, food, and beverages such as fruits, vegetables, grains, bark, roots, stems, flowers, cocoa, tea, and are therefore sources of dietary flavonoids (Panche *et al*., 2016).
They 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 because 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, they have 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).
The *in vitro* antioxidant activities flavonoids and phenols are well established. It has been documented that some flavonoids showed antioxidant activities more than a nutritional antioxidant for example vitamin C (Tsimogiannis and Oreopoulou, 2004; Soobrattee *et al*., 2005). However, the relationship between *in vitro* and *in vivo* antioxidant activities of the flavonoids and phenolics have not been properly established and documented. Therefore, it will be necessary to study the *in vivo* antioxidant activities of some flavonoids from plants, with antioxidant and anti-diabetic activities, in relation to their effects on progression and complications arising from of diabetes mellitus regarding its hepartoprotective function.

Materials and Methods

Materials

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 Chinese 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 Chinese Food grade Chemical Hunan Hua Kang Biotechnology.

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 Animal house College of Health Sciences, Kogi State University, Anyingba, Kogi State. The rats were housed in well-ventilated clean Aluminium cages in the Advanced laboratory animal house of the Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, and fed with starter mass (vital feed) and clean water for two weeks to acclimatization laboratory conditions prior to commencement of study. The rats were maintained under standard laboratory conditions of temperature and humidity, at 12 hours light and 12 hours dark cycle daily. After acclimatization, these animals were weighed and divided into 14 groups of 6 animals each.

Methods

Induction of diabetes mellitus

Diabetes mellitus was induced by administration of single intraperitoneal injection of 50 mg streptozotocin /kg body weight of in 8 hours-fasted rats (Burceli*net al.,* 1995). Fasting blood glucose level monitoring commenced 24 hours after induction and was determined with a glucometer. 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 and placed in fourteen groups of six animals each. They were subsequently maintained with grower's mash (vital feed) and clean water *adlibitum*.

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/kg body weight. Groups 10, 11, 12 were administered with 1.5 mg Vitamin C, 2.31 IU Vitamin E and 5 mg Glibenclamide/kg body weight respectively while groups 13 (Diabetic control) and 14 (Normal control) were administered with almond oil. The food grade flavonoids and vitamin E were separately dissolved in almond oil administered orally to the rats daily for a week's period using dosing needle. The dosage calculated for each rat was based on the weight of animal and dose specified for its group. The volume calculated for 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 via ocular puncture using capillary tubes. Sera were harvested from the blood collected and used to assay for some serum markers enzymes (ALT, AST and ALP), albumin, protein, lipid profile parameters.

Collection and preparation of liver tissue homogenate

Liver 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 % of the tissues respective homogenates were prepared by homogenizing the tissues using mortar and pestle (Brostrom and Jeffay, 1970; Priora*et al.,* 2010) in 10 mM phosphate buffer saline (PBS) of pH 7.4. The homogenates were centrifuged in a refrigerated centrifuge at 15,000 Xg for 15 minutes. The supernatants were collected and stored below -20 ^{o}C until used. The supernatants were used for the determination of alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), total cholesterol (TC), triacylglycerol (TG), high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c), protein and albumin.

Preparation of serum for some biochemical parameters

Blood samples collected from the animals were centrifuged at 3000 X g for 5 minutes in an refrigerated centrifuge. The supernatants (serum) were stored below -20 ^{o}C until used. Serum samples were used for the determination of alanine amino transferase (ALT), aspatate amino transferase (AST), alkaline phosphatase (ALP), albumin, protein and lipid profile parameters.

Biochemical parameters

The following biochemical parameters were carried out.

Determination of aspartate transaminase (AST) and alanine transaminase (ALT) activities

AST and ALT were determined using the spectrophotometric method of Bergermeyer*et al.*, 1978.

Procedure:

This assay is based on the principle that AST and ALT catalyse the transfer of amino group from L-aspartate/L-alanine to α-ketoglutarate to yield oxaloacetate/pyruvate respectively. Oxaloacetate/pyruvate oxidizes NADH to NAD^{+} in the presence of malate dehydrogenase/lactate dehydrogenase. The decrease in absorbance at 340 nm in a spectrophotometer due to the oxidation of NADH is monitored and is proportional to

AST/ALT activity. One ml of ALT or AST reagent was added into a test tube and allowed stand for 3 minutes to equilibrate to 37°C. A volume of 0.10 ml of specimen was added to the ALT or AST reagent and mixed gently, and the solution maintained at 37°C. Then, the absorbance was read three times at 60 seconds interval at 340 nm.

Calculation:

The mean change in absorbance readings were calculated thus (ΔA/min).

Therefore, ALT or

AST activity (IU/L) = $\frac{\Delta A/min. \times TV \times 1000}{Є \times SV \times LP}$

Where: ΔA/min = Average absorbance change per minute

TV =Total reaction volume (ml)

1000 = Conversion of IU/mL to IU/L

Є = Millimolar absorptivity of NADH (6.22)

LP = Light path (cm)

Note: Any samples with values above 500 IU/L were diluted 1:1 with normal saline, and re-assayed, and the results mulplied by two.

Determination of alkaline phosphatase (ALP) activity

ALP was assayed using the spectrophotometric method of Schlebusch*et al.* (1974).

Procedure**:**

At alkaline pH, ALP catalyzes the hydrolysis of p-nitrophenyl phosphate to yellow coloured p-nitro phenolate and phosphate; the change in absorbance measured at 405 nm is directly proportional to the enzyme activity.

p-Nitrophenyl phosphate + H_2O $\xrightarrow{\text{Alkaline Phosphatase}}$ p-Nitrophenol + H_3PO_4

One ml of ALP reagent was added into a test tube and allowed to equilibrate to 37 °C. The spectrophotometer was blanked with water at 405 nm. A volume of 0.025 ml of specimen was added to the reagent and mixed gently while the solution was still maintained at 37°C. Then, the absorbance was read three times at 60 seconds interval at 405 nm.

Calculation:

The mean change in absorbance readings were calculated thus (ΔA/min). Therefore, ALP activity (IU/L) = $\frac{\Delta A/min. \times TV \times 1000}{Є \times SV \times LP}$

Where: ΔA/min = Average absorbance change per minute

TV =Total reaction volume (ml)

1000 = Conversion of IU/mL to IU/L

Є = Millimolar absorptivity of NADH (18.75)

LP = Light path (1cm)

Note: Any samples with values above 800 IU/L were diluted 1:1 with normal saline, and re-assayed and the results multiplied by two.

Determination of triglyceride (TG) level

The serum TG level was estimated by GPO-POD enzymatic colorimetric reaction, according to the method described by Fossati*et al*. (1982).

Procedure:

The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoeimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Triglyceride + H_2O $\xrightarrow{\text{Lipase}}$ Glycerol + fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol – 3- phosphate + ADP

Glycerol-3 - phosphate + O_2 $\xrightarrow{\text{Glycerol phosphate oxidase}}$ dihydroxyacetone phosphate + H_2O

$2H_2O_2$ + 4 –aminophosphenazone + 4 chlorophenol $\xrightarrow{\text{Peroxidase}}$ quinomeimane + HCL+ $4H_2O$

Test tubes were labeled: Blank, Standard, Control, and Sample. One ml of working reagent was added into all the test tube and allowed to equilibrate at 37°C for 4 minutes. Then, 0.01 ml of sample or standard or control was added to the working reagent, mixed and incubated at 37°C for 10 minutes. The spectrophotometer was zeroed with the reagent blank at 500 nm and the absorbance of all the tubes were read and recorded.

Calculation:

$$\text{Triglyceride (mmol/l)} = \frac{\text{Abs. of unknown}}{\text{Abs. of standard}} \times \text{Concentration of standard (2.26 mmol/l)}$$

Determination of total cholesterol level

The serum TC level was estimated by enzymatic colorimetric method described by Naito (1984_a).

Procedure:

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinonemine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Cholestrol-ester + $H_2O \xrightarrow{\text{Cholesterol Esterase}}$ cholesterol + fatty acid

Cholesterol + $O_2 \xrightarrow{\text{cholesterol oxidase}}$ Cholesten-3- one + H_2O_2

$2H_2O_2$+phenol+4-Aminoantipyrine $\xrightarrow{\text{peroxidase}}$ Quinone + H_2O

Test tubes were labeled: Blank, Standard, Control and Sample. One ml of cholesterol reagent was added into all the test tube and allowed to equilibrate at 37°C for 2 minutes. Then, 0.01 ml of sample or standard or control was added to the working reagent, mixed and incubated at 37°C for 10 minutes. The spectrophotometer was zeroed with the reagent blank at 500 nm and the absorbance of all the tubes read and recorded.

Calculation:

$$\text{Total cholesterol (mmol/l)} = \frac{\text{Abs. of unknown}}{\text{Abs. of standard}} \times \text{concentration of standard (5.18 mmol/l)}$$

Determination of high-density lipoprotein cholesterol (HDL-C) level

The serum HDL-C level was estimated by precipitation and CHOD-POD enzymatic colorimetric reaction, according to the method as described by Grove (1979) and Naito (1984_b).

Procedure**:**

Low density lipoprotein (LDL-C) and (VLDL-C) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL-C (high density lipoprotein) fraction, which remains in the supernatant, is determined.

Stage 1: Precipitation reaction:

A volume of 0.2 ml of sample was added into a tube containing 0.5 ml of precipitant (sodium phosphotungstate in MgCl) and mixed thoroughly. It was allowed to stand at room temperature for 15 minutes and thereafter centrifuged at 4000 rpm for 15 minutes.

Stage 2: Colour development:

A volume of 0.050 ml of clear supernatant or standard was transferred into 1.0 ml of working reagent (R_2)/cholesterol reagent mixed and incubated at 37 °C for 10 minutes. The spectrophotometer was zeroed with the reagent blank at 500 nm and the absorbance of all the tubes were read and recorded.

Calculation:
High density lipoprotein cholesterol (mmol/l) = Abs. of unknown x Conc. of std (1.34 mmol/l)
Abs. of standard

Determination of low-density lipoprotein cholesterol (LDL-C) level
The LDL-C was estimated by computation, according to the methods described by Friedewald*etal*. (1972).
LDL-cholesterol (mmol/L) = total cholesterol – HDL-cholesterol – triglyceride/2.2.
Atherogenic index = Log1o (TG/ HDL-c) (Myat *et al*. 2018)

Determination of total protein
Total protein was determined according to the method of American Association for Clinical Chemistry (Doumas*et al.* 1981) and National committee for clinical Laboratory Standards (NCCLS, 1979)
Procedure:
The enzymatic reaction sequence employed in the assay of total protein in an alkaline pH is as follows:
Protein + Cu^{++} ——→ Cu-Protein complex
Protein in the serum forms a violet coloured complex when reacted with cupric ion in alkaline solution. The intensity of the violet colour is proportional to the amount of protein present when compared with a solution of known protein concentration (standard). Test tubes were labeled blank, standard (BSA), control, and treatment groups accordingly, and 3.0 ml of the Total protein reagent was pipetted into each test tube. Similarly, 0.05 ml (50 µL) of the blank, standard, and serum samples for control and treatment groups were added to appropriate test tubes and mixed by inversion. The tube was allowed to stand at room temperature (25-30 ^{o}C) for 10 minutes. The instrument was zeroed with the reading of the reagent blank at 540 nm. The absorbance of the standard, control, and treatment groups were taken at the same wavelength.
Calculation**:**
Total protein (g/dL) = Absorbance of the unknownX Concentration of the standard
Absorbance of standard

Determination of serum albumin
Procedure
Serum albumin binds selectively to the dye bromocresol green at the pH 4.2. The increase in absorbance of the resulting albumin-dye complex at 630 nm is proportional to the albumin concentration. Test tubes were labeled blank, standard (BSA), control, and treatment groups accordingly, and 1.5 ml of the albumin reagent was pipetted into the respective test tubes. Then 0.01 ml (10 µL) of the blank, standard (BSA), and samples from control, and treatment groups were added to the corresponding test tubes, mixed and allowed to stand for 5 minutes at room temperature before taking the reading at 630 nm. The spectrophotometric reading was zeroed with the blank before readings for standard (BSA), control, and treatment groups were taken.
Calculation:
Albumin (g/dL) = Absorbance of the unknownX Concentration of the standard
Absorbance of standard

Statistical analysis

Analysis of variance (ANOVA), Duncan multiple range test and least significant difference of the grouped data were performed using SPSS 21 software. Results were presented as Mean ± Standard deviation of replicate (n=4) determinations. The differences between the means were considered significant at $p < 0.05$.

Results

From Table 1, there was a dose dependent decrease in the activity of alkaline phosphatase (ALP) in quercetin treated animal groups (Q100, Q200 and Q400) and this decrease was significantly lower ($p<0.05$) when compared with normal control, diabetic control, glibenclamide and vitamin C treated animal groups. In rutin treated animal groups (R100, R200 and R400), a dose dependent increase in the activity of ALP was observed. The value was significantly higher ($p<0.05$) when compared with normal control, glibenclamide and vitamin E treated animal groups. Luteolin treated animal groups (L100, L200 and L400), shows a dose dependent increase which is significantly higher ($p<0.05$) when compared with ALP activity of Vitamin E treated animal group. L400 treated animal group was significantly higher ($p<0.05$) when compared with normal control, glibenclamide and vitamin E treated animal groups and significantly lower ($p>0.05$) when compared with diabetic control and vitamin C animal groups. The highest and least in ALP activity was seen in R400 and Q200/400 respectively.

In quercetin treated animal groups (Q100, Q200 and Q400), there was a dose dependent decrease in aspartate amino transferase (AST) activity. However, there was no significant difference ($p>0.05$) in the activity values of these treated groups when compared with the control groups and the standard animal groups. The rutin and luteolin treated animal groups show dose dependent increase in the activity of AST. In rutin treated animal groups (R100, R200 and R400), a significant increase ($p<0.05$) in AST activity was observed when compared with normal control, vitamin C and glibenclamide animal groups. The luteolin treated animal groups (L100, L200 and L400) shows no significant difference ($p>0.05$) in AST activity when compared with vitamin C, vitamin E, glibenclamide normal control and diabetic control animal groups.

The highest and least in AST activity was seen in R400 and L100 /200/respectively.

A dose dependent decrease in alanine amino transferase (ALT) activity similar to AST was observed in quercetin treated animal groups (Q100, Q200 and Q400). No significant decrease ($p>0.05$) in AST activity of Q400 treated animal group was observed when compared with normal and diabetic control animal groups. Conversely, a dose dependent increase in ALT activity was observed in rutin treated animal groups (R100, R200 and R400), the activity value in R400 was not significantly lower ($p>0.05$) when compared with vitamin C, vitamin E, glibenclamide, diabetic and normal control animal groups. No change in ALT activity was observed among the luteolin treated animal groups (L100, L200 and L400). The ALT activities of the animal groups are not significantly higher ($p>0.05$) when compared with vitamin C, vitamin E, and glibenclamide.

The highest ALT activity was seen in L400 and Q100. The lowest ALT activity was seen in R200. The liver protein concentration of Q200 treated animal group was significantly higher ($p>0.05$) when compared with normal control, diabetic control and glibenclamide treated animal groups.

The rutin treated animal groups (R100, R200 and R400), were significantly lower ($p>0.05$) in protein concentration when compared with vitamin C, vitamin E, glibenclamide, diabetic and normal control animal groups. The luteolin treated groups (L100 and L400), were significantly higher ($p>0.05$) than glibenclamide and diabetic control and significantly lower

($p>0.05$) than vitamin C and vitamin E but not normal control. The highest and least in protein concentration is seen in Q200 and Q100 respectively.

A dose dependent increase in the albumin concentration of quercetin treated animal groups (Q100, Q200 and Q400) and luteolin treated animal groups (L100, L200 and L400) was observed though the quercetin treated animal groups are significantly lower ($p<0.05$) in albumin concentration when compared with normal control, vitamin E and glibenclamide. The albumin concentration of animal group at dose level L400 was significantly higher ($p<0.05$) when compared with the normal control, diabetic control, vitamin C and glibenclamide. No significant increase was observed when compared with vitamin E treated group. The albumin concentration of the rutin treated animal group R400 was significantly higher ($p<0.05$) when compared with the vitamin C and diabetic control and significantly lower when compared with normal control but not Glibenclamide. The highest and least in albumin concentration was seen in L400 and R200 respectively.

Table 1: Effects of quercetin, rutin, and luteolin on some liver function parameters of the rat liver tissue

Treatments	ALP (U/g)	AST (U/g)	ALT (U/g)	Protein (mg/g)	Albumin (mg/g)
Q100	0.08 ± 0.01^{a}	0.20 ± 0.01^{abcd}	0.20 ± 0.01^{c}	68.10 ± 2.88^{a}	45.79 ± 0.18^{a}
Q200	0.06 ± 0.01^{a}	0.19 ± 0.02^{ab}	0.20 ± 0.01^{bc}	107.48 ± 0.59^{i}	54.11 ± 0.32^{d}
Q400	0.06 ± 0.01^{a}	0.19 ± 0.03^{abc}	0.19 ± 0.02^{abc}	73.41 ± 2.22^{cd}	54.17 ± 1.98^{d}
R100	0.27 ± 0.01^{d}	0.21 ± 0.01^{bcd}	0.18 ± 0.01^{ab}	74.44 ± 2.48^{d}	54.80 ± 0.08^{d}
R200	0.27 ± 0.04^{d}	0.22 ± 0.01^{cd}	0.18 ± 0.01^{a}	70.64 ± 0.11^{b}	45.08 ± 1.46^{a}
R400	0.29 ± 0.02^{d}	0.22 ± 0.01^{d}	0.19 ± 0.01^{abc}	71.67 ± 0.19^{bc}	60.99 ± 0.33^{e}
L100	0.11 ± 0.01^{b}	0.18 ± 0.01^{a}	0.20 ± 0.02^{bc}	97.84 ± 0.22^{h}	45.45 ± 0.46^{a}
L200	0.11 ± 0.01^{b}	0.18 ± 0.01^{a}	0.20 ± 0.01^{bc}	94.81 ± 0.39^{g}	51.54 ± 0.93^{c}
L400	0.12 ± 0.01^{bc}	0.19 ± 0.01^{ab}	0.20 ± 0.01^{c}	97.85 ± 0.05^{h}	65.26 ± 1.11^{g}
Vit. C	0.15 ± 0.02^{c}	0.19 ± 0.01^{ab}	0.19 ± 0.01^{abc}	113.98 ± 0.03^{j}	50.66 ± 0.56^{c}
Vit. E	0.08 ± 0.01^{a}	0.18 ± 0.01^{a}	0.19 ± 0.01^{abc}	115.25 ± 0.60^{j}	64.35 ± 1.94^{g}
Glib	0.11 ± 0.02^{b}	0.18 ± 0.02^{a}	0.19 ± 0.01^{abc}	86.27 ± 2.08^{f}	61.83 ± 0.11^{ef}
Dia. Ctrl	0.15 ± 0.02^{c}	0.20 ± 0.01^{abcd}	0.20 ± 0.01^{bc}	83.78 ± 0.25^{e}	48.61 ± 0.50^{b}
Nor. Ctrl	0.11 ± 0.01^{b}	0.18 ± 0.01^{a}	0.20 ± 0.02^{bc}	98.85 ± 0.82^{h}	62.79 ± 0.21^{f}
Oneway F	80.572	5.202	2.05	593.215	225.719
Anova P	0.000	0.000	0.040	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
ALP = Alkaline Phosphatase
AST = Aspartate Amino Transferase
ALT = Alanine Amino Transferase

From Table 2, the quercetin treated animal groups (Q100, Q200 and Q400) shows decrease in Pancreatictotal cholesterol (TC) Level. The decrease was significant ($p<0.05$) when compared with vitamin C, vitamin E, diabetic control and normal control. Among the rutin treated animal groups, R400 shows a significant decrease ($p<0.05$) in TC level when compared with vitamin C, diabetic control and normal control. However, this value was not significantly lower ($p>0.05$) than vitamin E. A dose dependent increase in TC level was

observed as the concentration of luteolin increased from 100 to 400mg/kg body weight. The TC level significantly decreased ($p<0.05$) in animal groups treated L100 and L200 respectively when compared with vitamin C, vitamin E, diabetic and normal control groups. The TC level of L400 treated animals was lower than the normal control though not significant ($p>0.05$). The least TC among the flavonoid samples was seen in Q100 treated animals. There was a dose dependent increase in pancreatictriacylglyceride(TG) Level of the quercetin treated animal groups (Q100, Q200 and Q400). These values are significantly lower ($p<0.05$) when compared with diabetic control, normal control, vitamin C, vitamin E and glibenclamide animal groups. They are also significantly lower ($p<0.05$) than the TG level in rutin and luteolin animal groups. It was also observed that the TG level decreased as the dose of rutin changed from 100 to 400 mg/kg body weight. R400 shows a significantly lower ($p<0.05$) TG level when compared with diabetic control, normal control, vitamin C, vitamin E and glibenclamide animal groups. The luteolin treated animal groups with concentrations 100 and 200 mg/kg body weight shows significantly lower ($p<0.05$) TG value when compared with diabetic control, normal control, vitamin C and glibenclamide animal groups but significantly higher ($p<0.05$) than the TG value of the vitamin E animal group. The group that recorded overall least result in TG concentration among the flavonoid groups is Q100.

A decreased in pancreatichigh-density lipoprotein- cholesterol (HDL-c) Level was observed in quercetin treated animal groups (Q100, Q200 and Q400) in a dose dependent manner. Animal groups treated with 100 mg quercetin /kg body weight was significantly higher ($p<0.05$) HDL-c concentration when compared with vitamin C, vitamin E, glibenclamide and diabetic control but significantly lower ($p<0.05$) than the normal control. In animal group treated with rutin (R200) at dose level 200 mg/kg body weight, the HDL-c concentration was significantly higher ($p<0.05$) than the vitamin C, vitamin E, glibenclamide, normal control and diabetic control. There was no significant difference ($p>0.05$) in HDL-c concentration when compared with normal control.

There was a dose dependent increase in HDL-c concentration of the luteolin treated animal groups (L100, L200 and L400). The L400 treated animal shows a HDL-c value significantly higher ($p<0.05$) when compared with diabetic control. The group that recorded the highest HDL-c concentration was R200 and the value was significantly higher ($p<0.05$) than all the flavonoid treated animal groups, vitamin C, vitamin E, glibenclamide, normal control and diabetic control.

low density lipoprotein–cholesterol (LDL-c) of quercetin level treated animal groups (Q400) was significantly lower ($p<0.05$) when compared with diabetic control, vitamin E, and glibenclamide. LDL-c of R100 treated animals was significantly lower ($p<0.05$) when compared with vitamin E, glibenclamide and normal control groups. However, the value was not significantly higher ($p>.05$) when compared with vitamin C. The L400 treated animal group was the least in LDL-c concentration and the value was significantly lower ($p<0.05$) than all the flavonoid groups, vitamin E, glibenclamide, normal control and diabetic control groups.

From Table 3, there is no significant increase in serum albumin concentration of Q200 treated animals when was compared with vitamin C and diabetic control. At dose concentration of 400 mg Rutin/kg body weight, the Serum albumin concentrations significantly increased when compared with that of normal control, diabetic control, vitamin C and vitamin E treated animal group.The luteolin treated animal group at dose concentration of 400 mg Luteolin/kg body weight was significantly higher than vitamin C and diabetic control animal groups in serum albumin concentration.

Table 2: Effects of quercetin, rutin, and luteolin on some lipid profile of the rat liver (μmol/mg)

Treatments	TC	TG	HDLc	LDLc	Athrogenic index
Q100	3.60 ± 0.24^{b}	0.96 ± 0.02^{a}	0.72 ± 0.01^{d}	1.92 ± 0.27^{ef}	0.12
Q200	3.62 ± 0.15^{b}	1.09 ± 0.06^{b}	0.57 ± 0.02^{c}	1.96 ± 0.12^{f}	0.28
Q400	2.64 ± 0.10^{a}	1.12 ± 0.00^{b}	0.34 ± 0.03^{ab}	1.18 ± 0.10^{c}	0.52
R100	5.87 ± 0.15^{f}	3.94 ± 0.03^{h}	0.92 ± 0.02^{e}	$1.01 \quad 0.14^{bc}$	0.63
R200	6.58 ± 0.12^{h}	3.94 ± 0.16^{h}	1.08 ± 0.10^{f}	1.56 ± 0.34^{d}	0.56
R400	4.86 ± 0.03^{d}	2.46 ± 0.08^{e}	0.72 ± 0.03^{d}	1.68 ± 0.07^{de}	0.53
L100	3.73 ± 0.05^{b}	1.69 ± 0.02^{d}	0.37 ± 0.01^{ab}	1.67 ± 0.06^{de}	0.66
L200	4.60 ± 0.17^{c}	1.62 ± 0.02^{d}	0.38 ± 0.04^{ab}	2.59 ± 0.21^{g}	0.63
L400	5.22 ± 0.04^{e}	5.14 ± 0.05^{j}	0.44 ± 0.04^{b}	0.64 ± 0.05^{a}	1.07
Vit. C	5.28 ± 0.04^{e}	3.94 ± 0.03^{h}	0.38 ± 0.05^{ab}	0.96 ± 0.06^{bc}	1.02
Vit. E	4.98 ± 0.15^{d}	2.98 ± 0.15^{f}	0.56 ± 0.04^{c}	1.44 ± 0.19^{d}	0.72
Glib	3.68 ± 0.04^{b}	1.46 ± 0.08^{c}	0.34 ± 0.02^{ab}	1.88 ± 0.10^{ef}	0.63
Dia. Ctrl	6.09 ± 0.03^{g}	4.12 ± 0.07^{i}	0.28 ± 0.01^{a}	1.68 ± 0.08^{de}	1.17
Nor. Ctrl	5.36 ± 0.08^{e}	3.64 ± 0.07^{g}	0.92 ± 0.19^{e}	0.80 ± 0.14^{ab}	0.60
Oneway F	364.883	1356.398	65.962	44.544	
Anova P	0.000	0.000	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
Vit. C = Vitamin C (Ascorbic acid)
Vit. E = Vitamin E (α-Tocopherol)
Glib. = Glibenclamide
Dia. Ctrl= Diabetic Control
Nor. Ctrl = Normal Control
TC = Total Cholesterol
TG = Triacylglyceride
HDLc = High Density Liporoptein-Cholesterol
LDLc = High Density Liporoptein-Cholesterol
100,200, 400 = 100,200, 400 mg/kg body weights respectively.

The value was not significantly higher than vitamin E and normal control in serum albumin concentration. Among the flavonoid treated animal groups, L400 was the highest in serum albumin concentration.

The serum protein concentration of the Q200 treated animal group was significantly higher than the serum protein concentration in standards (vitamin E, vitamin C and glibenclamide) and control groups (normal and diabetic control) and other flavonoid groups. The rutin treated animal group at dose concentration of 100 mg rutin /kg body weight was significantly higher than vitamin C and normal control animal groups in serum protein concentration but not significantly higher when compared vitamin E, glibenclamide and diabetic control groups. The L100 treated animal group was significantly higher in serum protein concentration when compared with standards (vitamin E, vitamin C and glibenclamide) and control groups (normal and diabetic control).

Table 3: Effects of quercetin, rutin, and luteolin on serum albumin and protein

Treatments	ALBUMIN	PROTEIN
Q100	26.68 ± 1.84^{a}	69.92 ± 2.38^{b}
Q200	29.97 ± 1.42^{bc}	86.61 ± 0.32^{h}
Q400	26.97 ± 1.25^{a}	70.98 ± 1.79^{bc}
R100	30.25 ± 1.76^{bc}	74.85 ± 1.25^{f}
R200	29.08 ± 0.63^{b}	67.60 ± 0.83^{a}
R400	33.64 ± 0.53^{e}	72.38 ± 0.62^{cde}
L100	29.29 ± 0.87^{b}	77.85 ± 0.28^{g}
L200	25.52 ± 0.16^{a}	73.27 ± 0.94^{def}
L400	31.49 ± 0.26^{cd}	70.11 ± 0.39^{b}
Vit. C	29.53 ± 0.84^{b}	71.76 ± 1.34^{cd}
Vit. E	31.38 ± 0.10^{cd}	73.66 ± 0.63^{ef}
Glib	32.70 ± 0.85^{de}	73.24 ± 0.73^{def}
Dia. Ctrl	29.31 ± 0.61^{b}	74.22 ± 0.41^{f}
Nor. Ctrl	30.85 ± 2.03^{bc}	71.50 ± 0.13^{bc}
Oneway F	16.467	73.427
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

From Table 4, there was a significant decrease ($p<0.05$) in serum alanine amino transferase (ALT) activity of quercetin (Q200) treated animal group of 200 mg/kg body weight when compared with diabetic control, vitamin E and glibenclamide animal groups. The serum alanine amino transferase (ALT) activity in all the rutin (R100, R200, R400) treated animal groups at 100, 200, 400 mg/kg body weight were significantly lower ($p<0.05$) when compared with diabetic control, vitamin E and glibenclamide animal groups. There was dose dependent decrease in alanine amino transferase (ALT) activity of all the luteolin (L100, L200, L400) treated animal groups of 100, 200, 400 mg/kg body weight. Their ALT values were significantly lower ($p<0.05$) than ALT activity in diabetic control, vitamin E and glibenclamide animal groups. The least in ALT activity was observed in group treated with Q400.

The serumaspartate amino transferase (AST) activity in all the quercetin, rutin and luteolin treated animal groups were significantly lower ($p<0.05$) when compared with diabetic control, vitamin E and glibenclamide animal groups. The lowest in AST activity among flavonoid treated groups was R200.

There was dose dependent decrease in serum alkaline phosphatase (ALP) activity of quercetin and Luteolin treated animal groups. However, the values were significantly higher ($p<0.05$) than the activity in normal control group. The least in ALP activity was observed in group treated with L400 and was significantly lower ($p<0.05$) when compared with the ALP activity in diabetic control, vitamin C, vitamin E and glibenclamide animal groups. The ALP activity in R100 was significantly lower ($p<0.05$) when compared with diabetic control, vitamin E and glibenclamide animal groups.

Table 4: Effects of quercetin, rutin, and luteolin on some serum marker enzymes

Treatments	ALT U/L	AST U/L	ALP U/L
Q100	32.53 ± 2.50^{d}	44.57 ± 0.48^{f}	369.92 ± 0.27^{i}
Q200	39.60 ± 3.28^{e}	43.40 ± 0.36^{e}	357.31 ± 6.80^{fgh}
Q400	22.17 ± 1.64^{b}	45.88 ± 0.36^{g}	308.61 ± 0.73^{d}
R100	32.81 ± 0.87^{d}	47.12 ± 0.31^{hi}	310.58 ± 0.34^{d}
R200	32.18 ± 0.25^{d}	39.14 ± 0.56^{c}	351.21 ± 0.82^{ef}
R400	32.30 ± 0.98^{d}	37.59 ± 0.59^{b}	352.04 ± 1.06^{efg}
L100	29.66 ± 8.45^{d}	46.05 ± 0.32^{gh}	344.83 ± 0.23^{e}
L200	28.40 ± 0.39^{cd}	46.50 ± 0.53^{gh}	258.91 ± 1.55^{c}
L400	24.83 ± 3.15^{bc}	41.29 ± 1.20^{d}	210.88 ± 0.36^{b}
Vit. C	21.89 ± 0.08^{b}	36.28 ± 0.26^{a}	359.74 ± 13.53^{gh}
Vit. E	38.27 ± 1.05^{e}	47.75 ± 0.22^{i}	364.47 ± 5.57^{hi}
Glib	40.81 ± 1.32^{e}	48.05 ± 0.27^{i}	369.91 ± 0.48^{i}
Dia. Ctrl	38.83 ± 1.21^{e}	50.25 ± 1.96^{j}	370.64 ± 0.64^{i}
Nor. Ctrl	14.37 ± 0.52^{a}	42.71 ± 0.77^{e}	151.67 ± 12.54^{a}
Oneway F	29.436	126.645	603.463
Anova P	0.000	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
ALP = Alkaline Phosphatase
AST = Aspartate Amino Transferase
ALT = Alanine Amino Transferase

From Table 5, there was a dose dependent decrease in Serum Triacylglyceride (TG) level in quercetin and rutin treated animal groups. The serum TG concentrations of quercetin (Q100, Q200 and Q400) treated animal groups were significantly lower when compared with that in diabetic control group. No significant decrease was observed in Q400 treated animals when compared with normal control. No significant difference in serum TG concentration of Q400 treated animals and glibenclamide treated animal group. There was a non-significant decrease in serum TG concentrations of R400 and L200 treated animal groups when compared with diabetic control group. The least serum TG concentrations among the flavonoid treated animal groups were Q100 and Q200.

There was a dose dependent increase in serum total cholesterol (TC) in rutin and luteolin treated animal groups. Conversely, quercetin decreased in a dose dependent manner. The TC of the flavonoid treated animal groups was significantly lower than the serum TCs in diabetic control and vitamin E treated animal group. The TC of the Q400 and L100 treated animal groups was significantly lower than the serum TCs in standards (vitamin E, vitamin C and glibenclamide) and control groups (normal and diabetic control). Among the flavonoid treated animal groups, the least in total cholesterol (TC) was L100 treated animal group

There was a dose dependent increase in serum high density lipoprotein-cholesterol (HDL-c) of all the flavonoid treated animal groups. The HDL-c of Q100 treated animal group was significantly higher than that of diabetic control. The HDL-c of R400 treated animal group was significantly higher than that of vitamin C, glibenclamide, diabetic control. The value was higher than normal control but not significant. The HDL-c of L400 treated animal group

was significantly higher than that of standard group, control groups and flavonoid treated animal groups.

The serums low density lipoprotein-cholesterol (LDL-c) of quercetin treated animal groups decreased in a dose dependent manner. The quercetin (Q400) group was significantly lower ($p<0.05$) when compared with that of vitamin C, vitamin E, glibenclamide, normal control and diabetic control group. The rutin and luteolin treated animal groups show a dose dependent increase in serum LDL-c. The R100 treated animal group was significantly lower when compared with vitamin E and diabetic control glibenclamide animal groups. The L100 treated animal group was significantly lower when compared with standard control groups, diabetic control, and normal control animal groups, and the least among the flavonoid groups.

Table 5: Effects of quercetin, rutin, and luteolin on serum lipid profile (mMole/L)

Treatments	TG	TC	HDLc	LDLc	Atherogenic Index
Q100	0.80 ± 0.01^{b}	2.74 ± 0.07^{ef}	0.47 ± 0.02^{b}	1.47 ± 0.05^{g}	0.23
Q200	0.80 ± 0.02^{b}	1.95 ± 0.09^{b}	0.47 ± 0.01^{b}	0.68 ± 0.09^{c}	0.23
Q400	0.78 ± 0.01^{b}	1.53 ± 0.16^{a}	0.51 ± 0.02^{bc}	0.25 ± 0.19^{b}	0.18
R100	1.10 ± 0.05^{f}	2.58 ± 0.04^{e}	0.58 ± 0.02^{de}	0.91 ± 0.06^{d}	0.28
R200	0.90 ± 0.02^{de}	2.59 ± 0.22^{e}	0.60 ± 0.06^{e}	1.10 ± 0.29^{de}	0.18
R400	0.89 ± 0.06^{cd}	2.75 ± 0.06^{ef}	0.64 ± 0.03^{f}	1.21 ± 0.13^{ef}	0.14
L100	0.96 ± 0.01^{de}	1.50 ± 0.04^{a}	0.54 ± 0.01^{cd}	0.04 ± 0.03^{a}	0.25
L200	0.94 ± 0.03^{de}	2.17 ± 0.06^{c}	0.53 ± 0.01^{c}	0.71 ± 0.06^{c}	0.25
L400	0.97 ± 0.02^{e}	2.85 ± 0.22^{f}	0.81 ± 0.04^{g}	1.08 ± 0.20^{de}	0.08
Vit. C	0.51 ± 0.05^{a}	1.58 ± 0.02^{a}	0.55 ± 0.02^{cd}	0.53 ± 0.05^{c}	-0.03
Vit. E	0.47 ± 0.04^{a}	3.12 ± 0.10^{g}	0.65 ± 0.01^{f}	2.01 ± 0.12^{h}	-0.14
Glib	0.80 ± 0.06^{b}	2.70 ± 0.04^{ef}	0.57 ± 0.04^{de}	1.34 ± 0.09^{fg}	0.15
Dia. Ctrl	0.96 ± 0.11^{de}	3.62 ± 0.03^{h}	0.40 ± 0.02^{a}	2.27 ± 0.15^{i}	0.38
Nor. Ctrl	0.83 ± 0.03^{bc}	2.38 ± 0.09^{d}	0.61 ± 0.02^{ef}	0.95 ± 0.11^{d}	0.13
Oneway F	59.205	129.740	54.332	122.459	
Anova P	0.000	0.000	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
TC = Total Cholesterol
TG = Triacylglyceride
HDLc = High Density Liporoptein-Cholesterol
LDLc = High Density Liporoptein-Cholesterol

Discussion

From Table 1, luteolin at dose, 400 mg, maintained normal liver enzyme levels (ALP, AST and ALT), protein when compared with the normal control. The albumin level was significantly ($p<0.05$) higher than the normal control. The serum enzymes (ALP, AST and ALT), markers of liver function in Q400 and L400 treated groups were significantly ($p<0.05$) lower than the diabetic control. The liver enzyme activities observed in non-Diabetic rats (or normal control group) indicates a normal and healthy liver. Values lesser than what is observes in normal control group is indicative of a compromised integrity of the hepatocyte membrane especially for ALT activity. Elevated serum enzyme activity is expected in the serum because of leached into the blood. The liver function parameters ALP, AST, ALT, protein, and albumin have age long been used as a useful indicators or indices to ascertain the functionality of the liver. They have successfully been used in diagnosis and in monitoring of progression of some liver related disease conditions and inflammatory processes. These parameters are equally used in toxicity monitoring of xenobiotics (Pharmaceuticals, neutraceuticals, health care products, cosmetics, food, plant extracts and environmental toxicants and host of others). AST and ALT are present in many cells, notably among them are the cells of the liver, heart, muscle, kidney and others. Abnormal serum concentration of these enzymes is an indication of assault on the membrane of cells of origin and leakage into the ECF of the blood. The liver activities of these enzymes in L400 group are comparable to the normal control group of the liver homogenate. Enzyme activities of L400 animal group and normal control, ALP (0.12 ± 0.01^{bc}/0.11 ± 0.01^{b}) , AST (0.19 ± 0.01^{ab}/0.18 ± 0.01^{a}), ALT (0.20 ± 0.01^{c} /0.20 ± 0.02^{bc}) are normal when compared with the normal control. The protein synthetic function of L400 was not compromised. Protein (97.85 ± 0.05^{h} /98.85 ± 0.82^{h}) and Albumin (65.26 ± 1.11^{g} /62.79 ± 0.21^{f}) when compared with normal control. Luteolin at 400 mg/kg body weight could confer mild hepatoprotective and antioxidant effect on the liver since all the enzyme activities are the same as the normal control. The protein and albumin synthesizing ability of the Liver is not impaired at L400 administered animals.

In Table 2, Quercetin at all concentrations brought about the reduction in TC, TG, HDLc and decreased atherogenic index at Q100 (0.12). Rutin increased the level of HDLc at the three dose levels and improved the atherogenic index by 0.56 at R200. The atherogenic index of R400 treated animal group was 0.53. Lipid biosynthesis in the liver is regulated by a family of transcription factors the sterol regulatory element binding proteins (SREBPs) namely, $SREBP_1$ and $SREBP_2$ (Shimano, 2001). $SREBP_1$ regulates enzymes of fatty acid synthesis while $SREBP_2$ regulates cholesterol biosynthesis and uptake by regulating biosynthesis of HMG-CoA reductase (Wong *et al.,* 2015). It may be likely that quercetin and rutin affected the expression of $SREBP_1$ and $SREBP_2$.. There may be down-regulation of these proteins. This affects the level of HDL-c and triacylglycerol produced by the liver and brings about reduction in liver TG (as VLDL), liver HDL and LDL (since it level depends on the circulating VLDL). The liver uptake of LDL-c may also be hampered via down regulation of $SREBP_2$..Flavonoid may affect the expression of SREBP or steroid regulatory element binding protein activating protein (SCAP) or directly affect steroid regulatory element in the promoter of the genes involved in lipid biosynthesis. Wong *et al*. (2015), reported that luteolin inhibit expression of $SREBP_2$ transcription factor in the hepatocyte cell line WRL and $HepG_2$. They also reported that luteolin prevented nuclear translocation of $SREBP_2$ hence decrease transcription of HMG CoA reductase (Wong *et al.,* 2015) an enzyme in cholesterol biosynthetic pathway. Quercetin and luteolin may also have affected LDL-c receptor expression in the liver hence reduced LDL-c in the liver. The level of hepatic HDL-c and LDL-c therefore directly affect the TC of the liver. The atherogenic index of Q100 and R400 are 0.12 and 0.53 respectively were desirable. VLDL is synthesized in the liver and its role is to transport triacylglyceride formed from excess dietary carbohydrate to extrahepatic tissues

where they are acted upon by the action of lipoprotein lipase at the capillary endothelium of the target tissues to release glycerol and fatty acids, the later which are used for the provision of energy in the muscle, stored in the adipose tissue, used as milk fat in the lactating mammary gland. LDL is produced in circulation supposedly at the liver sinusoid and delivers cholesterol to peripheral tissues. About 50-75 % of cholesterol is cleared by the liver via its LDL-receptor. LDL has a negative correlation with the development of atherosclerosis. HDL is on the other hand synthesized by the liver. It picks cholesterol ester from other tissues and lipoproteins and delivers it to the liver in a reverse cholesterol transport via scavenger receptor class B1 (SRB1). In the liver about 50 % of HDL-c may return to the liver. There is a positive correlation of HDL with the development of atherosclerosis. The ratio of high-density lipoprotein to low density lipoprotein (HDL-c / LDL-c) and atherogenic index is a tool for assessing the tendency of an organism to develop atherosclerosis and stroke.

In Table 3, there is no evidence of leakage of Albumin in Luteolin treated animal group as their concentration increased to L400 but the value did not vary significantly with the normal control and may be the best dose in hepatoprotection function. The albumin level of R400 group was significantly higher than the normal control. L400 greatly restored protein and albumin synthetic ability in the hepatic cells. The total protein level of the serum rather decreased as the concentration increased in luteolin treated animal groups (L100 to L400) but the value did not vary significantly ($p>0.05$) with the normal control. There is a reduction in the serum albumin and total protein of quercetin and rutin treated animal in group Q100, Q400 and R200. The Q200 and L100 shows elevated protein concentration. The ability of the liver to synthesize various proteins including albumin is also a measure of liver functionality of the liver. The half-life of the serum albumin is about 20 days (Thapa and Anuj, 2007). The serum albumin level at any point in time is an indicator to the rate at which it is synthesized, distributed, and degraded.

In Table 4, Quercetin at Q400 shows hepatoprotective and antioxidant properties on the oxidative damage because of diabetes mellitus condition since the liver and serum level shows reduced enzyme activity when compared with the diabetic control. The serum activities of ALT and ALP of Q400 and L400 groups were significantly ($p<0.05$) lower than the enzyme activity observed in diabetic control in all the flavonoid groups. The leakage of marker enzymes into the serum was an indication of tissue injury. The AST in R400 and L400 was significantly ($p<0.05$) lower than the normal control. ALP is a marker enzyme for plasma membrane and endoplasmic reticulum. Low serum enzyme activity is an indication of an intact liver cells membrane since their activity was similar to the normal control. This shows that the Luteolin at this concentration was able to constantly scavenge to some extent these ROS, because of diabetes mellitus condition of the rats. Any elevation in the serum activity of these enzymes could be because of leakage from another tissue location which are yet unknown.

In Table 5, there was a progressive decrease in kidney creatinine level in quercetin treated animal groups. This could be because of reduced glomerular filtration rate (GFR). The urea concentration of the kidney equally decreased. This also indicates reduced functionality of the kidney as the concentration of quercetin increased from 100 to 400 mg /kg body weight.

The progressive increase in concentration of creatinine as the dose of administered rutin and luteolin increased from 100 to 400 mg/kg body weight is a positive attribute on kidney functionality. It shows that rutin and uteolin can improve or reduce oxidative stress damage on kidney as a result of diabetes mellitus at a higher dose. However, the urea concentration of the kidney in 400 mg rutin /kg body weight of the animal was higher than the controls.

Conclusion

The effect of the food grade flavonoids on liver function, lipid profile and antioxidant protection were determined. The food grade flavonoids treated animal groups at 400 mg Quercetin, 100 mg Rutin and 400 mg Luteolin had better hepatoprotection. Similarly, the serum level of these enzyme markers also indicates that the concentrations mentioned above protected the liver better. The Albumin and protein of animals treated with 400 mg Quercetin is comparable with the normal control. The Albumin and protein of animals treated with 200 mg Rutin and 400 mg Luteolin are also comparable with normal control. The Atherogenic Index (AI) of serum lipid profile, of the flavonoid treated animal groups were significantly reduced but the least values were seen in animals treated with 400 mg Quercetin (AI=2.80), 400 mg Rutin (AI=3.34) and 100/400 mg Luteolin (AI=1.78/2.52 resp), are greatly lowered and therefore may have no cardisovascular health implications at any dose in comparison with diabetic control (AI=8.05). The AI of the liver homogenate lipid profile also shows that they decreased significantly in comparison with diabetic control. it is observed that Quercetin is more effective at low dose (100mg /kg), Rutin at medium and high doses (200 /400 mg/kg) while luteolin has been mostly found to be more effective at high dose (400 mg/kg).

Recommendations

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.

References

Abbasi, A., Bakker, . J., Corpeleijn, E., van der A. D. L., Gansevoort, R. T., Gans, R. O. (2012). Liver function test and risk prediction of incident type 2 diabetes: Evaluation in two independent cohorts. PLos One. 2012; 7:e51496.

Bastaki, S. (2005). Diabetes mellitus and its treatment. *International Journal of Diabetes and Metabolism,* **13** (3): 111-134.

Bergermeyer, H. U., Scheibe, P. and Wahlefeld, A. W. (1978). Methods for aspartate and alanine amino transferase. *Clinical Chemistry,* **125**:1487.

Bora, K., Borah, M., Chitia, H., Nath, C. K., Das, D. and Ruram, A. A. (2016). Presence of concurrent derangements of liver function test in type 2 diabetes and their relationship with glycemic status: A retrospective observational study from Meghalaya. *Journal of laboratory physicians*, **8** (1): 30-35. Doi: 10.4103/0974-2727.176227

Brostrom, C. O. and Jeffay, H. (1970). Protein catabolism in rat liver homogenates: a re-evaluation of energy requirement for protein catabolism. *The Journal of Biological Chemistry*, **245** (16): 4001-4008.

Burcelin, R., Eddouks, M., Maury, J., Kande, J., Assan, R. and Girard, J. (1995). Excessive glucose production, rather than insulin resistance, accounts for hyperglycemia in recent onset streptozotocin-diabetic rats. *Diabetologia,***38** (3): 283-290. doi:10.1007/BF00400632.

Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., Fernandes, J. D. d-R., Ohlrogge, A. W. and Malanda, B. (2018). Diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research in Clinical Practice*, **138**: 271-281.

Dos-Santos, J. M., Tewari, S. and Mendes, R. H. (2019). The role of oxidative stress in development of Diabetes and its complications. *Journal of Diabetes Research*, **2019. h**ttps://doi.org/10.1155/2019/4189813

Doumas, B. T., Bayse, D. D., Carter, R. J., Peters, T. and Scaffer, R. (1981). A candidate

referenceMethod for determination of total protein in serum. I. Development and evaluation.*Clinical Chemistry,* **27** (10):1642-1650. **h**ttps://doi.org/10.1093/clinchem/27.10.1642

Fossati, P., Prencipe, L. and Lorenzo, P. (1982). Serum triglyceride determination colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*. **28:** 2077 – 2080.

Friedewald, W. T, Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultra-centrifuge. *Clinical Chemistry,***18**: 499–502.

Getnet. T., Sintayehu, A., Alebachew, F. Molla, A. (2019). Prevalence of liver function test abnormality and associated factors in type 2 diabetes mellitus: a comparative cross-sectional study. *EJIFCC***,** 30 (3): 303-316.

Grove, T. H. (1979). Effect of reagent pH on determination of HDL-c by precipitation with Na-phosphotungsate-magnesium. *Clinical Chemistry*. **25**: 560.

Hanley, A. J., Williams, K., Festa, A., Wagenknecht, L.E., D'Agostino, R. B., Haffner, S. M. (2005). Liver markers and development of metabolic syndrome. *Diabetes*, **54** (11): 3140-3147.

Jayakar, B. and Suresh, B. (2003). Antihyperglycemic and hypoglycemic effect of *Aporosa lindleyana* in normal and alloxan induced diabetic rats. *Journal of Ethnopharmacology*, **84** (2-3):247-249. doi:10.1016/s0378-8741(02)00328-8.

Kayar, Y., Ilhan, A., Kayar, N. B., Unver, N., Coban, G., Ekinci, I., Hamdard, J., Pamukcu, O. and Eroglu, H. (2017). Relationship between poor glycemic control and risk factors, life style and complications. Biomedical Research, **28** (4): 1581-1586.

Kumar, S. and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An Overview. *The Scientific World Journal,* **2013***,* p. 16 http://dx.doi.org/10.1155/2013/162750..

Kunutsor, s.K., Apekey, T. A. and Walley, J. (2013). Liver aminotransferases and risk of incident type 2 diabetes: A systematic review and meta-analysis. American journal of epidemiology, **178**: 159-171.

Leeds, J. S., Forman, E. M., Morley, S., Scott, A. R., Tesfiaye, S. and Sanders, D. S. (2009). Abnormal liver function tests in patients with type 1 diabetes mellitus: prevalence, clinical correlations and underlying pathology. *Diabetesmedicine*, **26** (12): 1235-1241. Doi:10.1111/j.1464-5491.20009.02839.x

Naito, H. K. (1984_a). *Cholesterol. Clinical Chemistry*. A. Kaplan (ed). Toronto, Princeton: The C. V. Mosby Co St. Louis. pp. 437 and 1194-1206.

Naito, H. K. (1984_b). High-density lipoprotein (HDL) cholesterol. A. Kaplan *etal.* (eds). *Clinical Chemistry*. Toronto, Princeton: The C. V. Mosby Co St. Louis. pp. 437 and 1207-1213.

National Committee for Clinical Laboratory Standards (NCCLS) Approved Standards (1979). ACS-1. Specification for standardized protein Solution (Bovine serum Albumin). 2nd edn. National Committee for Clinical Laboratory Standards. 771 E Loncaster Ave., Villanova. PA 19-85.

Ni, H, oe, H. H. and Htet, A. (2012). Determinants of abnormal liver function testsin diabetes patients in Myanmar. International journal of Diabetes research, **1:** 36-41.

Panche, A. N., Diwan, A. D. and Chandra, S. R, (2016). Flavonoids: An overview. *Journal of Nutritional Science,***5**: e47.

Doi: 10.1017/jns.2016.41. PMCID: PMC5465813. PMID:28620474

Priora, R., Coppo, L., Salzano, S., Simplicio, D. P. and Ghezzi, P. (2010). Measurement of mixed disulfides including glutathionylated proteins. *Methods in Enzymology,* **473**: 149-159. https://doi.org/10.1016/S0076-6879(10)73007-X.

Ruiz-Cruz, S., Chaparro-Hernández, S., Hernández- Ruiz, K. L., Cira-Chávez, L. A., Estrada-Alvarado, M. I., Ortega, L. E. G., Ornelas-Paz, J. d-J. and Mata, M. A. L. (2017). Flavonoids: important biocompounds in foods. www.intechopen.com. doi:10.5772/67864. Assessed, 04/07/2020

Schlebusch, H., Rick, W., Lang, H. and Knedal, M. (1974). Standards in the activities of clinically important enzymes. *Deutsche MedizinischeWochenschrift,***99**: 765-766.

Shibabaw, T., Dessie, G. and Molla, M.D. (2019). Assesment of liver marker enzymes and its association with type 2 diabetes mellitus in Northwest Ethiopia. *BMC research notes,***12;** 707. https://doi.org/10.1186/s13104-019-4742-x

Shimano, H. (2001). Sterol regulatory element-binding protein (SREBPs): Transcriptional regulators of lipid synthetic. *Progress in Lipid Research*, **40**: 439-452.

Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I. and Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents. *Mechanism and Actions. Mutation Research/ Fundamental and Molecular Mechanisms of Mutagenesi*s, **579** (1-2):200-213. doi:10.1016/mrfmmm.2005.03.023

Tabatabaei-Malazy, O., Larijani, B. and Abdollahi, M. (2013). A novel management of diabetes by means of strong antioxidants combination. *Journal of Medical Hypothesis and Ideas,* **7** (1):25-30. https://doi.org/10.1016/j.jmhi.2012.12.002

Takian, A. and Kazempour-Ardebili, S. (2016). Diabetes dictating policy: An editorial Commemorating world health day 2016. *International Journal of Health Policy and Management,* **5** (10):571-573. doi.10.15171/ijhpm.2016.79

Thapa, B. R. and Anuj, W. (2007). Liver function test and interpretation. *Indian Journal of Pediatrics*, **74** (7):663-671.

Tsimogiannis, D. I. and Oreopoulou, V. (2004). Free radical scavenging activity of 5, 7,3'4' hydroxy susbstituted flavonoids. *Innovating Food Science and Emerging Technology*, **5**: 523-528.

Ucler R., Kartal, M., even, I., Alay, M., Bayram, I. and Olmez, S. (2015). Glycogenic hepartopathy in type 1 diabetes mellitus. *Case report in hepartolog,* **2015:** Id236143. https://doi.org/10.1155/2015/236143

Wong, T. Y. Klein, R. Couper, D. J., Cooper, I. S., Shahar, E., Hubbard, L. D., *et al.* (2001). Retinal microvascular abnormalities and incident stroke: The atherosclerosis risk in communities study. *Lancet,* **358**:1134-1140.

Wong, Y. T., Lin, S. M. and Leung, L. K. (2015). the flavone luteolin suppresses $SREBP_2$ expression and post- translational activation in hepatic cells. *Plos One,* **10** (8) e0135637, doi. 10.1371/journal.pone.0135637. PMCID: PMC4547722. PMID:26302339.

World health organization WHO, (2016). Global report on diabetes Geneva, Switzerland: WHO press.https://who.int/

World health organization WHO, (2020). Diabetes. Geneva, Switzerland: WHO press. https://who.int/news-room/fact-sheets/detail/diabetes

Yener, S., Ozer, E., Yuce, O., Bayraktar, F. and Yesil, S. (2009). Liver manifestation of poorly controlled type 1 diabetes mellitus: hepartic glycogenosis. *Endocrine abstracts* **20:270**