WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 12, 8-24. Research Article ISSN 2277– 7105

ANTIOXIDANT AND LIPID PROFILE EFFECTS OF TANNIC ACID IN STREPTOZOTOCIN INDUCED DIABETIC MALE WISTAR ALBINO RATS

Mba, O.J.*[1], Edward, U.I.[2] and Umeh, V.A.[1]

[1]Department of Biochemistry, College of Natural Science, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

[2]Centre for Molecular Bioscience and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

Article Received on
06 August 2020,

Revised on 26 August 2020,
Accepted on 16 Sept. 2020

DOI: 10.20959/wjpr202012-18722

***Corresponding Author**
Mba, O.J.
Department of Biochemistry, College of Natural Science, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

ABSTRACT

This study was aimed to evaluate the antioxidant and lipid profile effects of tannic acid in streptozotocin induced diabetic male wistar albino rats. Fifty four (54) male albino rats of mean weight 140 g were used for this study. The animals for the study were grouped into nine (9) groups of six (6) rats each. Groups (1), (2) and (5) were the control groups. Groups (3), (4) and (6) were the test groups, while groups (7), (8) and (9) were the groups that received the extract only. Group 1 is the normal control that received feed and water only, group 2 is the negative control group that was induced with diabetes without treatment, and group 5 is the positive control that was treated with 50 mg/kg body weight of the standard drug (glibenclamide). Test groups (3), (4) and (6) were orally given 100, 200 and 400 mg/kg body weight of tannic acid. Groups (7), (8) and (9) were the groups that received the extract only (100, 200, and 400 mg/kg body weight of tannic acid). All the rats used in this study were initially subjected to diabetes by single intraperitoneal induction of 65 mg/kg body weight of streptozotocin except the normal control group and the groups that received the extract only. Treatment lasted for 21 days and after which the animals were sacrificed under mild anesthesia (10% formasaline). Blood samples were collected in the plain bottle for the analyses on the antioxidant and lipid profile effects of tannic acid in diabetic rats by assessing biochemical parameters such as total cholesterol, high density lipoprotein, low density lipoprotein, triacylglycerol, very low density lipoprotein, superoxide dismutase, catalase,

malondialdehyde and reduced glutathione. From the result obtained, there was a significant ($p< 0.05$) increase between the negative control and the test groups that received 100, 200 and 400 mg/kg body weight of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg b.wt of tannic acid) for catalase (CAT) and malondialdehyde (MDA), while there was a significant ($p< 0.05$) increase between the negative and the test groups that received that 200 and 400 mg/kg b.wt of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg b.wt of tannic acid) for reduced glutathione (GSH). For superoxide dismutase (SOD), there was a significant ($p< 0.05$) decrease between the negative and the test groups that received 100, 200 and 400 mg/kg b.wt of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg b.wt of tannic acid) Also there was a significant ($p< 0.05$) decrease in triacylglycerol (TAG), total cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) between the negative control and the test groups that received 100, 200 and 400 mg/kg b.wt of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg b.wt of tannic acid), while there was a significant ($p< 0.05$) increase between the negative and the test groups that received that 200 and 400 mg/kg b.wt of tannic acid and the groups that received the extract only (100 mg/kg b.wt of tannic acid) for high density lipoprotein (HDL).The study indicates that tannic acid may have exerted antioxidant and hypolipidemic effects in streptozotocin induced diabetic male wistar albino rats, and may also be used pharmacologically in the management of hyperlipidemia and diseases implicated by free radicals.

KEYWORDS: Antioxidants; Catalase; Reduced glutathione; High density lipoprotein; Low density lipoprotein; Superoxide dismutase; Total cholesterol.

INTRODUCTION

In diabetes mellitus, chronic hyperglycemia produces multiple biochemical sequence and diabetes induced oxidative stress that plays an important role in the symptoms and progression of the disease.[1] Free radicals have been concerned in the causation of several disorders.[2,3] Increased oxidative stress has been postulated in the diabetic state which coexists with a reduction in the antioxidants status.[4] Tissue antioxidant states have altered in diabetes resulting in increased oxidative damage of membranes and tissue.[5,6,7] Oxidative stress has been strongly associated with tissue damage in diabetic individuals.[8] Streptozotocin (STZ), which is widely used to induce experimental diabetes in animals, induces its diabetes mainly by inducing oxygen free radicals, thereby damaging the pancreas.

STZ can induce partial destruction of b-cells in rats, resulting in insulin resistance-like symptoms, which pathologically would be very similar to human type 2 diabetes mellitus.[9] Antioxidants play a major role in the protection against molecular oxidative damage. Disturbances of antioxidant defence systems in diabetes have been demonstrated, including alteration in the activities of antioxidant enzymes and impaired glutathione (GSH) metabolism. Plant-derived herbal remedies are apparently effective, produce minimal or no side effects in clinical experience and are of relatively low costs as compared with oral synthetic hypoglycemic agents.[10] The antioxidant system includes Superoxide dismutase (SOD), Catalase (CAT), reduced Glutathione (GSH), Malondialdehyde (MDA) and indirectly glutathione reductase. The protective role of antioxidant enzymes is well known and has been investigated extensively in diabetic patients and experimental diabetic animals.[11]

Arteriosclerosis or coronary artery disease is a condition characterized by deposits of lipids, mainly cholesterol on the inner walls of the arteries. These deposits narrow the arterial channels and partly block the normal flow of blood through them.[12] The decrease in blood flow and oxygen can result in stroke, partial paralysis, loss of speech and sometimes death.[13] Arteriosclerosis is the main cause of mortality and morbidity in western countries and progressively increasing in developing countries.[14] Low fat diet is often prescribed for the management of arteriosclerosis as there are no specific treatments for the ailment.[15]

The present study was undertaken to study the antioxidant and lipid profile effects of tannic acid in streptozotocin induced diabetic male wistar albino rats.

2. MATERIALS AND METHODS

2.1 Chemical

Tannic acid powder extra pure (100 g) and Streptozotocin (500 mg) was purchased from sigma chemical (Bristol Scientifc Company). Assay kits for the estimation of total cholesterol, high density lipoprotein, low density lipoprotein, triacylglycerol, very low density lipoprotein, superoxide dismutase, catalase, malondialdehyde and reduced glutathione were purchased from Randox UK. All other chemicals used for this study were of analytical grade.

2.2 Animals

Male albino rats of mean weight 130 g were used for the present investigation. The rats were maintained on standard pellet diet and tap water ad libitum. They were kept in aluminum

cages in the animal house. Ethical approval was sought from the COLNAS ethical committee before commencement of study.

2.3 Induction of experimental diabetes

Diabetes was induced by injection of a single intra-peritoneal dose of Streptozotocin solution. Streptozotocin (65 mg/kg body weight) was dissolved in ice cold citrate buffer immediately before use. Diabetes was confirmed by glucose estimation. Animal with blood glucose value above 200 mg/dL was selected for the experimental study.

2.4 Experimental design

Eighteen (18) mice were used for the acute toxicity study. Lethal dose (LD_{50}) determination was conducted using the Lorke's method. Fifty four (54) male albino rats of mean weight 130 g were used for this study. The animals for the study were grouped into nine (9) groups of six (6) rats each. Groups (1), (2) and (5) were the control groups. Groups (3), (4) and (6) were the test groups, while groups (7), (8) and (9) were the groups that received the extract only. Group 1 is the normal control that received feed and water only, group 2 is the negative control group that was induced with diabetes without treatment, and group 5 is the positive control that was treated with 50 mg/kg body weight of the standard drug (glibenclamide). Test groups (3), (4) and (6) were orally given 100, 200 and 400 mg/kg body weight of tannic acid. Groups (7), (8) and (9) were the groups that received the extract only (100, 200, and 400 mg/kg body weight of tannic acid). All the rats used in this study were initially subjected to diabetes by single intraperitoneal induction of 65 mg/kg body weight of streptozotocin except the normal control group and the groups that received the extract only. Treatment lasted for 21 days and after which the animals were sacrificed under mild anesthesia (10% formasaline). Blood samples were collected in the plain bottle for the analyses on the antidiabetic effects of tannic acid in normal and diabetic rats by assessing the plasma glucose, plasma insulin, glycated hemoglobin and other biochemical parameters such as total cholesterol, high density lipoprotein, low density lipoprotein, triacylglycerol, very low density lipoprotein, superoxide dismutase, catalase, malondialdehyde and reduced glutathione.

2.5 Evaluation of the various parameters studied

2.5.1 Determination of reduced glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of.[16]

2.5.2 Catalase assay

Determination of catalase activity was according to[17] method.

2.5.3 Superoxide dismutase (SOD)

This was determined using the method.[18]

2.5.4 Lipid peroxidation (Malondialdehyde-MDA)

Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, malondialdehyde (MDA) as described by.[19]

2.5.5 Determination of total cholesterol

Total cholesterol was determined according to.[20]

2.5.6 Determination of triacylglycerol

Triglyceride was determined using enzymatic test glycerol-phosphate oxide method.[21]

2.5.7 Determination of High Density Lipoprotein (HDL)

HDL-Cholesterol was determined using method of.[22]

2.5.8 Determination of Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) Cholesterol

LDL and VLDL- cholesterol was calculated from Friedewald equation as stated by.[23]

2.6 Statistical analysis

The data were expressed as mean ± standard deviation and analyzed using statistical package for the social sciences (SPSS 22.0). Comparison was made between the test groups and the control groups using One way Anova and $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

3.1 Effects of GSH on streptozotocin induced diabetic rats

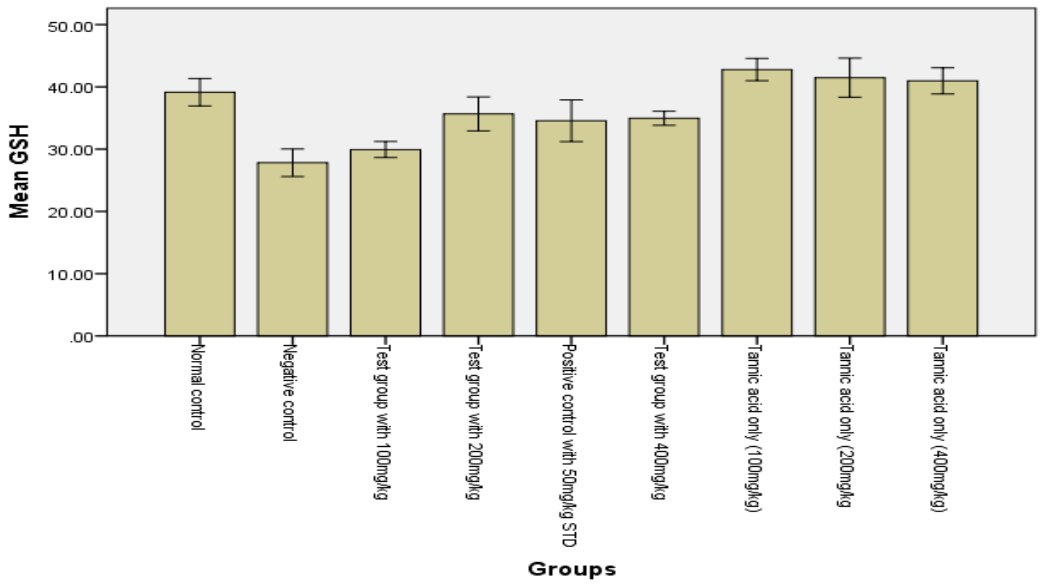


Fig. 1 Mean values comparison of GSH between the negative control, test groups and the group that received the extract only.

There is a significant increase ($p< 0.05$) between the negative control and the test groups that received 200 and 400 mg/kg body weight of tannic acids and the group that received the extract only (100, 200 and 400 mg/kg body weight of tannic acid).

3.2 Effects of SOD on streptozotocin induced diabetic rats

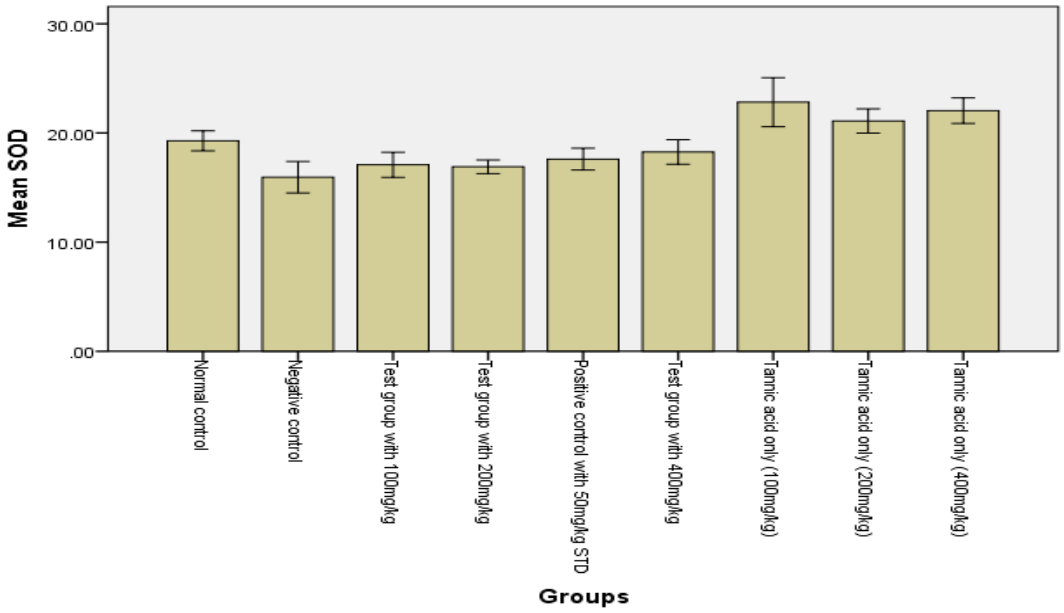


Fig. 2: Mean comparison of SOD between the negative control, test groups and the groups that received the extract only.

There is a significant increase ($p < 0.05$) between the negative control and the test group that received 400 mg/kg body weight of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg body weight of tannic acid).

3.3 Effects of MDA on Streptozotocin induced diabetic rats

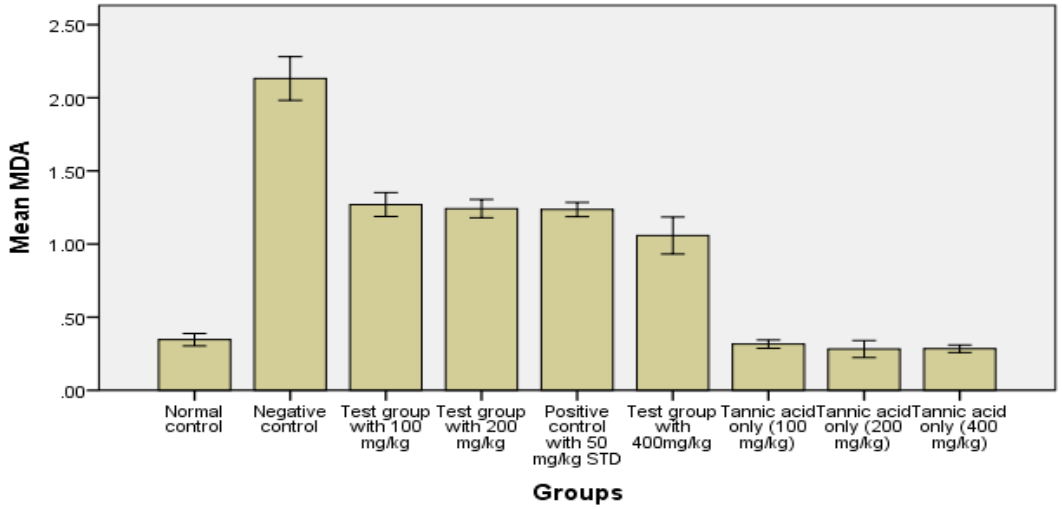


Fig. 3: Mean comparison of MDA between the negative control, test groups and the groups that received the extract only.

There is a significant decrease ($p < 0.05$) between the negative control, the test groups that received 100, 200 and 400 mg/kg body weight of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg body weight of tannic acid).

3.4 Effects of Catalase on streptozotocin induced diabetic rats

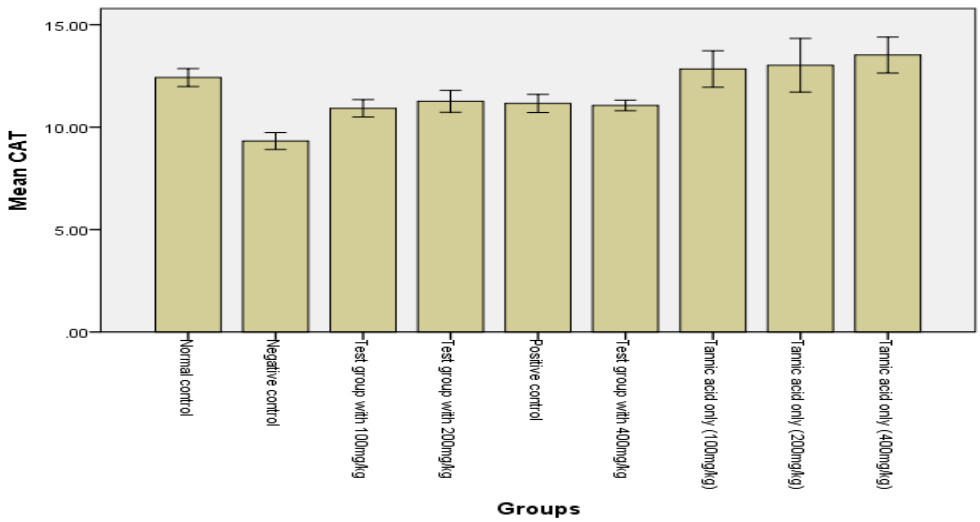


Fig. 4: Mean comparison of the negative control, test groups and the group that received the extract only.

There is a significant increase ($p < 0.05$) between the negative control and the test groups that received 100, 200 and 400 mg/kg body weight of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg body weight of tannic acid).

3.5 Effects of HDL on Streptozotocin induced diabetic rats

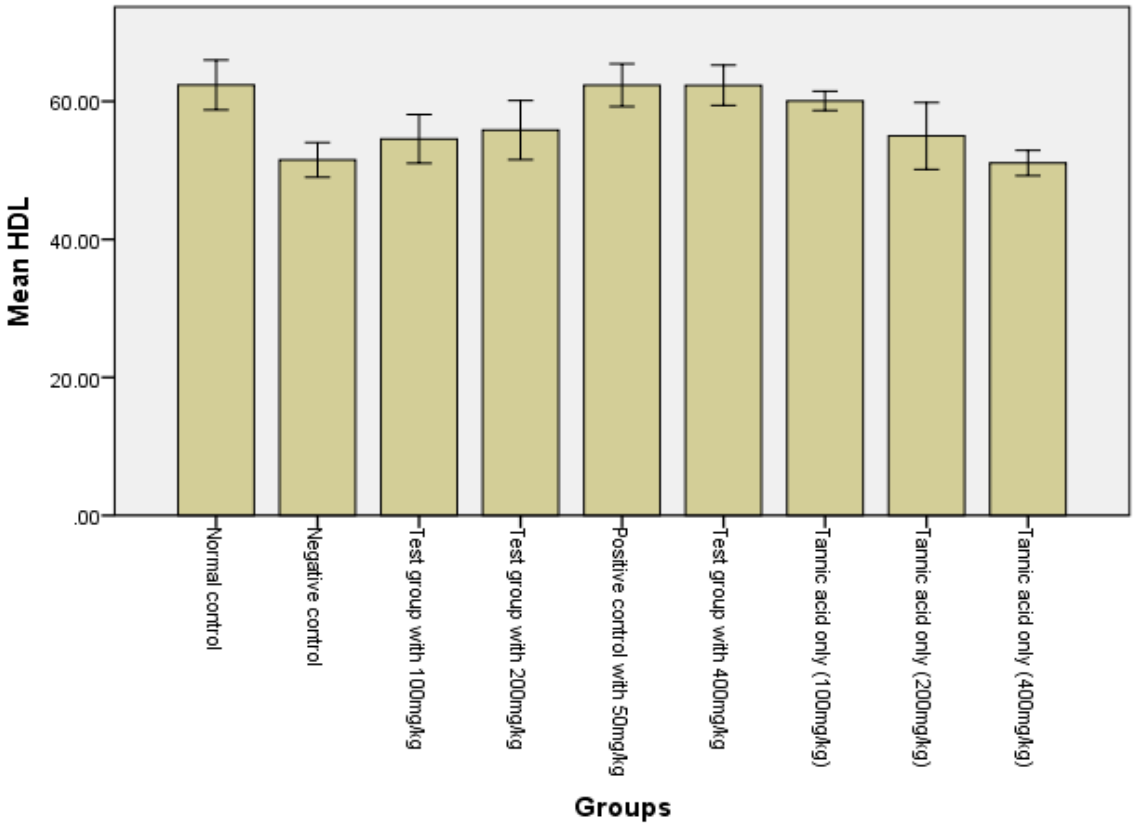


Fig. 5: Mean comparison of HDL between the negative control, test groups and the groups that received the extract only.

There is a significant increase ($p < 0.05$) between the negative control and the test groups that received 200 and 400 mg/kg body weight of tannic acid and the group that received the extract only (100 mg/kg body weight tannic acid).

3.6 Effects of Triacylglycerol on streptozotocin induced diabetic rats

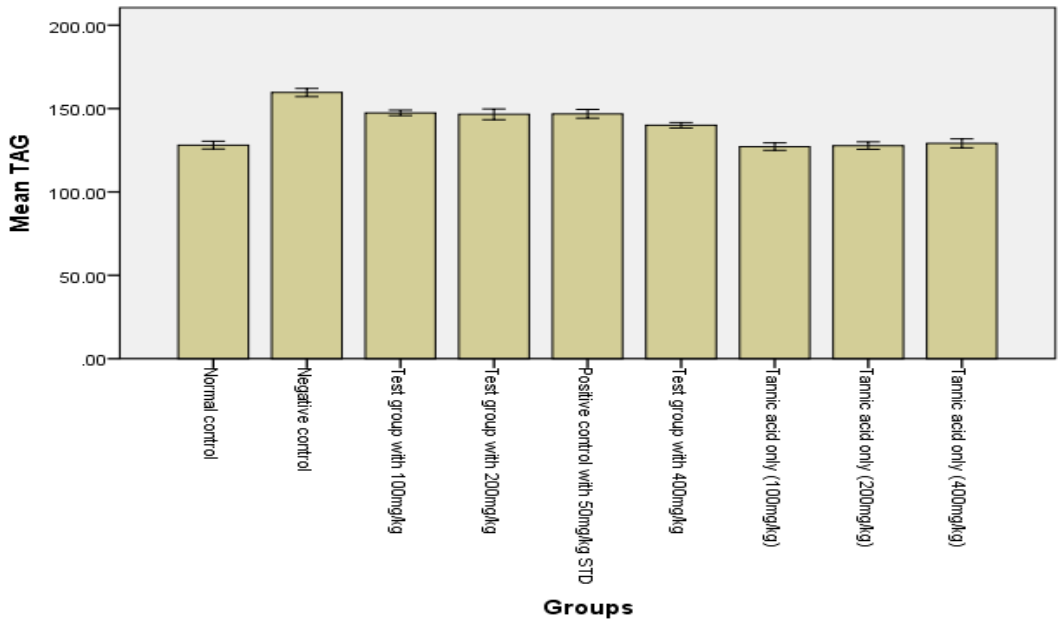


Fig. 6: Mean comparison of TAG between the negative control, test groups and the group that received the extract only.

There is a significant decrease ($p < 0.05$) between the negative control and the test groups that received 100, 200 and 400 mg/kg body weight of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg body weight of tannic acid).

3.7 Effects of Total cholesterol on streptozotocin induced diabetic rats

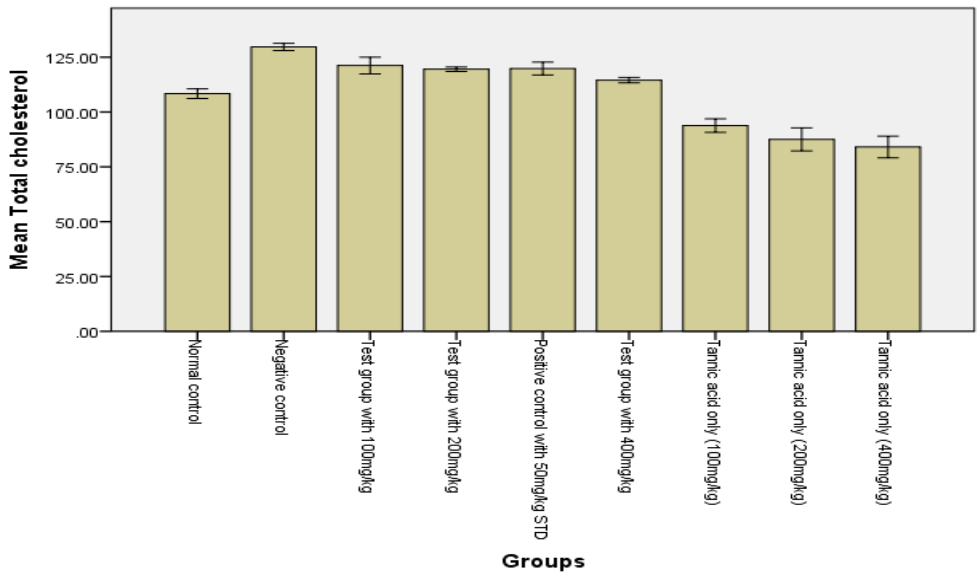


Fig. 7: Mean comparison of Total cholesterol between the negative control, test groups and the group that received the extract only.

There is a significant decrease ($p < 0.05$) between the negative control and the test groups that received 100, 200 and 400 mg/kg body weight of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg body weight of tannic acid).

3.8 Effects of Low density lipoprotein on streptozotocin induced diabetic rats

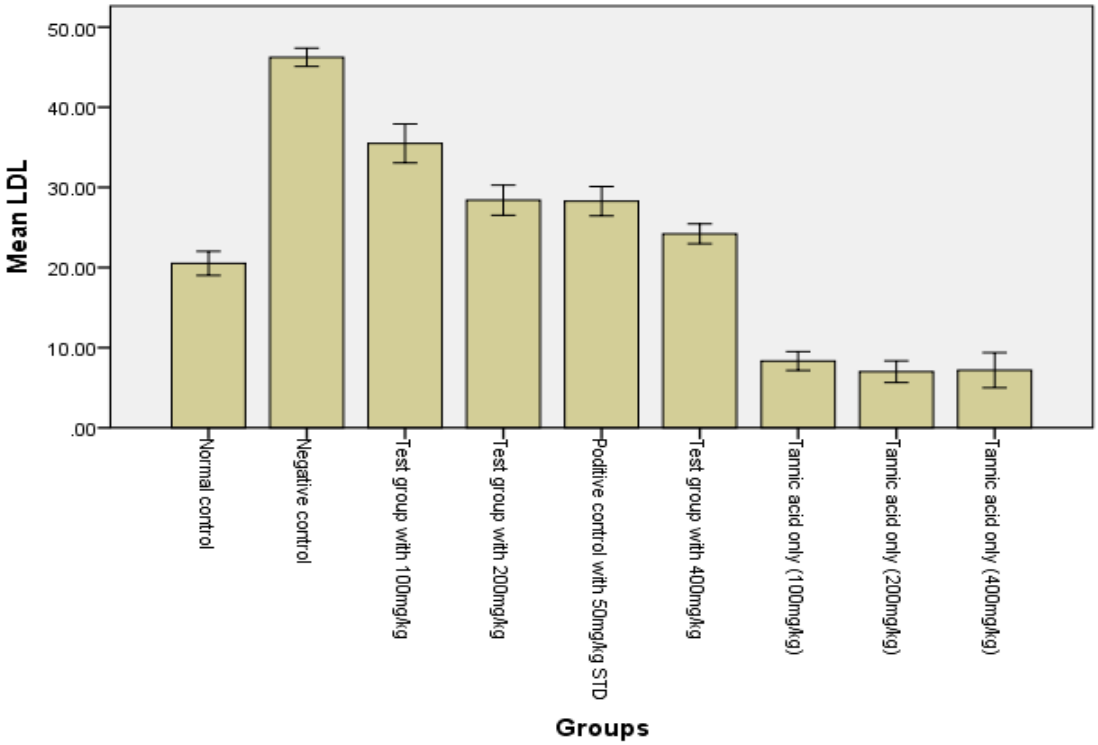
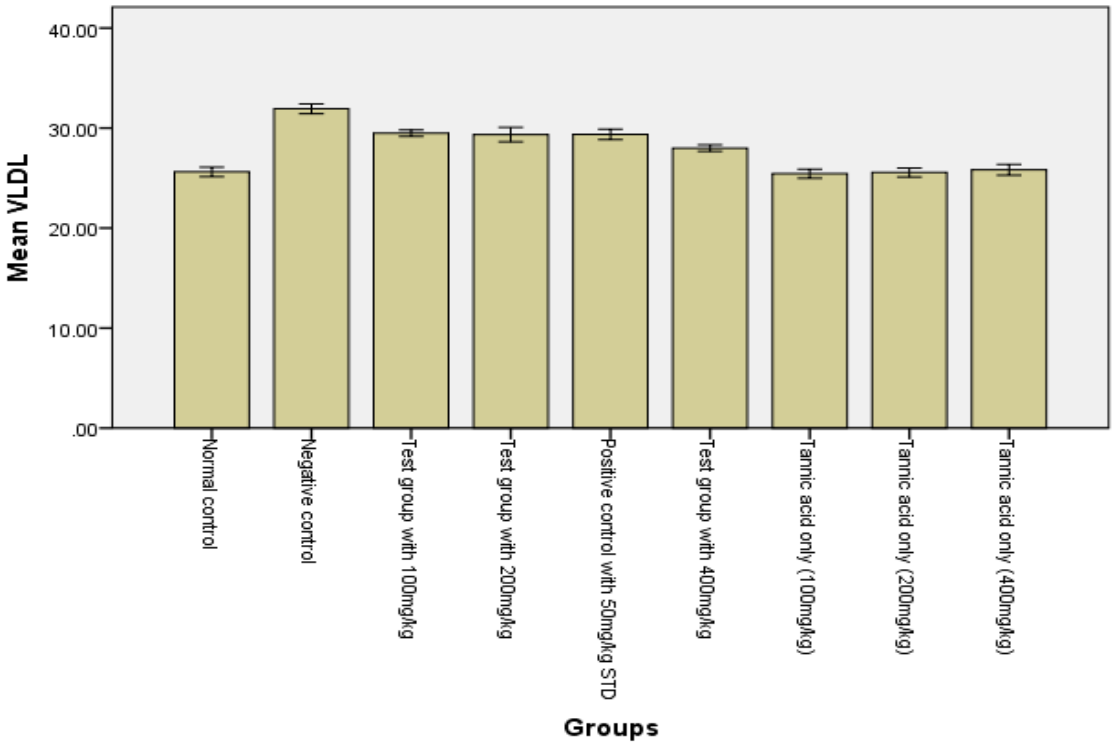


Fig. 8: Mean comparison of LDL between the negative control, test groups and the group that received the extract only.

There is a significant decrease ($p < 0.05$) between the negative control and test groups that received 100, 200 and 400 mg/kg of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg of tannic acid).

3.9 Effects of very low density lipoprotein (VLDL) on streptozotocin induced diabetic rats



Mean comparison of VLDL between the negative control, test groups and the group that received the extract only

There is a significant decrease ($p > 0.05$) between the negative control and test groups that received 100, 200 and 400 mg/kg of tannic acid and the groups that received the extract only (100, 200 and 400 mg/kg of tannic acid).

DISCUSSION

Use of tannic acid in food application is far more wide spread and significant amounts are used as process aids in beer clarification, aroma compound in soft drinks and juices. Tannic acid is applied directly to treat sore throat and tonsils, spongy or receding gums, cold sores and fever blister.[12] Tannic acid can medicate bleeding, chronic diarrhea, dysentery, bloody urine, painful joints, persistent coughs, and cancer. Vaginally, Tannic acid can be used as a doughe for white or yellowish discharge, i.e, leucorrhea.[13] Tannic acid is a good source of antihyperlipidimic and antihyperglycemic agent.[14,15]

Lipid peroxidation is one of the characteristic features of chronic diabetes. Lipid peroxide mediated damage has been observed in the development of both type 1 and type 2 diabetes mellitus. It has been observed that insulin secretion is closely associated with lipoxygenase

derived peroxides.[21] Low level of lipoxygenase peroxide stimulates the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation leading to cellular infiltration and islet damage in type 2 diabetes.[22] Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzymes and receptors.[23] Its products are harmful to most of the cells in the body associated with variety of diseases.[24] The present study showed a significant elevation of plasma and liver TBARS content in diabetic rats. The increased TBARS content of diabetic rats suggests peroxidative injury may be involved in the development of diabetic complications. Tannic acid could significantly reduce the liver lipid peroxidation products level in diabetic rats. This indicates that tannic acid is a potent inhibitor of the oxidative damage of liver tissue. MDA is a product of lipid peroxidation.[25] Extensive lipid peroxidation leads to disorganization of membrane by peroxidation of unsaturated fatty acids which also alters the ratio of polyunsaturated to other fatty acids. This would lead to a decrease in the membrane fluidity and the death of the cell.[25] In this study, MDA level showed a dose-dependent decrease close to normal level. This implies that tannic acid could have antioxidant properties by decreasing lipid peroxidation. This is consistent with earlier studies by.[26]

In diabetes oxidative stress damage, the pancreatic tissue there by further reducing insulin secretion. In the present study, the activity of SOD, CAT, GSH were significantly reduced in liver of diabetic induced rats. The previous report has shown that the activities of SOD, CAT and GSH were lowered in tissue of diabetic rats.[25] The observed decrease may be due to the utilization of non protein thiols by increased oxygen free radicals produced in hyperglycemia condition. Oral administration of tannic acid to diabetic rats significantly improved the antioxidant defense mechanism, which suggests its role in the protection of vital tissue from oxidative damage during diabetic condition. Reduced glutathione is a potent free radical scavenger GSH within the islets of β-cell and is an important factor against the progressive destruction of the β-cell following partial pancreatectomy.[26] Depletion of GSH results in enhanced lipid peroxidation. This causes increased GSH consumption and can be correlated to the increase in the level of oxidized glutathione (GSSG). Treatment of tannic acid resulted in the elevation of the GHS levels, which protect the cell membrane against oxidative damage by regulating the redox status of protein in the membrane.[27] The plasma lipid peroxide level in STZ-induced diabetes is generally thought to be due to pathological changes to tissue that increase the production and liberation of lipid peroxides into circulation.[28] Treatment with

tannic acid brought back lipid peroxidative markers to near normal levels, which could be as a result of improved glycemic control and antioxidants status. GSH, being the most important biomolecule against chemically induced toxicity can participate peroxides in the presence of GPX. GSH also functions as free radical scavenger and in the repair of free radical caused biological damage.[29] Reduced glutathione, a direct free radical scavenger, also reported to protect the cellular system against the noxious effect of lipid peroxidation.[30]

The role of free radicals in disease initiation cannot be overemphasized. Most free radicals such as hydroxyl radical (OH^-), the superoxide radical (O_2-), lipid peroxide radicals and hydrogen peroxide (H_2O_2) are being implicated in some disease conditions. These include: cancer, gastrointestinal inflammation, asthma, cataracts, cardiovascular disease, diabetes mellitus, liver disorder, periodontal disease, and other inflammatory processes.[30] These reactive oxygen species (ROS) are generated as a result of normal biochemical metabolism in the body which is due to high level of exposure to xenobiotics.[29] Pathological conditions result when the generation of ROS induced by stimuli in the organism exceeds the antioxidant capacity of the organism.[28] The harmful effect of these reactive species in normal metabolic processes which leads to disease condition is a consequence of their interaction with some biological compounds within and outside the cells. Recently, many natural and synthetic free radical scavengers and antioxidants have been employed in protecting biomolecules against free radical mediated damages.[30] In this present research, there was a significant ($P<0.05$) increase in reduced glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) in the test groups and the groups that received the tannic acid only (100, 200 and 400 mg/kg) when compared to the negative control.

The decreased levels of plasma GSH in diabetes could be due to its increased utilization in trapping the oxyradicals.[31] In our study, diabetic rats exhibited decreased level of GSH, which might be due to increased utilization for scavenging free radicals and increased consumption by GPX. Treatment with tannic acid can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH and detoxifies the free radicals generated. The decrease of GSH may hence be responsible for low GPX activity in diabetic tissues. It has been proposed that antioxidant that maintains the concentration of GSH may restore the cellular defense mechanisms, block lipid peroxidation and thus protect the tissue against oxidative damage.[31]

In the present study, the activity of high density cholesterol (HDL), triacylglycerol (TAG), total cholesterol, low density cholesterol (LDL), and very low density cholesterol (VLDL) were significantly increased in diabetic induced rats and significantly decreased ($p< 0.05$) in the treated groups (100 mg/kg, 200 mg/kg and 400 mg/kg b.wt of tannic acid) and the groups that received the groups only (100 mg/kg, 200 mg/kg and 400 mg/kg b.wt of tannic acid), which shows that the tannic acid was able to lower the lipid profile of streptozotocin induced diabetic rats, can be ascribed to the phytochemical constituents of the tannic acid, which implies that tannic acid can be used to prevent cardiovascular complications arising from hyperlipidemia.[32]

CONCLUSION

In conclusion, the present study provides that tannic acid exhibits antioxidants and hypolipidemic properties in streptozotocin induced diabetic rats by decreasing the levels of lipid peroxidation products and increasing the activity of antioxidants and also by decreasing the levels of lipids.

Recommendation

It is advised that further detailed investigation is necessary to find out it´s mechanism of action and establish its therapeutic potential in the treatment of diabetes.

Limitation of the study

The duration of this study was not more than 28 days. Moreover, our present findings were in rats and therefore cannot be directly interpreted that these effects observed in rats will be exactly and/or physiologically the same in humans. Therefore, our findings are subject to further research and verification especially in humans.

Ethical approval

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 85- 23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropiate ethics committe.

ACKNOWLDGEMENT

We sincerely appreciate all that made this work successful.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chandirasegaran GC, Elanchezhiyan KG, Sethupathy S. Efficacy of Berberine chloride on hyperglycemia in Streptozotocin induced diabetic rats, Int. Res. J. Pharm, 2016; 7(9): 14–18.
2. Wilson RL: Free radicals and tissue damage, mechanistic evidence from radiation studies. In: Biochemical mechanisms of liver injury. New York: Academic Press, 1998: 123-127.
3. Lawrence JC, Eric PD, Joyle AD, Donald DL, Mark AY: Effect of antioxidant treatment on streptozotocin induced diabetic rats on endoneurial blood flow, motor nerve conduction velocity and vascular reactivity of epineural arterioles of the sciatic nerve. Diabetes, 2001; 50: 1927-1937.
4. Collier A, Wilson R, BradlyH, Thomson JA, Small M: Free radical activity in Type 2 Diabetes. Diabetic Med, 1990; 7: 27-30.
5. Genet S, Kale R, Baquer NZ: Alteration in antioxidant enzymes and oxidative damage in experimental diabetic rat tissue: effect of vanadate and fenugreek (Trigonella foenum graecum). Mol Cell Biochem, 2002; (1-2): 7-12.
6. Saxena AK, Saxena P, Kale RK, Baquer NZ: Impaired antioxidant status indiabetic rat liver: effect of vanadate. Biochem Pharmacol, 1993; 45(3): 539-542.
7. Lou MF: Redox regulation in the lens. Prog Retin Eye Res, 2003; 22(5): 657-682.
8. Henriksen EJ, Diamond-Stanic MK, Marchionne EM. Oxidative stress and the etiology of insulin resistance and type 2 diabetes.Free Radic Biol Med, 2011; 51: 993– 9.
9. Zhang F, Ye C, Li G, Ding W, Zhou W, Zhu H et al. The rat model of type 2 diabetic mellitus and its glycometabolism characters. Exp Anim, 2003; 52: 401–7.
10. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of Annona squamosa L. in experimental animals. J Ethnopharmacol, 2005; 99: 75– 81.
11. Govindasamy Chandramohan, Khalid S AI-Numair, Kodukkar Viswanathan pugalendi, Restoration of altered Plasma erythrocyte and liver antioxidant levels by 3hydroxymethyl Xylitol in Streptozotocin-diabetic rats International journal of integrative Biology, 2009; 5: 176.
12. Haines T.H. Do sterols reduce proton and sodium leaks through lipid bilayers? Prog. Lipid Res, 2001; 4: 299- 324.
13. Olson RE. Discovery of the lipoproteins, their role in fat transport and their significance as risk factors. Jour. Nutr, 1998; 128(2): 439- 443.

14. Barter P, Gotto AM, Maroni, JC, Szarek, J, Grundy SM, Kastelein, JP, Bittner, V. HDL Cholesterol, VLDL cholesterol and cardiovascular events. New Engl. Jour. J. Med, 2007; 357(13): 1301 – 1301- 1309.
15. Meyers CD, Kamanna VS, Kashyap ML. Niacin therapy in atherosclerosis. Current Opinion in Lipidology, 2004; 15(6): 659- 665.
16. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys, 1959; 82: 70-77.
17. Aebi H. Catalase. Bergmeyar (eds). Methods in enzymatic analysis, New York, Academic Press. 1974; 674-684.
18. Xin Z, Waterman D, Hemken R, Harmon R. Effects of copper status on neotrophils function, superoxide dismutase and copper distribution in steers. J. Dairy. Sci, 1991; 74: 3078-3085.
19. Wallin B, Rosengren B, Shetzer HG, Camej G. Lipoprotein oxidation and measurement of TBARS formation in a single microlitre plate; Its use for the evaluation of antioxidant. Anal. Biochem, 1993; 208: 10-15.
20. Abell, L.L.; Levy, B.B.; Brodie, B.B. and Kendall, F.E. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. Journal of biological chemistry, 1952; 195: 357-366.
21. Jacob NJ, Dammark PJ. Lipids. Annual Biochemistry and Biophysic, 1960; 88: 250-255.
22. Ochei J. Kolhatkar AE. Laboratory science. 6th edition Mc Gram Hill, New Delhi, 2007; 190- 198.
23. Burtis CA, Ashwood ER, Bruns OE. Fundamentals of clinical chemistry. 6th edition. Elsevier, Haryana, 2008; 539- 555.
24. Cox, L., Cox, J., Ecobeauty: Scrubs, rubs, masks, and bath bombs for you and your friends. Berkely (Calif). Ten Speed Press, 2009; 9: 219-224.
25. Govington T.R. Tannic Acid. Handbook of Nonprescription Drugs. Washington: Web MD. Rwtrieved, 2013.
26. Govindasami Chandirasegaran, Chakkaravarthy Elanchezhiyan, Kavisa Ghosh, Subramaniam Sethupathy. Berberine chloride ameliorates oxidative stress, inflammation and apoptosis in the pancreas of Streptozotocin induced diabetic rats. Biomedicine & Pharmacotherapy, 2017; 95: 175–185.
27. Babby,A., Elanchezhiyan, C., Suhasini S. and Chandirasegaran, G. Antihyperglycemic effect of Tannic Acid in Streptozotocin Induced Diabetic Rats, International Journal of Current Research, 2014a; 6(3): 5396-5398.

28. Boubekri N, Amrani A, Zama D, Dendougi H, Benayache F, Benayache S. In vitro antioxidant and in vivo antidiabetic potential of n-butanol extract of Chrysanthemum fuscatum in streptozotocin induced diabetic rats. Int J Pharm Sci Rev Res, 2016; 41(2): 2149.
29. Jayaprasad B, Sharavanan PS, Sivaraj R. Antidiabetic effect of Chloroxylon swietenia bark extracts on streptozotocin induced diabetic rats. Beni-Suef University Journal of Basic and Applied Sciences, 2016; 5(1): 61-69.
30. Walsh MF and Pek SB. Possible Role of Endogenous Arachidoni Acid Metabolites in Stimulated Release of insulin and Glucagon from the Isolated, Perfused Rat Pancreas. Diabetes, 2005; 33: 926-936.
31. Alphosus OO, Chizaramok OA. Renal and Hepatic Antioxidant Status of Hy perglycemic Rats Treated with Single and Combinatorial Herbal Formulations. Pharmacognosy Communications, 2015; 5(2): 148-59.
32. Xing FZ, Tan BW: Antihyperglycemic and antioxidants properties of Andrographis paniculata in normal and diabetes rats. Clin. Exp. Pharmacol. Physiol, 2000; 27: 358-363.
33. Ihm SH, Yoo HJ, Park SW, Ihm J. metabolism, 1999; 48: 1141-1145.
34. Inove M, Saito Y, Hirato E. Regulation of redox status of plasma protein by mechanism and transport of glutathione and related compounds. J. Protein Chem, 1987; 36: 169-173.
35. Murugan P, Pari L. Effect of tetrahydrocurcumin on plasma antioxidants in Streptozotocin- nicotinamide experimental diabetes. J. Basic. Clin. Physiol. Pharmacol, 2006; 17(4): 231-244.
36. Pinakini K, Shankar, VK, Namita R. Evaluation of Antidiabetic of Ginkgo Biloba in Streptozotocin induced Diabetic Rats. Iran. J. Pharmacol. Ther, 2000; 4: 16-19.
37. Whiting PH, Kalansooriya A, Holbroo I, Haddad F, Jennings PE. The relationship between chronic glycemic control and oxidative stress in type 2 diabetes mellitus. Br. J. Biomed. Sci, 2008; 65: 71-74.
38. Mishra N, Singh N. Blood viscosity, lipid profile, and lipid peroxidation in type-1 diabetic patients with good and poor glycemic control. N Am J Med Sci, 2013; 5(9): 562-6.
39. Kweiterovich PO. The metabolic pathways to high density lipoprotein, low density lipoprotein and triacylglycerides: a current review. The American Journal of Cardiology, 2000; 12: 5- 10.