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Effects of Calcitriol Supplementation on Renal, Liver and Lipidperoxidation Biomakers, on Wistar Albino Rats that received 10% Fructose

Ibrahim Lai[1], Yakubu Sadau[1*], Fatima Umar Maigari[2]
[1]Department of Human Physiology,
College of Medical Sciences,
Gombe State University
Gombe, Nigeria

[2]Department of Biochemistry,
Gombe State University,
Gombe, Nigeria
Email: ysadau@gmail.com

Abstract
Calcitriol is formed in the kidney as the end product of vitamin D metabolism; Low calcitriol status has been implicated in the development of cardiovascular risk factors and other disoders. The study was designed to examine the effects of calcitriol treatment on indices of renal and liver biomarkers and lipid peroxidation on Wistar Albino rats. Animals were randomized into four groups of five rats each and supplemented with to 125 µg/Kg body weight calcitriol for three weeks after five weeks of fructose drinking. Group I: Control; Rats received water, Group II: Rats feed + 125 µg/Kg body weight of Calcitriol, Group III: Rats received 10% fructose solution, Group IV: Rats received10% fructose solution and125 µg/Kg body weight of Calcitriol. All the parameters were determined using commercial kits according to the manufacturers' instructions. Results showed that rats that received fructose exhibited a significant increase in urea, creatinine, liver enzymes activities and lipid peroxidation index while the activities of Superoxide dismutase (SOD) and Catalase (CAT) were significantly reduced when compared with the control. Calcitriol treatment significantly reduced renal failure makers and activities of liver enzymes. However, the activities of antioxidant enzymes were significantly increased in rats that received fructose. It was found that calcitriol treatment has shown an improved reno-hepatic protection, enhanced the activities of the antioxidant enzymes and prevented lipid peroxidation.

Keywords: Antioxidant enzymes, Calcitriol, liver enzymes, lipid peroxidation

INTRODUCTION
Dietary and nutritional imbalances have long been recognized as the key risk factors responsible for the upsurge of Type 2 Diabetes mellitus (T2DM) but the underlying mechanisms remain elusive (International Diabetic Federation, 2013). T2DM is characterised by significant hyperglycaemia resulting from compromised insulin utilisation and insufficient compensatory insulin production. It is an important health problem due to its high morbidity, mortality and increased global prevalence (International Diabetic Federation, 2013). In animal model of experimental diabetes, high fructose diet has been shown to promote oxidative cellular damage and exerts detrimental effects by reducing antioxidant defenses as well as induces the generation of free radicals (Lustig *et al*., 2010;

*Author for Correspondence

Chou *et al*., 2015).Fructose supplementation on the Wistar Albino Rats increases of hepatic acetyl CoA lead to increased production of very low-density lipoprotein and triglycerides which is associated with type 2 diabetes (Teff *et al*., 2009) Fructose causes Metabolic Syndrome, metabolic syndrome is a cluster of conditions- increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels that occur together increasing risk of heart disease, stroke and diabetes (Sánchez-Lozada *et al*., 2007). Numerous studies have shown the increase in insulin resistance upon ingestion of fructose (Hwang *et al*., 2007). Although fructose does not stimulate insulin in the short term, the insulin resistance and obesity induced by long term fructose feeding in experimental animals induces hyperinsulinemia (Elliott *et al*., 2002). Hyperinsulinemia may then lead to hypoglycemia or type 2 diabetes.

Calcitriol [1, 25(OH) $_2$ D3] which is a synthetic compound that is identical with the most active metabolite of vitamin D, 1, 25-dihydroxycholecalciferol, formed in the kidney as the end product of vitamin D metabolism, it is active in the regulation of the absorption of calcium from the gastrointestinal tract and its utilization in the body (Guines*et al*., 2000). Adverse effects of over administration of calcitriol include hypercalcemia, hypercalciuria, and hyperphosphatemia (Jones *et al*., 1994). Low calcitriol status has been implicated in the development of cardiovascular risk factors such as hypertension, dyslipidaemia, insulin resistance as well as type 2 diabetes (Johnson, *et al*., 2007; Pilz *et al*., 2013). Calcitriol supplementation has been reported to improve β-cell function, increase insulin secretion (Mitri *et al*., 2011), as well as enhances insulin sensitivity (Fu *et al*., 2010; Al-Daghari *et al*., 2012). It has also been shown to maintain glucose homeostasis and protects β-cells from oxidative stress induced tissue damage (Manna and Jain, 2012). Increased activity ofrenin angiotensin system (RAS) impairsβ-cell function and peripheral insulin sensitivity but calcitriol suppresses the RAS activity (Cheng *et al*., 2011). Studies investigating the effects of calcitriol supplementation on diabetes and its complications are often conflicting and inconsistent. There is insufficient information on the effects of calcitrol supplementation on biomarkers of renal and liver functions in fructose-induced T2DM in Albino Wistar rats. Therefore, this study investigated the effects of calcitriol administration on indices of renal and liver toxicity, antioxidant enzymes and lipid peroxidation in fructose-drinking rats.

MATERIALS AND METHODS

Animals

Twenty (20) adult male Wistar rats weighing between 130 – 200 g were obtained from College of Medical Sciences, Gombe State University, Gombe, Nigeria and were kept in the animal house of the Department of Human Physiology,maintained under standard conditions (room temperature 24-27C^{O} and humidity 60-65%) with 12 h light and dark cycle. They were fed withstandard laboratory diet dry pellets (Vital feed UAC) and given access to drinking water *ad libitum*. The rats were kept for two weeks prior to the commencement of the study, during which the animals were accustomed to routine handling and acclimatize to the new environment, as well as to stabilize them from the stressful effect which they may have been subjected tofrom where they were purchased to the experimental site (Adenkola*et al*., 2011).

Preparation of Fructose Solution and Experimental Design

Animals were fed with 10% fructose solution prepared daily and served as rats' drinking water for five weeks as previously described by Neeharika *et al*., (2012). Calcitriol treatment started 5 weeks after the beginning of fructose administration at a dose of 125µg/ml.

Animals were randomised into four groups of five animals each and the study lasted for 8 weeks as follows:
Group I: Control received normal food+ Distilled water
Group II: Received normal food+ 125 µg/kg body weight of Calcitriol
Group III: Received normal food+ 10% fructose solution
Group IV: Received normal food+ 10% fructose solution + 125 µg/kg body weight of Calcitriol

The study was conducted in accordance with internationally-accepted principles for laboratory animal use and care. At the end of this experimental procedure, rats were anaesthetized with diethyl-ether vapour. Blood samples were collected through cardiac puncture (Ebunlomo *et al*., 2012). 4 ml of blood was collected in lithium heparin bottles. Blood samples were centrifuged at 3000 x g for 15 minutes. Plasma was collected into bottles using a Pasteur pipette and stored frozen for later analysis.

Enzymes Assay
Urea, estimatation was using urea Elisa Kit's according manufacturers' specification. Creatinine estimated by method of Zhibo *et al*., 2017. The protein concentration of various samples was determined using the Biuret method as described by Edem *et al.* (2012). The principle of the test was based on the formation of coloured complex between proteins and cupric ions in alkaline solution. The result was expressed in millimole/litre

The activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated as described by Reitman and Frankel (1957) using test kits (Randox, UK). ALT was estimated by monitoring the levels of pyruvate hydrazones formed with 2,4-dinitrophenyl hydrazine (DNPH) while AST was determined by monitoring oxaloacetate hydrazine formed with DNPH.The plasma levels of SOD and CAT in normal, fructose-drinking and treated groups were determined as described by Freitas *et al*. (2005). The method was based on SOD inhibition of auto-oxidation rate of Hematoxylin and CAT based on monitoring the consumption of H_2O_2 substrate. Lipid peroxidation biomarker malondialdehyde (MDA) level was assayed by measuring Thiobarbituric acid reactive substances (TBARS)formation (Freitas *et al*., 2005).

Statistical analysis
Data were collected and analysed using Statistical Package for the Social Sciences (SPSS) for windows version 20.0 (SPSS Inc., USA). Values were expressed as mean ± Standard error of mean (S.E.M.). Differences between means were evaluated for significant differences using one-way analysis of variance (ANOVA) with Turkey-Kramer multiple comparison post-hoc tests. P values < 0.05 were considered significant.

Results
Effects of calcitriol on biomakers of renal failure in Wistar Albino rats
The effects of three weeks calcitriol administration on urea and total proteins of male Wistar rats is presented on Figure 1. Urea, creatinine and total proteins were significantly elevated in blood of rats that received 10% fructose for three weeks compared to the control 2.90 ± 0.36vs2.20 ± 0.16, 41.00 ± 1.58vs35.80 ± 2.59,6.30 ± 0.38 vs5.80 ± 0.7, 2.18 ± 0.821respectively. These parameters were significantly reduced in animals treated with calcitriol compared to the fructose drinking rats (2.18 ± 0.82 vs2.90 ± 0.36, 36.00 ± 1.48 vs 41.00 ± 1.58, 5.70 ± 0.57 vs 5.70 ± 0.57

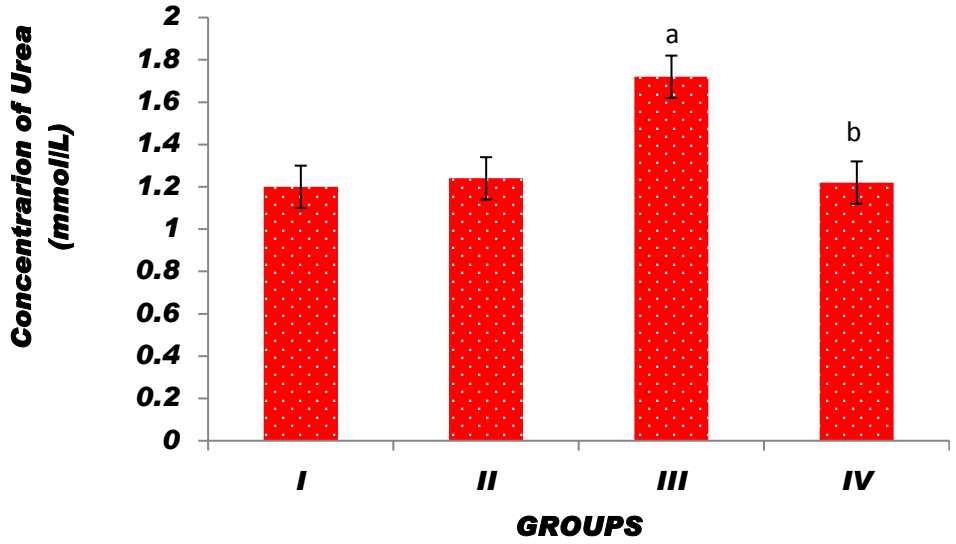

Fig 1: Effects of calcitriol treatment on urea concentration in rats that received 10% fructose solution
Results are expressed as mean ± SEM (n = 5). a = P≤0.05 vs control and b = P≤0.05 vs fructose-fed group.

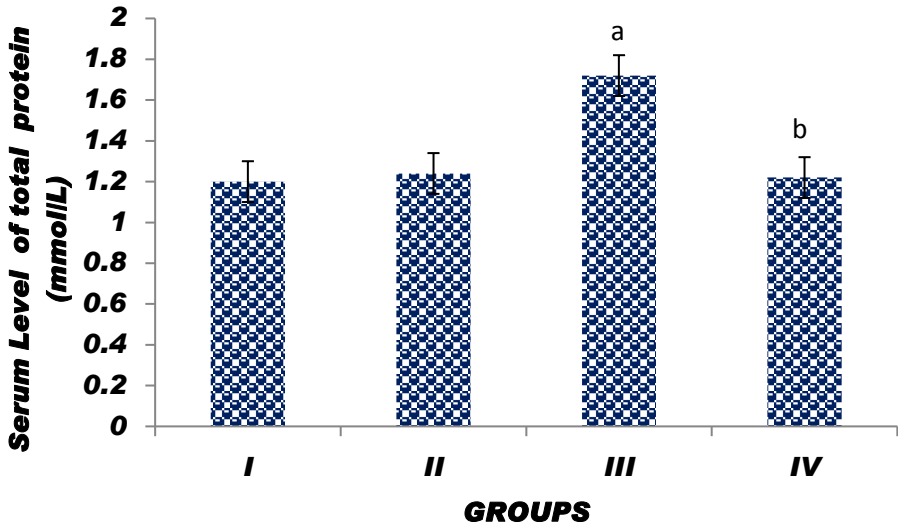

Fig 2: Effects of calcitriol treatment on total protein concentration in rats that received 10% fructose solution
Results are expressed as mean ± SEM (n = 5). a = P≤0.05 vs control and b = P≤0.05 vs fructose-fed group.

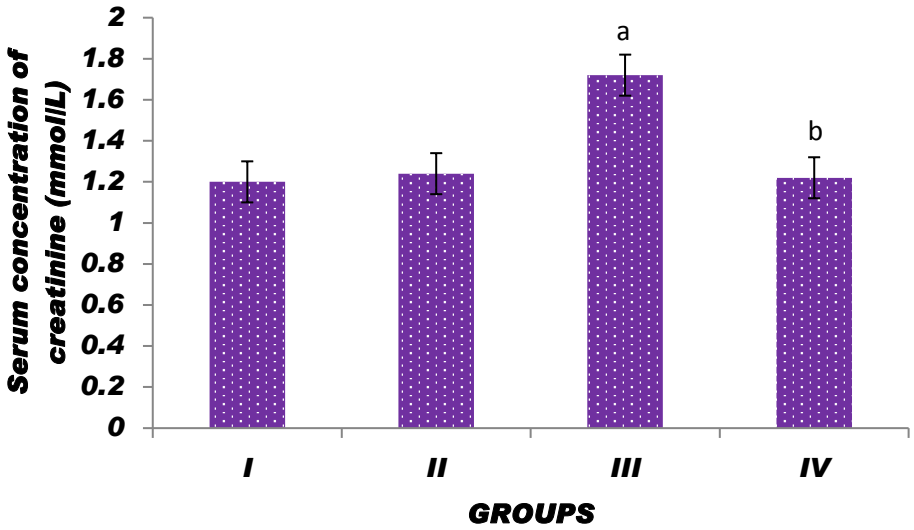

Fig3: Effects of calcitriol treatment on creatinine concentration in rats that received 10% fructose solution
Results are expressed as mean ± SEM (n = 5). a = P≤0.05 vs control and b = P≤0.05 vs fructose-fed group

Effect of calcitriol on liver enzymes in Albino Wistar rats

AST and ALT level in calcitriol treated rats were significantly reduced when compared with rats that received 10% fructose solution (7.00 ±0.13 vs 7.00 ±0.13, and 3.7 ± 1.34 vs 4.5 ± 1.60) and were statistically similar to those of the controls 7.00 ±0.13 vs 6.8 ± 1.30 and 3.7 ± 1.34 vs3.6 ±1.14 respectively.

Effects of calcitriol supplementation on antioxidant enzymes and lipid peroxidation

Fructose-fed rats exhibited significant decreased in the activities of SOD and CAT as compared to their respective controls (3.30 ± 0.16 vs 5.20 ± 0.22 and 7.40 ± 0.54 vs 8.50 ± 1.12 respectively). Treatment with calcitriol significantly increased the activities of the antioxidant enzymes and ameliorated the activity of enzymes in rats that received 10% fructose solution(5.70 ± 0.17 vs 3.30 ± 0.16 and 8.40 ± 1.06 vs 7.40 ± 0.54 respectively) as shown in table 1. correspondingly, rats that received 10% fructose solution had a significant (P<0.05) increase in plasma TBARS levels in comparison to the control (1.72 ± 0.24 vs 1.20 ± 0.18) and calcitriol treatment effectively prevented fructose-induced increased in TBARS levels in rats (1.22 ± 0.18 vs 1.72 ± 0.24). (Fig 4)

Table I: **Effects of calcitriol administration on liver enzymes and antioxidant enzymes activities in Albino Wistar rats**

GROUPS	I	II	III	IV
AST (U/L)	6.8 ± 1.30	6.8 ± 1.95	8.0 ± 1.58*	7.00 ±0.13#
ALT (U/L)	3.6 ±1.14	3.5 ± 1.00	4.5 ± 1.60*	3.7 ± 1.34#
SOD (mmol/L)	5.20 ± 0.22	5.70 ± 0.61	3.30 ± 0.16*	5.70 ± 0.17#
CAT (mmol/L)	8.50 ± 1.12	8.70 ± 0.99	7.40 ± 0.54*	8.40 ± 1.06#

Results are presented as mean ± SEM (N = 5). *p<0.05 vs control, and #p<0.05 vsrats that received 10% fructose solution

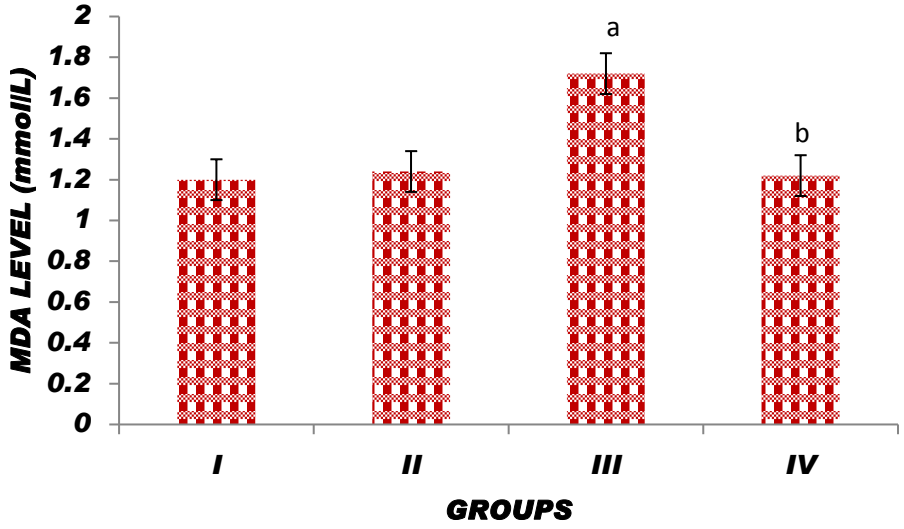

Fig 4: Effects of calcitriol treatment on MDA concentration on rats that received 10% fructose solution
Results are expressed as mean ± SEM (n = 5). a = P≤ 0.05 vs control and b = P≤ 0.05 vs fructose-fed group.

DISCUSSION

The present study investigated the effects of calcitriol treatment on indices of renal, liver function and lipid peroxidation biomarkers were investigated as well as the activities of antioxidant enzymes in an animal model that received 10% fructose solution is consistent with the results of previous studies (Hjelmeseath *et al*. (2010); Prabhu *et al*.(2013), the results of the present study revealed that urea; creatinine and total proteins were significantly elevated in blood of rats that received 10% fructose solution. A high level of these metabolites is significant markers of renal dysfunction (Lameire *et al*., 2005; Kesari *et al*., 2007; Dhanasekaran *et al*., 2009). Significant alterations in the serum levels of these markers are important indicators of kidney nephropathy (Gowda *et al*., 2010; Saka *et al*., 2012), hepatic insulin resistance, metabolic syndrome as well as type 2 diabetes (Cho *et al*., 2005). Calcitriol supplementation ameliorated the renal indices of toxicity in fructose-fed rats. The amelioration of renal indices suggested that calcitriol is effective in protecting the kidney against the deleterious effects of fructose diet and improved renal functions in rats that received 10% fructose solution. This finding is in agreement with previous studies that reported a renoprotective effect of calcitriol (De Zeeuw, *et al*., 2010; de Boer *et al*., 2012; Huang *et al*., 2012). Increased in total proteins in rats that received 10% fructose solution might be due to micro-protenuria, which is an important clinical marker of diabetic nephropathy and/or due to increased protein catabolism. A significant reduction in total plasma protein levels upon calcitriol administration indicated its favourable effects in reducing the alteration of protein metabolism and/or improved renal functions in rats that received 10% fructose solution. The results also showed that the serum liver enzymes (AST and ALT) were significantly higher in fructose-fed rats which might be due to toxic effects of fructose on the hepatocytes. Increase synthesis of the enzymes or leakages from damage tissues elevates enzyme concentrations in serum. Elevated serum AST and ALT levels are commonly used as sensitive markers of possible liver damage (Ramaiah, 2007). However, calcitriol treatment significantly decreased the liver enzymes in fructose-fed rats (AST and ALT). Thus, the result suggests that calcitriol was able to protect the liver against fructose-induced liver injuries. The result agrees with the findings of Jorde and Grimmes (2011) that calcitriol treatment decreased liver enzymes but disagrees with the results of Abbas *et al*.,

(2014) who reported no effect on serum liver enzymes following calcitriol supplementation. The activities of the enzymatic and non enzymatic antioxidant levels have been shown to be reduced during hyperglycaemia and in conditions of oxidative stress (Johansen *et al*., 2005; Dmytro and Volodymyr, 2013) which is similar to our observation. The results of the study showed fructose impaired radical scavenging activity and hence exposing proteins and lipids to peroxidation. However, calcitriol treatment enhances antioxidant activity and reduces lipid peroxidation in ratsthat received 10% fructose solution. This is probably in part by scavenging the reactive hydroxyl and peroxyl radicals and enhanced the production of endogenous antioxidant enzymes (SOD & CAT) depleted by fructose. Similar to our observation, studies have indicated that calcitriol may possess antioxidant properties and also strengthens the role of existing antioxidants by inhibiting inducible nitricoxide synthase [iNOS] Garcion *et al*., 1997) and increases levels of glutathione (Baas *et al*., 2000) in the body. The endogenous antioxidant enzymes have been shown to neutralize and prevent tissue damage caused by highly reactive oxygen species during diseases (Kumar and Ptiyadarsini, 2011). This finding is in line with the results of Dong *et al*., (2012) in which calcitriol treatment reduced fructose-induced oxidative stress.

CONCLUSION

In conclusion this studies have indicated that calcitriol possess antioxidant properties and also strengthens the role of existing antioxidants and that might be responsible to its' renoprotective, hepatoprotective due to its attenuation of fructose-induced oxidative stress, lipid peroxidation and decreased in biomarkers of renal and liver toxicity.

References

Abbas, S., Linseisen, J., Rohrmann, S., Buelens, J.W.J., Buijsse, B., Amiano, P., and Ardanaz, E. (2014). Dietary calcitriol and the risk of type 2 diabetes in European prospective investigation into cancer and Nutrition: the EPIC-Inter Act study. *European Journal of Clinical Nutrition*, 68: 196-202.

Al-Daghari, N.M., Al-Kharfy, K.M., Kumar, S., Chrousos, G.P., Al-Saleh, Y., Al-Attas, O.S., Sabico, S., and Al-Othman, A. (2012). Calcitriol supplementation as an adjunct therapy for Saudi patients with DMT2: an 18-month intervention study. *Cardiovascular Diabetologia*, 11:85.

Adenkola, A. Y., Ayo, J. O., Sackey, A. K. B. and Adelaiye, A. B. (2011).Ameliorative effect of ascorbic acid on rectal temperature of pigs transported by road for eight hours during the harmattanseason.*Agriculture and Biology Journal of North America*25: 734 – 741. DOI: 10.5251/ABJNA.

Baas, D *et al*. (2000).Rat oligodendrocytes express the calcitriol(3) receptor and respond to 1, 25 dihydroxycalcitriol(3).Glia, 31.1: 59–68.

Cheng, Q., Li, Y.C., Boucher, B.J., Leung, P.S., (2011). A novel role for calcitriol: modulation of expression and function of the local renin angiotensin system in mouse pancreatic islets. *Diabetologia,*54: 2077-2081.

Cho, H.K., Willett, W.C., and Stampfer, M.J. (2005). Dairy consumption and the risk of type 2 diabetes mellitus in men. *Annals of Internal Medicine*, 165: 997-1003.

Chou, C.L., Pang, C.Y., Lee, T.J.F., Fang, T.C. (2015). Beneficial effects of calcitriol on hypertension, Glucose Intolerance, Impairment of Endothelial-Dependent Vascular Relaxation and Visceral Adiposity in Fructose-Fed hypertensive rats. PLoS ONE, 10(3): e0119843.

De, Boer, I.H., Sachs, M.C., Cleary, P.A., Hoofnagle, A.N., Lachin, J.M., Molitch, M.E., Steffes, M.W., Sun, W., Zinman, B., Brunzell, J.D. (2012). Circulating calcitriol

metabolites and kidney disease in type 1 diabetes.*Journal Clinical Endocrinolology and metabolism*, 97: 2456-2465.

De Zeeuw, D., Agarwal, R., Amdahl, M., Audhya, P., Coyne, D., Garimella, T., Parving, H.H., Pritchett, Y., Remuzzi, G., Ritz, E. et al., (2010). Selective calcitriol receptor activation with paricalcitol for the reduction of albuminurea in patients with type 2 diabetes (AITAL STUDY): a randomised control trial. Lancet, 376(9752): 1543-1551.

Dhanasekaran, M., Baskar, A. A., Ignacimuthu, S., Agastian, P., and Duraipandiyan, V. (2009). Chemopreventive potential of Epoxy clerodanediterpene from tinosporacordifolia against diewthylnotrosamine-induced hepatocellular carcinoma*, Invest New Drugs*, 27:347-355.

Dmytro, G., and Volodymyr, L. (2013).Oxidative Stress Cause Consequence of Diseases. Precarpathian National University, Ivano- Frankivsk, Ukraine. www.intechopen.com

Dong, J., Wong, S.L, Lau, C.W., Lee, H.K., Zhang, L. (2012).Calcitriol protects renovascular function in hypertension by down-regulating angiotensin II type I receptors and reducing oxidative stress. European Heart Journal, 33: 2980-2990

Ebunlomo, A.O., Odetola,A.O., Bamidele,O.,Egwurugwu, J.N., Maduka,S.,andAnupe, J. (2012). Effects of Emilia praetermissa leaf extract on the heamatological biochemical parameters of stress induced ulcerated wister rats. African Journal of Biochemistry Research, 6(14): 185-189

Edem, V.F. Kosoko, A. Akinyoola, S.B. Owoeye, O. Rahamon, S.K. and Arinola, O.G. (2012). Plasma antioxidant enzymes, lipid peroxidation and hydrogen peroxide in wista rats exposed to Dichlorvos insecticide. *Archives of Applied Science Research*, 4 (4) : 1778 - 1781

Elliott. S.S., Keim, N.L., Stern, J.S., Teff, K., and Havel, P.J. (2002) Fructose, weight gain, and the insulin resistance syndrome *American Journal of Clinical Nutrition* 76: 911-922.

Fu, Z., Zhang, W., Zhen, W., Lum, H., and Nadler, J. (2010). Geinstein induces pancreatic β-cell proliferation through activation of multiple signaling pathways and prevents insulin-deficient diabetes in mice. *Endocrinology*, 151 (7): 3026-3037.

Freitas, R.W., Vasconcelos, S.M.M., Souza, F.C.E., Viana, G.S.B., Fonteles, M.M.F., (2005). Oxidative stress in the hippocampus after pilocarpine-induced status in epilepticus in Wistar rats. *Federation of European Biochemical Society Journal*, 272: 1307-1312

Gowda, S., Desai, P.BV., Kulkarni, S.S., et al., (2010). Markers of renal function tests.*North American Journal of Medical Sciences,* 2: 170-173.

Garcion, E et al. (1997). 1,25-Dihydroxycalcitriol3 inhibits the expression of inducible nitric oxide synthase in rat central nervous system during experimental allergic encephalomyelitis. Brain research. Molecular brain research, 45.2: 255–267.

Güneş, Y., Serpil, S., Peyman, Y., Gülay, D., and Atilla, E. (2000). The clinical and metabolic effects of calcitriol in the treatment ofpostmenopausal osteoporosis.*Romatizma*, (15) 2 : 93-98

Hjelmeseath, J., Roislien, J., Nordstrand, N., Hofso, D., Hager, H., and Hartmann, A. (2010).Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study. BMC, Endocrine disorders, 10:1-6.

Huang, Y., Yu, H., Lu, J., Guo, K., Zhang, L., Bao, Y., Chen, H., Jia, W. (2012). Oral supplementation with cholecalciferol 800 IU ameliorates albuminurea in Chinese type 2 diabetic patients with nephropathy. PolS One7: e50510.

Hwang I.S., Ho, H,, Hoffman, B.B., and Reaven, G.M. (2007). Fructose-induced insulin resistance and hypertension in rats. *Hypertension* 10: 512-516.

International Diabetes Federation (2013). One in ten will have diabetes by 2030. Available at http:/www. Idf.org/media-evens/press-releases diabetes-atlas-5th-edition

Johansen, J.S., Harris, A.K., Rychly, D.J., and Ergul, A. (2005). Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *CardiovascularDiabetologia*, 4: 5

Johnson, J., Rechard, J., and Timothy, G. (2007). The sugar fix: The high-fructose Fallout that Consuming fructose-sweetened beverages increases body adiposity in mice. *Journal of Obesity Research*, 13(7): 146-156.

Jones, C. L., Vieth, R., Spino, M., Ledermann, S., Kooh, S. W., Balfe, J., & Balfe, J. W. (1994). Comparisons between oral and intraperitoneal 1, 25-dihydroxyvitamin D3 therapy in children treated with peritoneal dialysis. *Clinical nephrology*, *42*(1), 44-49

Jorde, R., and Grimmes, G. (2011).Calcitriol and metabolic health with special reference to the effect of calcitriol on serum lipids.*Prog Lipids Res*, 50: 303-312.

Kesari, A. N., Kesari, S., Singh, S. K., Gupta, R. K., and Watal, G. (2007). Studies on the glycaemic and lipidemic effect of MurrayaKoenigii in experimental animals. *Journal of Ethopharmacology*, 112: 305-311.

Kumar, A., and Ptiyadarsini, K.I. (2011).Free radicals, oxidative stress and importance of antioxidants in human health.*Journal of Medical and Allied Sciences*, Vol. 1(2):53-60

Lameire, N., Van, B.W., Vaholder, R. (2005). Acute renalfailure. *Lancet,* 365:417-430

Lustig, R.H., (2010). Fructose: metabolic, hedonic and societal parallels with ethanol. *Journal of Dietary Association*.110: 1307-1321.

Manna, P., Jain, S.K. (2012).Calcitriolupregulates glucose transporter 4 translocation and glucose utilisation mediated by cystathioninelyase (CSE) activation and H2S formation in 3T3L1. *Biol. Chem.* 287: 42324-32.

Mitri, J., Murau, M.D., and Pittas, A.G. (2011).Calcitriol and type 2 diabetes: A systemic review. *European Journal of Clinical Nutrition*, 65:1005-1015.

Neeharika, V., Vamsi Krishna, R., and Madhava, R.B. (2012). Effects of Madhuritha on Dexamathasone and Fructose Induced Insulin Resistance in Rats. Scholars Research Library*, Journal of Natural Production and Plant Resources*, 2(2): 288-294.

Pilz, S., Kienreich, k., Rutters, F., De Jongh, R., Van Ballegoijen, A.J., Grubler, M., Tomaschitz, A., Dekker, J.M.(2013). Role of calcitriol in the development of insulin resistance and type 2 diabetes.*CurrDiab Rep*, 13: 261-270

Prabhu, V.V., Kanna, N., Guruvayoorappan, C. (2013). 1, 2-diazole prevents cisplatin-induced nephrotoxicity in experimental rats. Pharmacological Report, 65(4): 980-990.

Ramaiah, S.K. (2007). A toxicology guide to the diagnostic interpretation of hepatic biochemical parameters.Food Chemistry.*Toxicology*, 45: 1551-1557.

Reitman, S and Frankel, S, (1957). A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase.*Am.J.Clin.Pathol*, 28: 56-58.

Saka, W., Akhigbe, R., and Popoola, O., (2012).Changes in serum electrolyte, urea and creatinine in Aloe vera-treated rats.*Journal of Young Pharmacists*, 4(2): 78-81.

Sánchez-Lozada, L.G., Tapia, E., Jiménez, A., Bautista, P., and Cristóbal, M. (2007). Fructose induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats. *American JournalPhysiol Renal Physiol* 292: 423-429.

Teff, K.L., Grudziak. J., Townsend, R.R., Dunn, T.N., and Grant, R.W. (2009). Endocrine and metabolic effects of consuming fructose and glucose- sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. *Journal of Clinical Endocrinology and Metabolism* 94: 1562-1569.

Zhibo, G., Lei, C., Zhenqiang, X., Xiaoming, S., Dongfeng, S., and Gerd, A.K. 2017).Farnesoid X receptor activation protects the kidney from ischemia-reperfusion damage. *Scientific Report*, 7:9815 doi:10.1038/s41598-017-10168-6