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Comparative Effect of Daily Administration of Allium sativum and *Allium cepa* Extracts on Alloxan Induced Diabetic Rats

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Abstract: *Effective regulation of blood glucose level is the most important factor for reducing risks of diabetic complications and this must be done without any side effects or interference with normal physiological function of the body which is not usually achievable with the conventional diabetic drugs. This has resulted in the current shift to the use of herbal preparation which is presumed to be effective, cheap with no side effects. Most noted among these are onion and garlic. This present study was therefore conducted to compare the effects of daily administration of garlic and onion extracts on alloxan induced diabetic rat. Diabetes was induced in Female Wister rats weighing 150-200g by intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg body weight).Diabetes was confirmed after seven days in rats showing fasting blood glucose levels ≥ 200mg/dl. The diabetic rats were randomly allocated into three experimental groups which received garlic extract, onion extract or normal saline depending on the group. The extracts were administered orally for six weeks after which the animals were sacrificed and blood samples were collected for Biochemical analyses. From the results obtained it can be concluded that the extracts of fresh Allium sativum and Allium cepa significantly reduced the serum glucose with Allium sativum extract proving to be more potent while no significant difference was shown in their nephro- and hepato-protective activities.*

Keyword: *alloxan, diabetes, Garlic, herbal preparation, Onion*

I. Introduction

The current prevalence of diabetes mellitus in the third world countries especially Africa has been linked to the spread of Western life styles like physical inactivity combined with diets that are high in calories; processed carbohydrates, saturated fats and insufficient dietary fiber [1, 2].The estimated number of people with diabetes is expected to hit 380 million by 2025 and most of them will likely be found in Asia and Africa [3, 4].

Diabetes mellitus has been reported to be the major cause of blindness, kidney failure, lower-extremity amputation, cardiovascular diseases and premature mortality [5]. Effective control of blood glucose level is still the most important factor for reducing risks of diabetic complications [6] and this must be done without any side effects or any interference with normal physiological function of the body which is not usually achievable with the conventional diabetic drugs. This has resulted in the current shift to the use of herbal preparation which is presumed to be effective, cheap with fewer or no side effects [7];most noted among these are ginger, onion and garlic which appear to be most effective and least toxic [8].

Allium cepa belongs to the family Liliaceae and is widely cultivated throughout the world [9], its most popular modern uses include regulation of blood pressure, blood glucose ,blood cholesterol level[9,10] and as antiseptic[11]. Active ingredient in A. cepa include phenolic compounds (flavonoids, anthocyanins, phenolic acids and flavonols), organosulphur compounds, vitamins and some minerals [12, 13, 14], these compounds mediate the pharmacological effects of A. cepa. Thus, phenolic acids, such as caffeic, chlorogenic, ferulic, sinapic, p-coumaric acids, vanillic, syringic and p-hydroxybenzoic appear to be active antioxidants [15, 16]. Its vitamins especially vitamin C have a protective function against oxidative damage and a powerful quencher of singlet oxygen (O_2), hydroxyl (OH) and peroxyl (RO_2) radicals[17,18]. Garlic bulb contains chemically active substances such as enzymes, amino acids, minerals and sulphur containing compounds such as alliin (S-allyl cysteine sulphoxide (SACS) and allicin (diallyl disulphide) which are responsible for garlic's pungent odour and many of its medicinal effects [19]. Allicin which is associated with garlic action does not exist in garlic until it is crushed or cut where injury to the garlic bulb activates the enzyme allinase which metabolizes allin to allicin[20].Garlic has been found to have antibacterial and antifungal activity, reduce aortic plaque deposits[21],

inhibits vascular calcification in human patients with high blood cholesterol[22], reduce hyperlipidemia[23 ,24] and hyperglycaemia[25].The primary purpose of this research was to compare biochemical effects of Allium sativum and allium cepa extracts in alloxan induced diabetic rats.

II. Materials And Methods

2.1 Collection of Plant Samples

Plant samples were obtained from Masaka market in Nasarawa State and identified in Biological Sciences Department, Bingham University karu, Nasarawa State, Nigeria.

2.2 Preparation of Allium sativum (Garlic) and Allium cepa(onion)

The same method was used to prepare garlic and onion extract from their respective bulbs as follow: fresh healthy bulbs of garlic or onion weighing 50g were washed and peeled; the peeled bulbs were then cut into small pieces and homogenized in 70 ml cold, sterile 0.9% NaCl in the presence of some crushed ice. The homogenization was carried out in a blender at high speed using 30-second bursts for a total of 10 minutes. The homogenized mixture was filtered 3 times through cheesecloth, the filtrate was centrifuged at 2000 RCF for 10 minutes and the clear supernatant obtained was diluted to100 ml with normal saline. The concentration of this garlic or onion preparation was considered to be 500 mg/ml on the basis of the weight of the starting material (50g/100 ml). The aqueous extract of the garlic or onion was stored at 4 °C until use.

2.3 Experimental Animal

Female wister rats weighing 150-200g were purchased from Bingham University animal house Nasarawa state. The rats were approved for the experiment by the local committee and were housed in individual plastic cages with stainless steel at room temperature ($25\pm3^{o}C$) at 12 hr dark-light cycles. All the rats were allowed free access to the same diets and water and acclimatized for 14 days before the treatment.

2.4 Preparation of alloxan

Two grams of crystalline alloxan monohydrates were dissolved in 50mls of normal saline (0.9% NaCl solution) to yield a concentration of 40mg/ml.

2.5 Induction of diabetes

The rats were divided into two groups before the induction of diabetes: non-diabetic control group and experimental group (to be induced with alloxan) with the weights of all the rats taken before alloxan administration.

Diabetes was induced in the experimental rats after overnight fasting (12hrs) by intraperitoneal administration of 150mg of alloxan per kilogram body weight of rat (150mg/kg body weight).

After the induction, all the rats were allowed free access to the same feed and water and the alloxan-induced rats were carefully examined for the next 24hrs for evidence of allergic reactions, behavioural changes and convulsion.

After one week, blood was collected from the tail vein and diabetes was confirmed in alloxan treated rat with fasting blood glucose levels greater than 200mg/dl.

2.6 Experimental Design

The control and experiment animals were divided into different groups and treated accordingly:

Group 1 (non-diabetic group): normal control (receive 1ml normal saline through direct stomach intubation every day).

Group 2 (Diabetic control): receive 1ml normal saline through direct stomach intubation every day.

Group 3 (Garlic group): Diabetic rats receiving 400mg garlic extract per kilogram body weight daily.

Group 4 (Onion group): Diabetic rats receiving 400mg onion extract per kilogram body weight daily.

2.7 Preparation of samples for biochemical analysis

After six weeks of treatment with the different extracts, the body weights of all the rats were taken again and were allowed to fast for 12hr before they were sacrifice under sodium pentobarbitone anaesthesia. Whole blood was collected via cardiac puncture using sterile syringes and needles and emptied into plain bottles; this was allowed to clot for about two hours. The clotted blood was centrifuged at 3,500rpm for 30mins to recover the serum from clotted blood. Serum was separated with sterile syringes and needles and stored frozen until it is used for biochemical analysis.

2.8 Assays

The blood glucose in a protein free serum was determined as described by Sood [26]. Urea, creatinine and total bilirubin concentrations were determined by the methods of Patton and Crouch [27] Henry et al.[28] and Pearlman and Lee[29], respectively. Total cholesterol was measured by the procedure described by Allain et al.,[30]. Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were estimated with the Randox reagent kit using 2, 4-dinitrophenylhydrazine as substrate according to the method described by Reitman and Frankel[31]. Protein content was determined by the method of Lowry et al. [32] while Alkaline phosphatase was assessed as described by Principato et al.,[33]. All the assays were carried out at the Department of Biochemistry, Bingham University Karu Nasarawa state, Nigeria.

III. Result And Discussion

The administration of garlic extracts to the diabetic rats significantly reduced serum glucose compared to the diabetic group that did not receive the same treatment, although these reductions were not enough to bring it down to the normal rats' serum glucose levels (Table 1). This effect of garlic has been reported for both alloxan and streptozotocin- induced diabetic rats[25,34,35,36], this ability to reduce serum glucose level is associated with the presence of S-allyl cysteine sulphoxide, a sulphur-containing amino acid which has been shown to posses the potential to reduce the blood glucose in rats almost to the same level as insulin[37,38].These active constituents of garlic extract are believed to act as hypoglycaemic agent by increasing either the pancreatic secretion of insulin from the beta cell or its release from bound insulin[2,39,40], interferes with intestinal glucose absorption[41] and peripheral glucose utilization[42,43] and lastly by stimulating the few surviving β-cells to produce insulin or regenerates β-cells of the islets, since β-cells have been shown to have a potential for regeneration[41, 44,45].

Onion extract also significantly reduce the serum glucose level .This has also been reported before by Eyo et al.[2] .This action may be attributed to allyl propyl disulphide and other sulphur compounds in onions[2,46] which may act by mopping up the Reactive Oxygen Species (ROS) and free radicals which mediate the toxic action of alloxan on pancreatic beta cells[47,48,49,50].The best reduction in blood glucose level was however observed with garlic extract (70.23%) compare to onion(63.35%) while normal saline administered to the diabetic rats did not affect the glucose levels.

Table 1: Effect of Allium cepa and Allium sativum on Blood Glucose Level of Diabetic Rats

TREATMENT	DAY 1 (mg/dl)	DAY 28 (mg/dl)	% Change In Glucose Level
NORMAL CONTROL	77.00 + 7.35	79.00 + 6.00	+ 2.60
DIABETIC CONTROL	245.50 + 4.91	338.75 + 7.42	+ 37.98
ONION(400mg/kg)	231.25 + 3.40	84.75 + 1.85	-63.35
GARLIC (400mg/kg)	374.50 + 9.29	111.50 + 10.29	-70.23

All Values Are Expressed As Mean ± Standard Deviation of Four Observations

As shown in Table 2, alloxan induction produced significant increase in the hepatic marker enzymes (ALT, AST and ALP). The increase in these hepatic marker enzymes (ALT, AST, and ALP) levels is as a result of these enzymes leaking from liver cells cytosol into bloodstream[51] since the toxic action of alloxan is mediated through the generation of free radicals[47,48] which eventually damage the liver cells leading to the leakage of these enzymes which are located in the cell cytoplasm once the cellular membrane integrity is compromised; the serum concentrations of these enzymes are usually proportional to the damage[51,52]. In particular, the elevation of ALT an enzyme found mainly in the liver is an indication of liver damage. Significant reduction in the activities of these enzymes in garlic and onion extracts treated diabetic rats is an indication of their hepatoprotective activities [53, 54]. These protective effects of onion and garlic extracts may be associated with their inherent-antioxidant properties as reported by Rahman [49] which is believe to accelerate the regenerative capacity of the hepatocytes, cause stabilization of the cell membrane of the hepatocytes, and ultimately protect the liver cells against deleterious agents and free radical mediated toxic damages. For example,onion contains sulphur-containing compounds of which their oxidized thiols can trap electrons from other systems[35,55,56,57].Generally, from the results(TABLE 2),there is no significant difference between the hepatoprotective effects of garlic and onion in contrast to the observation of Micheal et al.,[58] and Tatfeng and Enitan[59] who reported that garlic protects liver from damages better than onion.

Table 2: Effect of Allium cepa and Allium sativum on the Liver of the Treated Diabetic Rats

BIOCHEMICAL PARAMETER	AST (U/I)	ALT(U/I)	ALP(U/I)
NORMAL CONTROL	35.50^{a} + 0.58	20.85^{a} + 0.98	22.26^{b} + 0.64
DIABETIC CONTROL	80.00^{c} + 5.77	37.65^{c} + 6.73	63.81^{d} + 8.90
ONION(400mg/kg)	40.50^{b} + 0.58	24.00^{b} + 1.15	15.40^{a} + 2.53
GARLIC (400mg/kg)	40.00^{b} + 0.50	23.50^{b} + 1.73	26.95^{c} + 0.63

All Values Are Expressed As Mean ± Standard Deviation of Four Observations.
Values with different superscript in the same column are significantly different at p< 0.05

Serum urea and creatinine are often regarded as reliable markers of renal function status Thus, elevations in the serum concentrations of these markers are signs of renal injury [60].Administration of both garlic and onion extracts led to reduction of these kidney biomarkers (Table 3).Their nephroprotective properties have been proposed to be mediated through antioxidant or free radical scavenging activities due to the high concentration of flavonoids and alkaloids they contain[61,62].These showed that the extract of garlic and onion enhanced the ability of the kidneys to remove these waste products from the blood as indicated by reduction in serum urea and creatinine levels and thus, confer protective effect on the kidney of diabetic rats[35].

Alteration in serum lipids profile is well known in diabetes [63, 64] including alloxan diabetic rats [65]. In this study, the untreated diabetic rats showed hypercholesterolemia, similar to the earlier reports of Abdul Rahman et al. [66] and Idogun et al. [67] among diabetic patients.

Studies with raw garlic and onion have shown that they significantly reduce the total serum cholesterol [50, 68].The hypocholestrolemic activities of garlic and onion are due to the presence of allicin and allyl propyl disulphide respectively together with their derivatives compounds [69] . Other non-sulphur components like saponins might also have contributed to their actions.These hypocholesterolemic effect is due to the abilities of the active substances to limit hepatic cholesterol biosynthesis[50,70,71,72], enhance cholesterol turnover to bile acids and its excretion through gastrointestinal tract or by inhibiting cholesterol absorption from intestinal lumen without changing HDL-cholesterol levels[22,23,24,50].

Table 3: Serum Levels of Urea, Creatinine, and Cholesterol In The Treated Rats.

TREATMENT	UREA (mg/dl)	CREATININE (mg/dl)	CHOLESTEROL (mg/dl)
NORMAL CONTROL	26.32^{a} + 1.50	0.45^{a} + 0.06	51.29^{a} + 0.33
DIABETIC CONTROL	103.57^{c} + 9.21	1.36^{d} + 0.16	128.70^{c} + 9.28
ONION(400mg/kg)	29.86^{b} + 1.79	0.67^{b} + 0.04	56.17^{b} + 0.77
GARLIC (400mg/kg)	29.00^{b} + 0.50	0.81^{c} + 0.01	55.10^{b} + 2.70

All Values Are Expressed As Mean ± Standard Deviation of Four Observations.
Values with different superscript in the same column are significantly different at p< 0.05

IV. Conclusion

From the results obtained it can be concluded that the extracts of fresh Allium sativum and Allium cepa significantly reduced serum glucose and can also protect liver and kidney in diabetic patients although the extract of garlic seems to be more effective in reducing the blood glucose.

Reference

[1]. N.S. Ugochukwu ,N.E. Babady, M. Cobourne and S.R Gasset , The effect of Gongronema latifolium extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats, Journal of Biosciences, 28(1),2003,1- 5.
[2]. J.E. Eyo, J.C. Ozougwu and P.C. Echi, Hypoglycaemic Effects Of Allium Cepa, Allium Sativum And Zingiber Officinale Aqueous Extracts On Alloxan-Induced Diabetic Rattus Novergicus Medical Journal Of Islamic World Academy Of Sciences 19(3),2011, 121-126.
[3]. J.C.Ozougwu,Anti-diabetic effects of Allium cepa (onions) aqueous extracts on alloxan-induced diabetic Rattus novergicus, Journal of Medicinal Plants Research, 5(7),2011, 1134-1139.
[4]. R. Samreen, Diabetes mellitus; Scientific Research and Essay, 4 (5), 2009, 367-373.
[5]. D. Gohdes, Diabetes in North American Indians and Alaska natives, pp 561 – 582. In: Diabetes in America. Ed by M.I. Harris, C.C. Cowie, M.P. Stem, E.J. Boyko, G.E. Reiber and P.H. Berineth. 8th Edition. United States Department of Health and Human Services, Public Health Services, National Institute of Health, Washington, DC. DHHS Publication Number 1995,561-582.
[6]. K. Min-Jung, K. Ji-Hye,C. Ha-Neul,K. Myoung-Jin,H. Jung-Hee, L. Jai-Heon and K. Jung-In, Hypoglycemic effects of Welsh onion in an animal model of diabetes mellitusNutrition, Research and Practice (Nutr Res Pract),4(6),2010,486-491
[7]. A.A. Osinubi, O.G.Ajayi, A.E.Adesiyun, Evaluation of the anti-diabetic effect of aqueous leaf extracts of Tripinanthus butungil in male spragne Dawley rats, Medical Journal of Islamic World Academy of Science, 16(1), 2006, 41 – 47.
[8]. K. Srinivasan, Plant foods in the management of diabetes mellitus: spices as beneficial antidiabetic food adjuncts**,** Int. J. Food Sci. Nutr., 56,2005,399-414.
[9]. M.A. Ikram, A review on chemical and medicinal aspects Of Allium cepa, Pak. J. Sci. Ind. Res., 14(5), 1971, 395-398.
[10]. P.T. Mathew and K.T. Augusti, (1975). Hypoglycaemic effects of onion, Allium cepa linn on diabetes mellitus: A preliminary report, Indian J. Physiol. Pharmacol., 19(4), 1975, 212-217.
[11]. R.C .Jain,Onion and garlic in experimental cholesterol induced atherosclerosis in rabbit, Indian J. Med. Res., 64(10),1976, 1509–1515.
[12]. C. Teyssier, M.J. Amoit, N. Mondy, J. Auger, R. Kahane and M.H. Siess, Effect of onion consumption by rats on hepatic drug-metabolizing enzymes, Food Chem. Toxicol., 39, 2001, 981-987.
[13]. A.M. Kamal and J.R. Daoud, Effect of onion and/or garlic as feed additives on blood,tissue constituents and growth performance in Muscovy ducks. Vet. Med. J., 51, 2002, 161-175.

[14]. K.E. Campos, Y.S. Diniz, A.C. Cataneo, L.A. Faine, M.J. Alves and E.L. Novelli, Hypoglycaemic and antioxidant effects of onion, Allium cepa: Dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. Int. J. food Sci. Nutr., 54,2003, 241-241.

[15]. R.A. Larson, The antioxidants of higher plants. Phytochemistry. 27,1998, 969-978.

[16]. S.M. Ibrahim, Y.A. Mostafa, Elhassaneen andM.A. El-Soadan, Dietary phytochemicals as chemopreventive for liver cancer. Bull. Pharma.Sci. 27,2004, 87-94.

[17]. E. Niki, Action of ascorbic acid as a scavenger of active and stable oxygen radicals, Am. J. Clin. Nutr. 54,1991, 1119s-1124s

[18]. L.C. Saalu,G.O. Ajayi,A.A. Adeneye, I.O. Imosemi and A.A.Osinubi, Ethanol seed extract of grape fruit (Citrus paradisi) as an effective attenuator of doxorubicin-induced oxidative stress in the rat heart, Int. J. Cancer Res., 5,2009, 44-52.

[19]. M.T.Murray and J.E. Pizzorno, Allium sativum (garlic). In: Textbook of natural medicine. 2nd Edition. (eds. J.E. Pizzorno Jr. and M.T. Murray), Edinburgh: Churchill Livingstone,1999,571-577.

[20]. E. Block, The chemistry of garlic and onions, Sci. Am., 252,1985, 114-119.

[21]. I. Durak , H.S. Oztürk, E. Olcay and C. Güven , Effects of garlic extract supplementation on blood lipid and antioxidant parameters and atherosclerotic plaque formation process in cholesterol-fed rabbits, J Herb Pharmacother 2 (2),2002, 19–32.

[22]. I. Durak , M. Kavutcu and B. Aytaç et al., Effects of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol,J. Nutr. Biochem., 15 (6), 2004, 373–7.

[23]. J. Kojuri, A.R. Vosoughi and M. Akrami (March 2007). Effects of Anethum graveolens and garlic on lipid profile in hyperlipidemic patients". Lipids Health Dis 1 (6),2007, 5.

[24]. H.F. Mader, Treatment of hyperlipidaemia with garlic-powder tablets:Evidence from the German Association of General Practitioners' multicentric placebo-controlled double-blind study, Arzneimittelforschung 40 (10),1990, 1111–6.

[25]. R.J Ojo, A.E. Memudu, C.O. Akintayo and I.S. Akpan, Effects of Pre-induction Administration of Allium sativum on Some Biochemical Parameters in Alloxan Induced Diabetic Rats, Research Journal of Applied Sciences, Engineering and Technology, 4(23),2012, 5129-5135.

[26]. R. Sood, Medical Laboratory Technology Methods and Interpretations. 5th edition, Jaypee Brothers Medical publishers Limited, New Delhi, India.1999

[27]. C.J. Patton and S.R. Crouch, Spectrophotometeric and kinetics investigation of the Berthelot reaction for determination of ammonia, Analytical Chemistry, 49,1997, 464- 469.

[28]. R.J. Henry, D.C. Cannon and J.W. Winkelman, Clinical Chemistry Principles and Techniques, 11th Ed. Happer and Row Publishers, New York.1974, 1629.

[29]. F.C.Pearlman and R.T.Y. Lee, Detection and measurement of total bilirubin in serum with use of surfactants as solubilizing agents. Clinical Chemistry. 20, 1974, 447–453.

[30]. C.C Allain, L.S. Poon, C.S.Chan, W. Richmond and P.C. Fu, Enzymatic determination of total cholesterol, Clinical Chemistry. 20(1), 1974, 470-475

[31]. S. Reitman and S. Frankel, A colorimetric method for the determination of glutamic oxaloacetic and glutamic pyruvic transaminases, Am. J. Clin. Pathol. 28, 1957, 56-66.

[32]. O.H. Lowry,N. J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurements with the folin phenol reagent. J. Biol Chem. 193, 1951, 265-275.

[33]. G.B. Principato, M.C. Asia, V. Talesa, G. Rosi and E. Giovannini, Characterization of the soluble alkaline phosphatase from hepatopancreas of Squilla mantis L. Comparative Biochemistry and Physiology, 80B,1985, 801–804.

[34]. S.K. Banerjee and S.K. Maulik,Effect of garlic on cardiovascular disorder: a review, Nutrition Journal, 1: 4 – 15, 2002.

[35]. F.M. El Demerdash, M.I.Yousef and E. Naga, Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol., 43(1),2005, 57-63.

[36]. J.R. JamisoN,Garlic (Allium sativum). In: Clinical Guide to Nutrition and Dietary Supplements in Disease Management. London: Churchill Livingstone, 2003: 541-546.

[37]. C.G. Sheela and K.T. Augusti, Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic Allium sativum Linn., Indian J Exp Biol., 1992; 30: 523–526.

[38]. C.G. Sheela, K. Kumud and K.T. Augusti, Anti-diabetic effects of onion and garlic sulphoxide amino acids in rats. Planta Med., 61,1995, 356-357.

[39]. M.S. Islam and H. Choi, Comparative effects of dietary ginger (Zingiber officinale) and garlic(Allium sativum) investigated in a type 2 diabetes model of rats. J Med Food, 11(1),2008, 152- 9.

[40]. Y. Adachi, J. Yoshida, Y. Kodera, A. Katoh, J. Takada and H. Sakurai, 2006. Bis (allixinato) oxovanadium (IV) complex is a potent antidiabetic agent: studies on structure-activity relationship for a series of hydroxypyronevanadium complexes. J Med Chem., 49(11), 2006, 3251-6

[41]. I.E. Juárez-Rojop , J.C. Díaz-Zagoya, J.L. Ble-Castillo, P.H. Miranda-Osorio, A.E. Castell-Rodríguez, C.A. Tovilla-Zárate, A. Rodríguez-Hernández, H. Aguilar-Mariscal, T. Ramón-Frías and D.Y. Bermúdez-Ocaña, Hypoglycemic effect of Carica papaya leaves in streptozotocin-induced diabetic rats. BMC Complementary and Alternative Medicine, 12,2012,236.

[42]. E. Porchezhian, S.H. Ansari , N.K. Shreedharan , Antihyperglycemic activity of Euphrasia officinale leaves. Fitoterapia,71,2000,522–526.

[43]. R. Gupta, A.K. Sharma, M.C. Sharma ,R.S. Gupta, Antioxidant activity and protection of pancreatic β-cells by Embelin in streptozotocin-induced diabetes. J Diabetes, 4,2012,248–256.

[44]. N.Z. Baquer, P. Kumar, A. Taha,R.K. Kale, S.M.Cowsik and P. McLean, Metabolic and molecular action of Trigonella foenum-graecum (fenugreek) and trace metals in experimental diabetic tissues. J Biosci 2011, 36,2011,383–396.

[45]. V.K. Kondeti, K.R. Badri, D.R. Maddirala, S.K. Thur, S.S. Fatima, R.B. Kasetti, and C.A. Rao, Effect of Pterocarpus santalinus bark, on blood glucose, serum lipids, plasma insulin and hepatic carbohydrate metabolic enzymes in streptozotocin-induced diabetic rats. Food Chem Toxicol.,48,2010,1281–1287

[46]. B. Andallu, V. Suryakantham , S.B lakshmi, Effect of mulberry (Morms indica L) therapy on plasma and erythrocyte membrane lipids in patients with Type 2 diabetes. Clinical Chemical Acta, 314,2001, 47 - 53.

[47]. T. Szkudelski, The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas Physiol Res 2001;50,2001,536-46

[48]. R. Ankur and A. Shahjad, Alloxan Induced Diabetes: Mechanisms and Effects. International Journal of Research in Pharmaceutical and Biomedical Sciences, 3 (2),2012, 819 -823.

[49]. K. Rahman, Garlic and aging: A new insights into an old remedy. Ageing Res Rev; 2,2003,39–56.

[50]. C. E. Ugwu and J. Omale, Comparative Effects Of Aqueous Garlic (Allium Sativum) And Onion (Allium Cepa) Extracts On Some Haematological And Lipid Indices Of Rats Annual Review & Research in Biology 1(3),2011, 37-44.

[51]. K. Somayeh, A. Sedigheh, J. Moshtaghian, M. Rafieian, A. Adelnia and F. Shamsi,Liver-Protective Effects Of Hydroalcoholic Extract Of Allium Hirtifolium Boiss. In Rats With Alloxan-Induced Diabetes Mellitus Arya Atherosclerosis Journal, 6(1),2010, 11-15.
[52]. S.E.Atawodi, Evaluation of the hypoglyce-mic, hypolipidemic and antioxidant effects of methanolic extract of "Ata-Ofa" polyherbal tea (a polyherbal) in alloxan-induced diabetic rats. Drug Invention Today ,3,2013,270-6.
[53]. S. Kumar, V. Kumar and O. Prakash, Antihyperglycemic, antihyperlipidemic potential and histopathological analysis of ethyl acetate fraction of Callistemon lanceolatus leaves extract on alloxan induced diabetic rats. J. Exp. Integr. Med., 1,2011,185-190
[54]. D.D. Bandawane, K.H. Bibave, A.V. Jaydeokar, U.S. Patil and M.G. Hivrale, Antihyperglycemic and Antihyperlipidemic Effects of Methanolic Extract of Holarrhena antidysenterica Bark in Alloxan Induced Diabetes Mellitus in Rats. Pharmacologia, 4, 2013, 95-106.
[55]. O.S. Ogunmodede, L.C. Saalu, B. Ogunlade, G.G. Akunna and A.O. Oyewopo, An Evaluation of the Hypoglycemic, Antioxidant and Hepatoprotective Potentials of Onion (Allium cepa L.) on Alloxan-induced Diabetic Rabbits, International Journal of Pharmacology, 8,2012, 21-29.
[56]. F. Fazlolahzadeh, K. Keramati, S. Nazifi, S. Shirian, S. Seifi, Effect of Garlic (Allium sativum) on Hematological Parameters and Plasma Activities of ALT and AST of Rainbow trout in Temperature Stress ,Australian Journal of Basic and Applied Sciences, 5(9),2011, 84-90.
[57]. A. Eidi, M. Eidi, E. Esmaeili, Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin- induced diabetic rats, Phytomedicine,13(9- 10),2006, 624-9.
[58]. A.O. Micheal, O.M. Folaranmi, O.D.Ajoke, A.G.Olatunji, A. Oluwole, and A. Adewumi, Effects of ginger (Zingiber officinale Roscoe) and garlic (Allium sativum L.) on rats Infected with Klebsiella pneumonea. The Inter J of Altern Med., 7(1), 2009,346–439.
[59]. Y.M. Tatfeng and S.S. Enitan, Effects of onion and garlic extracts on some immunologic cells. African Journal of Traditional, Complementary and Alternative medicine, 9(3),2012, 374-379.
[60]. R.D. Adelman, W.L. Spangler, F. Beasom, G. Ishizaki, G.M.Conzelman, Frusemide enhancement of neltimicin nephrotoxicity in dogs, The Journal of Antimicrobial Chemotherapy, 7(4),1981, 431 – 440.
[61]. N.J. Miller, C.A. Rice-Evans, The relative contribution of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink, Food Chem, 60,1997, 331 – 337.
[62]. A.A. Adeneye and A.S. Benebo, Protective effect of the aqueous leaf and seed extract of Phyllanthus amarus on gentamicin- and acetaminophen-induced nephrotoxic rats. Journal of Ethnopharmacology, 188, 2008, 318-323.
[63]. J.C. Ozougwu, Anti-diabetic effects of Allium cepa (onions) aqueous extracts on alloxan -induced diabetic Rattus novergicus. J Med Plants Res.,5(7),2011, 1134-1139.
[64]. M.W. Massing, C.A. Sueta, M. Chowdhury, D.P. Biggs, and R.J. Simpson,Lipid management among coronary artery disease patients in diabetes mellitus or advanced age. Am J Cardiol, 87,2001, 646–664.
[65]. P. Pushparaj, C.H. Tan, and B.K. Tan, Effect of Averrhoa bilimli leaf extract on blood glucose and lipids in streptozotocin diabetic rats. J. Ethnopharmacol., 72,2000, 69-76.
[66]. A. Rahman and F. Olufunsho, Hyperlipidaemia among Saudi diabetic patients–pattern and clinical characteristics. Ann Saudi Med, 15(3),1995, 240 – 243.
[67]. E.S.Idogun, E.L. Unuigbe, P.S. Ogunro, O.T. Akinola, A.A. Famodu, Assessment of serum lipids in Nigerians with type 2 diabetes mellitus complications. Pakistan J Med Sci, 23(5),2007, 708–712.
[68]. I.L. Effendy, D.L. Simmons, G.R. Campbell, J.H. Campbell, The effect of aged garlic extract "kyolic", on the development of experimental atherosclerosis, Atherosclerosis, 132, 1997, 37 42
[69]. L. Liu, and Y.Y. Yeh, S-alkeniyl cysteines of garlic inhibit cholesterol synthesis by deactivating HMG-CoA reductase in cultured rat hepatocytes. J. Nutr., 132(6), 2002,1129-1134.
[70]. N. Gupta and T.D. Porter, Garlic and garlic derived compounds inhibit human squalene monoxygenase. J. Nutr., 131, 2001, 1662-1667.
[71]. D.K. Singh and T.D. Porter, Inhibition of sterol 4 alpa-methyl oxidase is the principal mechanism by which garlic decreases cholesterol synthesis. J. Nutr., 136(3),2006, 759S- 764S.
[72]. H.A. Oboh and C.O. Omofoma , (2008), The Effects of Heat Treated Lima Beans (Phaseolus lunatus) on Plasma Lipids in Hypercholesterolemic Rats . Pakistan Journal of Nutrition ;7 (5),2008,636-639.