Effect of administration of *Ehretia anacua* aqueous extract on blood glucose level in alloxan - induced diabetic rat

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**Abstract**

The effects of crude aqueous extract of *Ehretia anacua* on alloxan induced diabetic rats was investigated. Male albino rats of weighing between 120 to 150 were used, divided into 6 groups of five animals per group. Group I received distilled water throughout the experiment and served as the control. Group II received 110 mg/kg of alloxan interperitoneally. Groups III, IV, V and VI received 110 mg/kg of alloxan and in addition administered with aqueous *Ehretia anacua* extract daily for 14 days. Blood glucose level was monitored at five days interval for fourteen days. Target organs (pancrease) was taken from each rat. The histopathological studies of the pancrease were examined. In alloxan - induced diabetic rats, blood glucose level was significantly increased compared with the control rats. Treating diabetic rats with 50, 100 and 200 mg/kg bw *Ehretia anacua* caused a significant decrease in the blood glucose level. The Photomicrograph of the histopathology examination of the pancrease (× 100) of the groups treated with alloxan showed poor architecture was destroyed whereas those treated with *Ehretia anacua* showed normal architecture. This illustrates the amelliorative effects of the extract on the alloxan-induced toxicity. It could be concluded from these results that, *Ehretia anacua* extract should be used in manufacture processes of the natural products as functional foods or as a dietary supplement with anti-diabrectic activity as hypoglycemic effect.

**Keywords:** *Ehretia anacua*; Diabetes; Alloxan; Blood Glucose; Histopathology

1. Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia caused by defective insulin secretion and / or action, resulting in long term multi-organ complications. Chronic hyperglycemia [1] (Caughron and Smith, 2002) causes damage to the eyes, heart, kidneys, nerves, and blood vessels. The current review focuses on herbal [2] (Lebovitz, 2001) drug preparations and plants used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses. On the other hand, high glucose level was found to increase the production of free radicals, as determined by cell damage markers. Increased oxidative stress has been implicated in the pathogenesis of diabetic complications and reduced levels of antioxidants are found in blood and tissue in both human and experiments diabetes [3]; [4], [5]. (Cuncio *et al.*, 1995; Baynes and Thorpe, 1999; Koleva *et al.*, 2002). There are several synthetic drugs that have been used over time for the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses. On the other hand, high glucose level was found to increase the production of free radicals, as determined by cell damage markers. Increased oxidative stress has been implicated in the pathogenesis of diabetic complications and reduced levels of antioxidants are found in blood and tissue in both human and experiments diabetes [3]; [4], [5]. (Cuncio *et al.*, 1995; Baynes and Thorpe, 1999; Koleva *et al.*, 2002). There are several synthetic drugs that have been used over time for the treatment of diabetes. These include insulin, sulfonylurea, biguanides, α-glucosidase inhibitors, and glinides, which are administered to achieve a better glycemic regulation. Unfortunately, many of these drugs have their limitations and comes with quite a number of adverse effects, such as lactic acidosis, low blood sugar, upset stomach, skin rash or itching, weight gain, kidney complications, upset stomach, tiredness or dizziness, metal taste [6] (Wild *et al.*, 2004) etc. Thus managing diabetes using synthetic drugs without side effects remains a challenge [6]; [7]. (Wild *et al.*, 2004; Rajagopalaet *et al.*, 2008). This however has drawn a lot of interest and attention to the curative

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claims and norms of medicinal plants all over the world, especially in underdeveloped countries in Africa and some parts of Asia [8]. (Gagliano et al., 2007).

The leaves of *Ehretia anacua* plant are used as an antidiabetic agent in Nigerian folk medicine. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes there is an increased demand by patients to use the natural products with anti-diabetic activity [9]. (Neuwinger, 2000). One such plant expected to have anti-diabetic activity is *Ehretia anacua*.

The core aim of the present study was therefore; to examine the effects of *Ehretia anacua* leaf aqueous extract on lipid profiles and hyperglycaemia in alloxan-induced diabetic rats

### 2. Justification for the Research

Diabetes mellitus is a complex and multifarious group of disorders characterized by hyperglycaemia that has reached epidemic proportions in the present century. Infection is a leading cause of morbidity and mortality among the diabetic population [10]. (Mottalib et al., 2017). Diabetes is also associated with vascular and renal dysfunctions characterized by hypertension, dyslipidaemia and arteriosclerosis [11]. (Emadian et al., 2015). Numerous studies have provided convincing evidence for the presence of oxidative stress, and its role in the pathogenesis of the complications of diabetes [11]. (Emadian et al., 2015).

The leaves of *Ehretia anacua* plant are used as an antidiabetic agent in Nigerian folk medicine. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes there is an increased demand by patients to use the natural products with anti-diabetic activity [9]. (Neuwinger, 2000). One such plant expected to have anti-diabetic activity is *Ehretia anacua*.

Based on ethnobotanical uses, research on the antidiabetic effects of *Ehretia anacua* leaf and the effects of the extract on the lipid profile via the assessment of the lipid profile seems quite lacking. Therefore, there is a need to investigate the effects of the extract on the blood glucose levels in alloxan-induced diabetic rats.

### 3. Material and methods

#### 3.1. Plant Materials

Samples of *Ehretia anacua* leaves were collected from a private farm in Ado Ekiti, air dried in the laboratory, pulverized and then stored in an airtight container.

#### 3.2. Reagents and Chemicals

All reagents and chemicals were all of analytical grade.

#### 3.3. Extraction of the extract

*Ehretia anacua* leaf was air-dried for 30 days at room temperature. The air-dried samples were ground to fine powder using a blender. 500 g of the powdered leaves was soaked in 2000 ml of distilled water for 72 hours. It was then filtered using a cheese cloth, and freeze-dried to obtain the dried extract. The extract was kept in a closed container and kept inside the fridge at 4°C for further studies.

#### 3.4. Animal’s protocol

30 male wistar albino rats weighing 120g – 150g was obtained from the animal house at The Federal Polytechnic, Ado-Ekiti. They were acclimatized in the animal house of the Department of Science Technology, The Federal Polytechnic, Ado-Ekiti for 2 weeks, housed in clean wire meshed cages under standard conditions temperature (24 ± 1°C), relative humidity, and 12 / 12-hour light and dark cycle. They were allowed to have free access to food (commercial palletized diet from Vital Feed Mill) and drinking water *ad libitum* daily. The rat beddings were changed and replaced every day throughout the experimental period.

#### 3.5. Experimental Design

30 male wistar albino rats were randomly divided into six groups (I-VI) of five animals in each group.
3.6. Animal treatment
The animal treatment is shown in the table below

| Groups                      | Treatment                                                                 |
|-----------------------------|---------------------------------------------------------------------------|
| Group A: Normal control (NC)| Distilled water only for 14 days                                         |
| Group B: Diabetic control (DC)| 110 mg/kg Alloxan alone for a single administration                      |
| Group C                      | 110 mg/kg Alloxan+100 mg/kg *Ehretia anacuas* for 14 days                 |
| Group D                      | 110 mg/kg Alloxan+200 mg/kg *Ehretia anacuas* for 14 days                 |
| Group E                      | 110 mg/kg Alloxan+ 21.4 mg/kg metformin for 14 days.                       |

3.7. Determination of Blood Glucose Level
The blood glucose level was determined using glucometer

3.8. Dissection of Rats
The rats were dissected and the pancreas were excised using scissors and forceps.

3.9. Histopathological Analysis
Histopathological Analysis was carried out according to the method of [12]. Avwioro (2010).

4. Results

Table 2 Effects of *Ehretia anacuas* leaf extract on the blood glucose level

| Group / Treatment | Initial level (before induction) | Induced glucose level | 5th day     | 10th day     | 14th day     |
|-------------------|----------------------------------|-----------------------|-------------|-------------|-------------|
| Normal control    | 98.33 ± 4.13                     | -                     | 106.23 ± 2.10 | 102.01 ± 5.12 | 96.14 ± 2.13 |
| Diabetic control  | 100.23 ± 8.33                    | 307.29 ± 10.5         | 402.72 ± 7.21 | 387.22 ± 9.20 | 394.50 ± 6.72 |
| Alloxan + S. L (150 mg/kg) | 107.75 ± 4.5                 | 340.14 ± 2.37         | 179.12 ± 8.76 | 102.81 ± 9.00 | 97.21 ± 2.45 |
| Alloxan + S. L (100 mg/kg) | 99.45 ± 3.3                   | 415.09 ± 0.92         | 203.20 ± 7.22 | 117.25 ± 7.05 | 100.43 ± 4.30 |
| Alloxan + S. L (50 mg/kg) | 118.12 ± 2.4                   | 478.42 ± 3.61         | 355.81 ± 4.50 | 205.80 ± 3.05 | 103.22 ± 5.10 |
| Alloxan + MET (21.4 mg/kg) | 105.53 ± 5.5                  | 457.76 ± 5.03         | 221.43 ± 5.20 | 106.54 ± 2.70 | 96.16 ± 4.05  |

4.1. Histopathological Examination on the Pancreas
Photomicrograph of a pancreas section stained by haematoxylin& eosin showing normal architecture, the parenchyma of the pancreas shows normal serous acinar and zymogenic cells (slender arrow) containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissues (blue arrow) and septa (red arrow) are seen. There are normal compact islets of langerhans (white arrow) consisting of round to oval collections of endocrine cells [Figure 1].

Photomicrograph of a pancreas section stained by haematoxylin& eosin showing poor architecture, the parenchyma of the pancreas shows severely thickened vessels and duct (red arrow), and also showing mild peri ductal infiltration (blue arrow). There are diffuse islets which are composed of trabeculae of endocrine cells interspersed between adjacent acini, the borders of the diffuse islets are ill defined (white arrow). There is moderate infiltration of inflammatory cells within the intra acinar space and also seen within the islet (slender arrow), there is mild vascular congestion (black arrow) [Figure 1].
Photomicrograph of a pancreas section stained by haematoxylin & eosin showing poor architecture, the parenchyma of the pancreas shows severely thickened vessels and duct (red arrow), and also showing mild peri ductal infiltration (blue arrow). There are diffuse islets which are composed of trabeculae of endocrine cells interspersed between adjacent acini, the borders of the diffuse islets are ill defined (white arrow). There is moderate infiltration of inflammatory cells within the intra acinar space and also seen within the islet (slender arrow), there is mild vascular congestion (black arrow).
Figure 3 Diabetic group (110mg/kg Alloxan) but treated with *Ehretia anacua* leaf at 100 mg/kg

Photomicrograph of a pancreas section stained by haematoxylin& eosin showing normal architecture, the parenchyma of the pancreas shows normal serous acinar and zymogenic cells (slender arrow) containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissues (blue arrow) and septa (red arrow) are seen. There are normal compact islets of langerhans (white arrow) consisting of round to oval collections of endocrine cells.

Figure 4 Diabetic group (110mg/kg Alloxan) but treated with *Ehretia anacua* at 200 mg/kg.

Photomicrograph of a pancreas section stained by haematoxylin& eosin showing normal architecture, the parenchyma of the pancreas shows normal serous acinar and zymogenic cells (slender arrow) containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissues (blue arrow) and septa are seen. There are normal compact islets of langerhans (white arrow) consisting of round to oval collections of endocrine cells.
5. Discussion

The treatment of DM remains a challenging issue. Researchers are exploring safe and effective medications to overcome the detrimental effects of insulin resistance-related metabolic derangement, including hyperglycaemia, hyperinsulinaemia, hyper-lipidaemia, oxidative stress, inflammation, atherosclerosis and other complications [13]. (DeFronzo et al., 2013). For patients with DM, no safe treatments yet exist apart from diet and lifestyle modifications [14]. (Elsheikh et al., 2013). However, combined pharmacological therapy is recommended to improve insulin sensitivity in the liver (metformin and pioglitazone) and its periphery (thiazolidinediones), together with other drugs such as betaine, atorvastatin, losartan and orlistat [15]. (Crawford et al., 2009). However, the clinical value of these treatments is very subjective. Patients taking these drugs should be closely monitored due to possible contraindications with DM medications and the vulnerable condition of the liver during the drug detoxification process [16]. (Adams et al., 2006). Antioxidant therapy is a potential future therapeutic strategy; increasing antioxidant levels in patients with DM may hopefully counter the effects of oxidative stress and inflammation, thereby reducing the severity of diabetic complications. A few plant-based products and vitamins have been investigated as ways of protecting against and possibly reversing damage believed to be caused by oxidative stress and inflammation [17]. (Seven et al., 2004). Vitamin E and betaine are just a few of the antioxidants which have shown good clinical implications in the reduction of DM severity and the protection of the organs from DM-induced damage [17]. (Seven et al., 2004). Therefore, Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain [18]. (Okigbo et al., 2008). Their properties could be based on the antioxidant, antimicrobial antipyretic, enzyme inhibitory effects of the phytochemicals present in them [19]. (Adesokan et al., 2008).

The present study was undertaken to investigate the antihyperglycemic activity of sandpaper leaf Ehretia anacua extract in diabetic model rats. Metformin was used as a standard drug for diabetic model rats. It is well established that the only available diabetes medication in the Biguanides class of drug is metformine [20]. (Hundal et al., 2000). Biguanides prevent the liver from producing glucose and help to improve the body sensitivity toward insulin. Metformin is commonly used as first line treatment for type 2 diabetes and may occasionally be prescribed in combination with insulin for people with type 1 diabetes. These pills stop the liver from making too much sugar (glucose). They also help the sugar get into the cells [20]. (Hundal et al., 2000).
On the other hand, insulin activate glucose uptake in various cells including muscles and adipocytes, stimulates hexose uptake, lipogenesis and inhibit lipolysis and stimulate protein synthesis. Administration of Metformin and extracts into rats almost normalized serum glucose levels. Our results demonstrate that all the extracts of sandpaper leaves and metformin showed significant antihyperglycemic effect in diabetic model rats. Administration of a dose of 150 mg/kg, 100 mg/kg and 50 mg/kg body weight of the extract produced a potent and strong antihyperglycemic effect in diabetic rats. The obtained results are supported by the finding of other investigators [21]; [22]. (Sharma et al., 1997; Aderibigbe et al., 2001). Antihyperglycemic activity that is found in diabetic rats indicates that the extracts may interfere with the intestinal glucose absorption in the gut by various mechanisms [23]; [24]. (Nahar et al., 2000; Vinik and Wing, 1990). It may be postulated that the extracts of sandpaper leaf might stimulate glycogenesis in the liver, which is enhanced by feeding [25]. (Creutzfeld et al., 1979). This effect was confirmed by Perpetus and Salgado, 2003 where they showed that blood glucose level of diabetic rats consuming mango flour for 90 days decreased 66% in comparison to control rats. It was also observed that hepatic glycogen level of those diabetic rats was 64% greater than control. The author claimed that this increase in glycogen level might have contributed to the reduction of blood glucose level in these animals.

According to certain studies, the aberrant signal which promotes glucose production in the liver during DM supposedly also enhances fatty acid oxidation due to a lack of fuel demand [26]. (Reid, 2006). However, other research has found that the liver stops oxidising fatty acids and uses them instead to synthesise triglycerides which then accumulate abnormally in the liver [26]. (Reid, 2006). In type 1 DM, insulin deficiency up regulates hormone-sensitive lipase in the adipose tissues, subsequently leading to increased lipolysis and the circulation of free fatty acids, which subsequently accumulate in the liver. These processes enhance the hepatic uptake of very-low-density lipoproteins and synthesis of triglycerides [26]. (Reid, 2006). Concurrently, elevated glucagon levels inhibit hepatic triglyceride output. Therefore, accumulation of fat in the liver may be due to an imbalance in the uptake, synthesis, export and oxidation of free fatty acids in the liver [27]. (Cohen et al., 2006). Aside from abnormalities in lipoprotein metabolism, an accumulation of hepatic fat in DM may be due to either hyperglycaemia-induced activation of the transcription factor carbohydrate-responsive element-binding protein and sterol regulatory element-binding protein 1c, the up regulation of the glucose transporter 2 protein with subsequent intrahepatic fat synthesis or a combination of these mechanisms [27]. (Cohen et al., 2006).

Poor architecture, mild peri ductal infiltration, mild vascular congestion, moderate infiltration of inflammatory cells within the intra acinar space and within the islet of pancreas, diffuse islets which are composed of trabeculae of endocrine cells interspersed between adjacent acini, the parenchyma of the pancreas shows severely thickened vessels and duct in the pancreas section of Wistar rats treated with alloxan only when compared with the pancreas sections of the control is indicative of alloxan related toxicity.

Administration of the extract Ehretia anacua improved the histo-architecture of the pancreas and by extension restored its functionality. Histoarchitectural distortion, such as, inflammatory cells infiltration observed in the pancreas sections is resultant of alloxan intoxication, while observed histoarchitectural preservation is consequent to treatment with plant extract. This finding is supportive of medicinal plant related studies that have reported hepatoprotective activity of plant extracts; [28]. (Al-Qarawiet al., 2012) treatment with plant extract decrease the severity of histopathological changes induced by acetaminophen. Extract of Ehretia anacua presented histoarchitectural preservation of the pancreas cells when compared with the control. Treatment with Ehretia anacua demonstrated the plant extract potentials as a free radical scavenger and lipid peroxidation inhibitor, thus helping to maintain the integrity and permeability of cell membranes and protects cells and tissues against oxidative stress induced by free radicals [29]. (Naik and Panda, 2012).

6. Conclusion

Medicinal plants are used in several countries to manage diabetes and diabetes complications and thought to be less toxic than the synthetic drugs. Phytomedicines are also easily available and affordable to many people. In conclusion, the extract of Ehretia anacua reduced the levels of glucose, and preserve the Histoarchitecture of the pancreas in experimentally induced diabetic rats. Therefore, the plant has hypopilidaemic effects on alloxan-induced damage in rats. Based on these findings, the results thereby lend credence to the ethnomedicinal use of the extracts in the management of diabetes at evaluated dosages and their use should be encouraged.
Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that No conflict of interest in this work.

Statement of ethical approval

All authors hereby declare that the research has been determined exempt from review by the Polytechnic animal research and ethics review committee and that the principles of the laboratory animal care were followed.

References

[1] Caughron, KF and EL Smith,.Definition and description of diabetes mellitus. South Med. J,2002; 95(1):35-49.
[2] Lebovitz, HE. Diagnosis, classification are pathogenesis of diabetes mellitus. J. Clin. Psychiatry.62 (Suppl) 2001; 27:5-9.
[3] Cunico, F, I Pegoraro, F Falleti, G Perrella and A Ceriello,. SOD and GSH inhibited the high glucose induced oxidative damage and the PDGF increased secretion in cultured human endothelial cells Thrombosis Homeostasis, 1995; 74:963973.
[4] Baynes, J. and S. Thorpe,. The role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes, 1999; 48:119.
[5] Koleva, I, T Beek, J Linssen et al. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Anal.,2002; 13:8-17.
[6] Wild, S, Roglic, G, Green, A, Sicree, A, and King, H"Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030" Diabetes care,2004; 27:1047-1053.
[7] Rajagopal, KandSasikala, K.Antihyperglycaemic and antihyperlipidaemic effects of Nymphaeastellata in Alloxan monohydrate-induced diabetic rats. Singapore Medical Journals,2008; 49:137-141.
[8] Gagliano, N., Grizzi, F., and Annoni, G. Mechanism of aging and liver functions.Dig Dis Sci,2007; 25:118-123.
[9] Neuwinger, HD. African traditional medicine: a dictionary of plant use and applications. Medpharm Scientific, Stuttgart, Germany.2000; 589 pp.
[10] Mottalib A, Kasetty M, Mar JY, Elseaidy T, Ashrafzadeh S, Hamdy O "Weight Management in Patients with Type 1 Diabetes and Obesity".Current Diabetes Reports.August 2017; 17 (10):92.
[11] Emadian A, Andrews RC, England CY, Wallace V, Thompson JL (November 2015). "The effect of acronutrients on glycaemic control: a systematic review of dietary randomized controlled trials in overweight and obese adults with type 2 diabetes in which there was no difference in weight loss between treatment groups".The British Journal of Nutrition.
[12] Avwioro O G 2010:Histochemistry and tissue pathology, principle and techniques, Claverianum press, Nigeria.
[13] DeFronzo RA, Eldor R, Abdul-Ghani M. Pathophysiologic approach to therapy in patients with newly diagnosed type 2 diabetes. Diabetes Care. 2013;36:S127–38.
[14] Elsheikh E, Henry LL, Younossi ZM. Current management of patients with nonalcoholic fatty liver disease. Expert Rev Endocrinol Metab. 2013;8:549–58. doi: 10.1586/17446651.2013.846212.
[15] Crawford JM, Iacobuzio-Donahue C. Liver and biliary tract. In: Kumar V , Abbas AK, Fausto N,Aster JC, editors.Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia, Pennsylvania, USA: Saunders; 2009; pp. 833–90.
[16] Adams LA, AnguloP .Treatment of non-alcoholic fatty liver disease.Postgrad Med J.2006;82:315–22.
[17] Seven A, Guzel S, Seymen O, Civelek S, Bolayirli M, Uncu M, et al. Effects of vitamin Esupplementation on oxidative stress in streptozotocin induced diabetic rats: Investigation of liver and plasma. Y onsei Med J. 2004;45:703–10.

[18] Okigbo, R.N., Eme, U.E. and Ogbogu, S. Biodiversity and conservation of medicinaland aromatic plants in Africa. Biotechnology and Molecular Biology Review; 2008; 3(6):127-134.

[19] Adesokan, AA, Yakubu, MT, Owoyele, BV, Akanji, MA, Soladoye, A and Lawal, OKEffect of administration of aqueous and ethanolic extracts of Enantiachloranthastem barkon brewer’s yeast-induced pyresis in rats. African Journal of Biochemical Research; 2008; 2 (7):165-169

[20] Hundal, R, Krssak, M, Dufour, S, Laurent, D, Lebon, V, Chandramouli, V, Inzucchi, S, Schumann, W, Petersen, K, Landau, B, and Shulman, G. Mechanism by which metformin reduces glucose production in type 2 diabetes. Diabetes care, 2000; 12:2063–9.

[21] Sharma SR, Dwivedi SK, SwarupD. Hypoglycemic potential of Mangiferaindica leaves in rats. Pharmaceutical Biol. 1997; 35:130-33.

[22] Aderibigbe AO, Emudianughe TS, Lawal BAS. Evaluation of the antidiabetic action of Mangiferaindicain mice. Phytototherapy Res. 2001; 15:456-58.

[23] Nahar N, Rokeya B, Ali L, Hassan Z, Nur-e-Alam M, Choudhury NS, Khan AKA, Mosihuzzaman M. Effect of three medicinal plants on blood glucose levels in nondiabeticand diabetic model rats. Diabetes Res. 2000; 35:41-49.

[24] Vinik A, Wing RR. In: Diabetes mellitus: Theory and practice. Rifkin H, Porte D Jr. (eds). 2nd ed. New York, Elsevier, 1990; pp 465-97.

[25] Creutzfeld W. The incretin concept today. Diabetologia 1979; 16:75-85.

[26] Reid AE. Non-alcoholic fatty liver disease. In: Feldman M, Friedman LS, Brandt LJ, editors.

[27] Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: Old questions and new insights. Science. 2011;323:1519–23.

[28] Al-Qarawi A, Mousa HM, Ali BH, Abdel-Rahman H, El-Mougy SA Protective effect of extracts from dates (Phoenix dactylifera L.) on carbon tetrachloride-induced hepatotoxicity in rats. Int J Appl Res Vet M, 2012; 2:176-80.

[29] Naik, S R and Panda, V SAntioxidant and hepatoprotective effects of Ginkgo bilobaphytosomes in carbon tetrachloride-induced liver injury in rodents. Liver Int2012; 27:393-9.