JMBR: A Peer-review Journal of Biomedical Sciences
December 2015, Vol. 14 No. 2 pp 22-30

ROLE OF ETHANOL LEAF EXTRACTS OF FICUS GLUMOSA ON FASTING BLOOD GLUCOSE AND LIVER FUNCTION TEST RESULTS OF DIABETES TREATED RATS

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ABSTRACT:
In Africa many plants and plant products are used traditionally for the managements of diabetes mellitus or its complication. But, science based evidence on some of this product safety need to be establish. We determine the effect of the Ethanol leaves extract of Ficus glumosa on fasting blood glucose level and liver enzymes of diabetes (DM) treated rats. Thirty (30) adult rats weighing (190 – 220) grams of about 8 to 10 weeks of age were used for this study in the month of December, 2014. The animals were randomly divided into six groups (n= 6/group) and DM was induced using single dose of streptozotocin 65mg/kg intraperitoneal (ip). Group 6 served as the control group receiving normal saline (5ml/kg) alone via intra-peritoneal route (ip), Group 1 (positive control), 2, 3, 4 and 5 were treated with the following for 7 days. Normal saline 5ml/kg, 100mg/kg, 200mg/kg and 400mg/kg of Ficus glumosa ethanol leaves extract respectively via ip while Group V received insulin 6 iu/kg. Fasting blood sugar levels were measured at one day intervals for 7 days. Rats were sacrifice for the Serum liver enzymes and liver tissue for analysis on the 7th day. The 100mg/kg and 400mg/kg of Ethanol leave extract of Ficus glumosa significantly lowered fasting blood glucose level and 400mg/kg show elevation in liver enzymes and reveal altered hepatocyte architecture (interphase hepatitis) compared with the control group 6 and lower dosage treatment group (2 and 3). The results of these study reveal that Ficus glumosa possesses hypoglycemic effect and at higher dose of 400mg/kg/day have effect on liver enzymes in diabetes rat.

Introduction

Diabetes mellitus (DM) is a metabolic disease with multiple etiology and many systemic complications, according to a recent estimate by the World Health Organization and International Diabetic Federation, There were 382 million people in the world with diabetes in the year 2013 and this is projected to increase to 592 million by 2035 [1]. This disease is associated with reduced life expectancy, significant mortality and diminished quality of life. In 2005 an estimated 1.1 million people died from diabetes and diabetes complications [1- 3]. Several studies have reported on the hypoglycemic property of Ficus glumosa in animal models of diabetes [4, 5]. They used organic solvent based extracts of the plant's stem bark but, the use of the leaf is the common practice in communities that have acclaimed to its hypoglycemic efficacy and it is therefore possible that the extracts may serve as a remedy by blocking or intercepting the activity of

KEYWORDS: *diabetes mellitus, liver enzymes, leaf extract and ficus glumosa.*

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environmentally acquired toxins such as mycotoxins, insecticides and pesticides [6]. The changes in the intensity of the hypoglycemic effect of Ficus glumosa over a predetermined time course will be informative in establishing its anti-diabetic and medical value in prolonged usage. The hypoglycemic efficacy of Ficus glumosa has only been compared with those of oral hypoglycemic drugs in the previous studies reviewed [7, 8]. Comparing its efficacy with that of insulin and the action on the liver enzymes will provide helpful information on its anti-diabetic benefits in type 1 DM although, Individuals with type-2 diabetes have a higher incidence of liver function test abnormalities. Liver function tests (LFTs) are commonly used in clinical practice to screen for normal liver physiology, monitor the progress of a known problem and effects of potentially hepatotoxic substances. Science based evidence to evaluate the chemical components, safety and effectiveness of traditional medicine need to be established for noble drug development. Although, research shows that some herbal medicine is effective for specific conditions further study of the safety and effective dose is still necessary [9-11]. The most common LFTs include the evaluations of serum Aminotransferases, Alkaline phosphatase (ALP), Aminotransferase (ALT) and Aspartate aminotransferase (AST). Mild chronic elevations of transaminases often reflect underlying insulin resistance [12] and loss of insulin effect on the liver leads to glycogenolysis and an increase in hepatic glucose production and hyperinsulinemia [13]. Hyperinsulinemia might directly lead to hepatic insulin resistance with associated increased lipogenesis and formation of fatty liver. The excess in free fatty acids found in the insulin-resistant state and the associated oxidant stress from reactive lipid peroxidation and paroxysmal β-oxidation is known to be directly toxic to hepatocyte and leads to injury and elevation of serum transaminases [14]. However, elevation of transaminases does not always correlate with histological changes in the liver, the elevation in ALT, a gluconeogenic enzyme whose gene transcription is suppressed by insulin, could indicate impairment in insulin signaling rather than purely hepatocyte injury. In contrast, aspartate aminotransferase (AST) and □-glutamyltransferase (GGT) serum concentrations are unrelated to changes in hepatic insulin action. Followed 451 non-diabetic Pima Indians for an average of 6.9 years to determine whether hepatic enzyme (ALT, AST, and ALP) elevations could be linked to the development of type-2 diabetes. After adjustment for age, sex, body weight, whole body insulin sensitivity and acute insulin response only elevated ALT. Research shows higher ALT as a risk factor for type-2 diabetes and indicates a potential role of increased hepatic gluconeogenesis in the pathogenesis of DM that presents with elevated ALT [13]. Many so called plant poisoning involves harmless species that have been treated with insecticides, weed killers, or fertilizers [15, 16]. Although no significant adverse effects have been reported for the local use of Ficus glumosa [17]. This study was to ascertain the hypoglycemic effect of ethanol leaf extract of Ficus glumosa and its safety on the parameters of liver function test (ALT, ASP and ALP) in diabetic rats. We hypothesized that Ficus glumosa possesses hypoglycemic properties with no effect on liver enzymes if properly administered.

Materials and Methods

Streptozotocin for the induction of DM was purchased from Sigma Aldrich (St. Louis, MO, USA) and Assay kits for the

assessment of liver enzymes were purchased from (Randox Laboratory Ltd, North West Life Science Specialties Vancouver, Canada). Glucose-test Strips for assessment of plasma glucose levels were from (Accu-Check Advantage II, Roche. Diagnostics GmbH Germany). All other chemicals and reagents used were of analytical grade.

Collection of Plant Materials

Fresh leaves of Ficus glumosa plant was obtained locally from a farmer in Nigeria. The plant was identified and authenticated at the Herbarium Unit of Biological Science, Ahmadu Bello University Zaria, Nigeria and a voucher specimen (No. 0412) deposited for reference.

Animals

Thirty five (30) adult male Wister rats age 8 to 10 weeks (190- 220) g were used in this study. The animals were kept in the cages under standard laboratory conditions with free access to food (standard pellets from Guinea Feeds, Plc., Nigeria) and water ad libitum, allowed one week to acclimatize.

Plant Extract preparation

The leaves of the plant were plucked from the stem and then dried in an ovum during the month of October, 2014. The dried samples were pulverized using a mortar and pestle and extracted with 70% ethanol using cold maceration. The mixture was filtered and the filtrate concentrated under vacuum using rotary evaporator at 60 ^{0}C to obtain the Ethanol Extract Residue (EER). Ethanol Extract Residue (4g) was soaked in 99% ethanol (100ml) for twenty four (24) hours [19]. The extract was then filtered, using a filter paper (Whatman size no.4) into a beaker and later poured into a volumetric flask and Stoppard. The filtrate (4% w/v) was then stored at negative 4 ^{0}C in a refrigerator for later use. From the stock solution (4% w/v or 4000mg/100ml), volumes containing (400, 200 and 100) mg were determined per kilogram (kg) of animal weight.

Induction of Diabetes Mellitus

The rats were fasted overnight for at least 16 hours before induction of diabetes. DM was induced by a single intraperitoneal injection of freshly prepared solution of streptozotocin 65mg/kg body weight. While the control rats were similarly handled using 0.9% normal saline and served as the control group. The rats were then kept in their cages for 48 hours before determination of baseline fasting blood sugar levels with free access to food and water at libitum [20, 21, and 22]. The animal with fasting blood sugar level of $\geq$ 200mg/dl are considered diabetes and used in this study.

Experimental Design

Group 1: DM rats receiving 0.9% normal saline (5ml/kg) diabetic control.
Group 2: DM rats receiving 100mg/kg body weight of the extract.
Group 3: DM rats receiving 200mg/kg body weight of the extract.
Group 4: DM rats receiving 400mg/kg body weight of the extract.
Group 5: DM rats receiving 6.0 iu/kg body weight short-acting insulin.
Group 6: Non DM rats receiving 0.9% normal saline (5ml/kg) normal control.
Animals received the aforementioned treatments via intraperitoneal rout (ip) for a period of one week.

Determination of blood glucose levels

Blood samples were collected from the tail vein of the rats on days 0, 1, 3, 5 and 7. Fasting blood sugar was done using

glucose-oxidized principle sand results were expressed as mg/dl [12, 23].

Determination of liver enzymes and histological investigation

Serum Alkaline phosphatase (ALP) activity was assayed by the method of Bassey- lowry-Block (1946) with modification. Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities were assayed by the method of Reitman and Frankel (1957) with some modification and the Histopathology of the liver samples were carried out at the Ahmadus Bello University Teaching Hospital (ABUTH), Pathology Laboratory using the method of Carleton (1967) with modification.

Statistical analysis

The data obtained were expressed as mean ± standard error of mean (SEM) and data was statistically analyzed using SPSS Version 20.0. One way analysis of variance (ANOVA) with multiple comparisons (Dunnett's method) and value of P was < 0.05.

Results and Discussion

Streptozotocin is a drug of choice used to induce diabetes in animals. In this study, we find out ethanol leaf extract of ficus glumosa to have significantly decrease fasting blood glucose level (Table. 1), some liver enzymes and histological study (Tab. 2 & 3) findings when admistered for a period of one week to diabetes rats (p value < 0.05).

Table 1:

Fasting blood glucose level of ethanol extract of Ficus glumosa treated and untreated DM rat

	Fasting blood glucose levels (mg/dl) Mean ± SEM				
Sample size (n)	n = 5	n = 5	n = 5	n = 5	n = 5
Samples collection days	Day 0	Day 1	Day 3	Day 5	Day 7
1. Diabetic Normal Saline (Negative Control)	481.5± 32.5*	413.0 ± 31.06 *	425.0 ± 38.9*	464.0 ± 42.5*	465.0 ± 44.5*
2. Diabetic on (100mg/kg Extract)	423.2 ± 33.1*	†336.4 ± 36.6*	†260.4 ± 28.0*	†224.0± 20.1*	†201.0± 20.5*
3. Diabetic on (200mg/kg Extract)	475.2 ± 34.2*	†358.6 ± 35.2*	ns 399.6 ± 39.6*	†313.4 ± 27.5*	†331.4 ± 38.0*
4. Diabetic on (400mg/kg Extract)	468.0 ± 19.4*	†346.6 ± 25.6*	†266.2 ± 09.9*	†204.0 ± 15.9*	†206.4 ±23.1*
5. Diabetic on (IP Insulin 6.0 IU)	484.2 ± 32.7*	ns 378.2 ± 09.7*	†317 ± 14.3*	†308.8 ± 07.5*	†307 ± 33.2*
6. Normoglycemic (Positive control)	98.4 ± 03.8	81.8 ± 04.00	90.6 ± 03.6	79.0 ± 03.4	85.6 ± 3.7

***P*< 0.05 vs. normoglycemic group; †*P*< 0.05 vs. diabetic negative control group; ns (not significant).**

The analysis of variance (*ANOVA*) showed that on day 0 before the starting of treatment (extract and insulin), fasting blood glucose (fbs) levels were not significantly different in all the DM groups. On

days 1, 3, 5 and 7 after starting of treatment fbs levels were significantly decreases in all the groups that received the different doses of extract compared with the untreated DM group 1 (control group) which is elevated. Blood glucose levels were significantly lower in the positive control group compared with group 1 and all the other treatment groups (days 0, 1, 3, 5 and 7). P **< 0.05**

Table 2

The liver enzymes results of *ficus glumosa* treated streptozotocin induced diabetes rats.

Treatment	Liver enzymes (IU/L) (mean ± SEM)		
	AST	ALT	ALP
1. Diabetic on NS	21.21 ± 1.62*	32.60 ± 2.21ns	69.60 ± 3.01ns
2. Diabetic (100mg/kg Extract)	ns 18.40 ± 4.40	ns 32.60 ± 8.20	†63.00 ± 16.0
3. Diabetic (200mg/kg Extract)	ns 18.80 ± 2.86	ns 36.80 ± 3.71	ns 70.20 ± 3.51
4. Diabetic (400 mg/kg Extract)	†24.60 ± 1.78	† 39.60 ± 3.14	†77.60 ± 3.70
5. Diabetic (I.P Insulin 6.0 iu)	ns 22.80 ± 3.31	†37.20 ± 3.89	ns 71.40 ± 3.40
6. Normoglycemic (control)	18.00 ± 1.30	33.60 ± 2.44	66.80 ± 4.81

P* < 0.05** vs. normoglycemic group; †P* < 0.05** vs. diabetic negative control group; ns (not significant); NS means normal saline. The analysis of variance (*ANOVA*) showed no significant difference in the serum levels of AST, ALP and ALT of the positive and the negative control groups. The dose of 400mg/kg of the extract significantly ($P < 0.05$) elevated AST, ALP and ALT compared with all the groups including the negative and control groups.

Table 3:
Summary results of the effect of *Ficus glumosa* on histopathological study of the liver

GROUPS	HEPATOCYTE	PORTAL TRACT	CENTRAL VEIN	OTHER FEATURES
Diabetic Normal saline:	-	-	-	-
Diabetic (100mg/kg Extract):	-	-	-	-
Diabetic (200mg/kg Extract):	-	-	-	-
Diabetic (400 mg/kg Extract):	Mild fatty change	-	-	-
Diabetic (I.P Insulin 6 I.U):	-	-	-	-
Normoglycemic rats:	-	-	-	-

The histopathological study results of the liver samples obtained from each of the six group, No abnormality represented as (a negative -ve sign means normal histological findings), some changes are observed in the group that received 400mg of extract as seen (Table. 2).

Diabetes Mellitus (DM) is characterized by multiple metabolic complications such as changes in normal food metabolism, altered enzymes functions and diabetes ketoacidosis (DKA) that continue to be the major cause of morbidity and mortality rate in developing countries [2, 13, 24]. Hyperglycemia remains the hallmark and the diagnostic indicator of DM [25]. This study showed that, the baseline fasting blood sugar level following the induction of hyperglycemia before the commencement of the treatment, the fbs of all the groups were significantly increased compared with the normal rats (negative control). Subsequently, following the administration of the extract the fbs on (3, 5 and 7) days, of the groups (i.e. Groups II, III and IV) that received the different doses of the extract (100, 200 and 400) mg/kg respectively, were significantly lower compared with the normal and diabetic groups that received 0.9% normal saline alone and even when compared with the DM group that received insulin (Table. 1). These results suggest that, Ethanolic leaves extract of Ficus glumosa possesses hypoglycemic effect in corroborating with previous studies that reported on the hypoglycemic effect of Ficus glumosa [4]. This might be due to the presence of some active components like Flavonoids, ceramides, saponins, Cardiac glycosides and steroids [18, 26]. The results of these study showed that, the intensity of the hypoglycemic effects at (100 and 400) mg/kg of the extract increased in a time dependent pattern compared with 200mg/kg or insulin (6.0iu), the fbs of rats that received 100mg/kg and 400mg/kg were lower in a time dependent pattern compared to either of the two groups that received 200mg/kg and insulin (6.0iu). This result is suggestive of an increasing anti-diabetic value of Ficus glumosa in prolonged usage (Fig. 2).

However, we observed that there were no significant differences between positive and the negative control groups in the serum levels of all the liver enzymes assessed except for AST, elevation of AST which is not a specific indicator of liver parenchyma damage, it is also present in other tissue (red blood cells, cardiac,

skeletal muscle). However, serum AST, ALT and ALP were all elevated in the group that received 400mg/kg of the extract compared with the other groups (Table. 2). The liver enzymes elevation need to be further evaluated because of the establish alloxan effect on the hepatocytes [31] This result is indicative of leakage of these enzymes into the serum due to probably hepatocyte damage caused by 400mg/kg dosed of the extract used which is in support of alloxan action on glucokinase activity hence, more investigation need to be carried out to ascertain the exact action of ficus glumosa on hepatocytes [32]. Putting together the results of liver enzyme (ALT, ASP, and ALP) assessments and the histopathological study results of the liver samples obtained in the present study (Table. 2 & 3), the liver sample from the group that received 400mg/ kg showed mild fatty change (Table. 1). In explaining this observation from our results, the effects may be due to the dosage of the extract 400mg/kg affecting the hepatocytes evidence from the total liver enzymes elevation [11, 16].

In conclusion; Ficus glumosa are seen to possess anti-hyperglycemic effect and the intensity of the effect appears to increase with prolonged used, in a step ladder fashion. However, at higher dose the extract are seen to be hepatotoxic evidence by the significance increase of the liver enzymes (AST, ALT and ALP), mild fatty changes appearing in the group treated with 400mg/kg of the extract (Table. 2 & 3). We advocate on more study to establish the exact cause of liver enzymes elevation and also to explore all the potential chemical components (volatile and non-volatile) of this extract to come up with new and noble finding.

Acknowledgements
The first author want to appreciate the Malaysian International Scholarship of Malaysian ministry of Education for all their support.

Author Contributions
The author's responsibilities were as follow; Mahaneem Binti Mohamed and Aliyu Mohammed, study design and general monitoring; Umar Zayyanu Usman experimentation, manuscript writing. All the authors participated in the animal handling and data analysis.

REFERENCES:

1. International Diabeteic Federation (IDF). Diabetes Atlas, Sixth edition. Chapter 2/ DM Global burden, 2014; 01- 06.

2. Amos A, Mc Carthy D and Zimmet P. The king global burden of diabetes and its complication part I. Diagnosis and classification of diabetes mellitus provisional report of WHO consultation. Diabet med, 1997; 15: 539-553.

3. Maritim AC, Sanders RA, and Watkins JB. Diabetes, oxidative stress and antioxidants. A Review, J. Biochem molecular toxicology. 2013; 17: 123 – 127.

4. Umar ZU, Moh'd A and Tanko Y. Effect of Ethanol leaf extract of Ficus Glumosa on Fasting blood glucose and Serum lipid profile in Diabetic Rats. Niger. J. Physiol. Sci. 2013; 28: 099 – 104.

5. Harris E H. Elevated Liver Function Tests in Type 2 Diabetes. Clinical Diabetes. 2011; 2: 115-119.

6. Schwinghammer TL and Kroboth PD. Basic concepts in pharmacodynamics Modeling. Journal of Clinical Pharmacology. 1988; 28: 388– 394.

7. El- sayed M El- sayed, Osama M Abo- Salem, Hamdy A Aliyu and Ahmed M Mansour. Potential antidiabetic and Hypolipidemic Effects of Propolis Extract in Streptozotocin Induced Diabetic Rats. Pak J. Pharm Sci. 2009; 22: 168 – 174.

8. Ayesha M, Anna S M, Campton S G, S O Samson and Cortett Richart. Fig-eating by vertebrate frugivoves a global review. Biological Reviews. 2001; 76(40): 529-572.

9. Tataranni P A. High alanine aminotrasferases is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetic care. 2002; 51: 1889 – 1895.

10. World Health Organization. Diabetes mellitus report of a WHO study Group, Technical Report Series. 2010; 727: 218 – 221.

11. Neuschwander-Tetri BA and Caldwell S. Nonalcoholic steatohepatitis. Summary of AASLD single topic conference, Hepatology. 2005; 37: 1202-1219.

12. Baskaran K, Ahamath BK, Shanmuga Sundaram, R.K and Shanmugasundaram E R. Anti-diabetic effect of a leaf extracts from Gymnema sylrestre in non insulin dependent diabetes mellitus patients. J. Ethnopharmacol. 2001; 30: 295-300.

13. Biagio Arcidiacono, Stefania Liritano, Aurora Nocera, Katiuscia Possidente, Maria T. Nevolo, Valeria Ventura, Daniela Foti, Eusebio Chiefari and Antonio Brunetti . Insulin Resistance and Cancer Risk: An Overview of the Pathogenetic Mechanisms. Experimental Diabetes Research, 2012; 789174: 12.

14. Valpato GT, Calderon IMP, Sinzato S, Campos KE, Rudge MVC, Damasceno DC. Effect of Morus nigra aqueous extract treatment on the maternal – fetal outcome oxidative stress status and lipid profile of Streptozotocin – Induced diabetic rats. Journal of Ethno pharmacology. 2011; 138: 691- 696.

15. Francois C, Fares C, Baiocchi Jean Michel. Safety of Desmodium adscendens extracts on hepatocytes and renal cells protective effect against oxidative stress. J. Intercult. Ethnopharmacol, 2015; 4(1): 1 – 5.

16. Sasidharan S, Chen Y, Saravanan D, Sundram KM and Yoga Latha. Extraction, Isolation and Characterization of Bioactive Compounds from plants extracts. Afri. J. Tradit Complementary Altern Med. 2011; 8: 1- 10.

17. Madubunyi I, Onoja Samuel A, Isaac. In vitro antioxidant and in vivo antidiabetic potential of the methanol extract of ficus glumosa Del (moraceae) stem bark in alloxan induced diabetic mice. Comparative clinical pathology. 2012; 21(4): 389.

18. Novatus F Mushi, Ester Innocent, Abdul Wkidukuli. Cytotoxic and antimicrobial activities of Substituted phenanthrenes from the roots of Combretum adenogenium steud Ex A. Rich (Combretaceae). J. Intercult. Ethnopharmacol, 2015; 52 – 56.

19. Braz J and Chem S. Antioxidant activity and composition of Propolis obtained by different methods of extraction. Journal of the Brazilian Chemical Society. 2011; 22: 103- 5053.

20. Chinedum Ogbonnaya Eleazu, KATE Chenidum Eleazu, Sonia Chukwuma and udeme Nelson Essien. Review of the mechanism of cell death resulting from Streptozotocin challenge in experimental animals, its practical use and potential risk to humans. Journal of Diabetes & Metabolic Disorders. 2013; 12: 12- 60.

21. Etuk EU. Animals models for studying diabetes mellitus. Agric and Biol J. of N. Am. ISSN Print: 2010; 2151- 7517.

22. Lenzen S. The mechanisms of alloxan and Streptozotocin – induced diabetes. Diabetologia clin and Expt Diabetes & Metabolism. 2007; 7: 886- 7

23. Aribal- Kocatuk P, Kavas G O, Buyukkaneini D I. Pretreatment Effect of Resveratrol on Streptozotocin- induced Diabetic Rats. Biol Trace Elem Research. 2007; 118: 244-9.

24. World Health Organization. Technical Report Series No. 844: Prevention of Diabetes mellitus. Studies Report Group. 2011; 10:18-23 and 93-97.

25. Bishayee H, Joner G, Mandrup T, Poulsen T, Christy M Zachau, and Chistiasen D. Relation between breast feeding and incidence rates of insulin dependent diabetes mellitus a hypothesis, Lancet. 2004; 8411: 1083- 6.

26. Xiali Guo, Bin Chen, Liping Luo, Xi Zhang Ximo Dai and Shangji Gong. Chemical Compositions and Antioxidant Activities of Water Extracts. J. Agricultural and Food Chemistry. 2011; 59: 12610- 12616.

27. Madubunyi K, Moovdechale, Hartmann, Anat and Baryoef, Offers. Response to comment on Early Domesticated fig in the Jordan Valley. Journal 0f Biological Science. 2010; 314:16836.

28. Sofowara A. Medicinal plants and Traditional Medicine in Africa. John Wiley publishers, New York 2nd edition. 1984; 128-132.

29. World Health Organization. Diamond Project Group on Epidemics. American journal J. of Epidemiology. 2007; 135: 803 – 816.

30. Karin Ekberg and Bo- lennart Johansson. Effect of C- Peptide on Diabetic Neuropathy in Patients with Type 1 Diabetes. 2008; 457912: 5.

31. Handen K, Boujbihan MA, Masmoudi H, Ayadi FM, Jamoussi K, Elfeki A. Combined vitamins (C & E) Insulin improve oxidative stress and pancreatic and hepatic injury in alloxa n diabetes rats. Biomedicine and Pharm. 2009. 63; 95- 99.

32. Zhang X, liang W, Mao Y, Li H, Yang Y, Tan H. Hepatic glucokinase activity is the primary defect in alloxan induced diabetes of mice. Biomedicine and Pharmacotherapy. 2009; 63 180- 186.