

## NEUROPROTECTIVE POTENTIAL OF MANGO (*MANGIFERA INDICA*) LEAVE EXTRACT IN ALLOXAN-INDUCED DIABETIC RATS

BALOGUN WASIU<sup>1</sup>, MORAKINYO AMOS O<sup>1</sup>, ADEYEMO KHADEEJAH A<sup>1</sup>, IMAM AMINU<sup>2</sup>, ISHOLA AZEEZ O<sup>2</sup>, COBHAM ANSA E<sup>3</sup>

### ABSTRACT

**OBJECTIVE:** To study the effects of *Mangifera indica* leaves on alloxan-induced diabetes checking the level of oxidative stress, behavioural observation and histology state of the prefrontal cortex in adult male Wistar rats.

**STUDY DESIGN:** Analytic Study

**PLACE AND DURATION:** The research work was carried out between 7th February and 3rd April of 2013, at the Department of Anatomy, University of Ilorin, Nigeria.

**METHODOLOGY:** Twenty five adult male Wistar rats were divided into five groups. Group B was induced with hyperglycaemia by alloxan (150 mg/kg body weight; Group B). Group C and D induced diabetic rats were treated with 10mg/kg of *Mangifera indica* and 20mg/kg of *Mangifera indica* respectively. A group of undiabetic rats were also treated with 20mg/kg of *Mangifera indica* (Group E). The control was given feed and water ad libitum (Group A). At 28 days of treatment, novel object recognition test was done, prefrontal histology was studied by the haematoxylin and eosin staining; while oxidative stress markers assayed in the prefrontal homogenate. Glucose oxidase method was used in measuring the blood glucose level.

**RESULT:** In contrast to the untreated diabetic group, the blood glucose concentration of the treated groups and control group showed was no significant difference ( $P>0.05$ ) at 28 days of treatment with *Mangifera indica*. Tissue Malondialdehyde and Superoxide Dismutase levels in the diabetic untreated group were statistically significant ( $P>0.05$ ) when compared with the control. Using the novel object recognition test, the untreated rats showed loss of short term memory. Histologically, there was improvement in the treated group compared to the untreated group.

**CONCLUSION:** *Mangifera indica* confers better protection against hyperglycaemia-induced oxidative stress and memory loss in brain of adult Wistar rats.

**KEYWORDS:** *Mangifera Indica*, Memory, Neurons, Alloxan, Diabetes

### INTRODUCTION

Diabetes Mellitus is a heterogeneous group of metabolic group disorder characterized by glucose intolerance and fasting hyperglycaemia<sup>1</sup>. It can also be defined as a disorder of carbohydrate metabolism resulting in high glucose level with other neurogenic and vascular complication affecting a variety of organs in the body<sup>2</sup>. Reactive oxygen species (ROS) production increases in diabetes owing to hyperglycemia which induces autoxidative glycosylation of cell membranes, destruction of the antioxidant systems, lipid peroxidation, and tissue injury<sup>3</sup>.

Although therapeutic drugs have long been used in treating diabetes mellitus in our continent and throughout the world, the mechanism of action of most of the drugs have not been

fully defined likewise their toxicity have not been fully known. Many botanical drugs are also being screened for their efficacy, safety and dosage in the management of diabetes mellitus. In developing countries, Medicinal plants play a central role in the diabetes mellitus management probably because resources are limited<sup>4</sup>. Management of diabetes by the infusion of insulin and the use of oral hypoglycemic drugs is not enough so patients are always exposed to long-term complications. Some herbs and spices have shown positive scientific results to be effective and non-toxic in the management of diabetes mellitus<sup>5</sup>.

*Mangifera indica* is a commonly used herb in ayurvedic medicine. Its potential as an antiseptic, anti-fungal, anthelmintic, tumor suppressant, anti-allergic, immunomodulation, hypolipidemic, hepatoprotective, gastroprotective have also been reported<sup>6</sup>.

Alzheimer's dementia and diabetes mellitus have both been linked with memory loss. This has made Neuroscientist to link Alzheimer's dementia to diabetes by naming it "type 3 diabetes" because insulin is also found in the brain<sup>7</sup>. Alzheimer's dementia have both type 1 and 2 diabetes as risk factor and recent work have reported that insulin has several functions in the brain including neurotrophic, metabolic and endocrine, being fully involved in memory and neuronal survival<sup>8,9</sup>. More work needs to be done to establish the regions of the brain that are affected in diabetes. The prefrontal cortex is involved in cognitive and memory functions.

This present work investigated if administration of *Mangifera indica* can ameliorate the effects of memory loss in the prefrontal cortex in an alloxan-induced diabetes Wistar rats using histological, behavioural and biochemical techniques.

1. Research Assistant
2. Assistant Lecturer

Department of Anatomy  
University of Ilorin, Nigeria

3. Assistant Lecturer  
Department of Anatomy  
University of Calabar, Nigeria

**Correspondence to:**

Balogun, Wasiu Gbolahan  
Department of Anatomy, PMB 1515, College of Health Sciences,  
University of Ilorin, Ilorin, Nigeria  
E-mail: ballonogodie@yahoo.com

## METHODOLOGY

The research work was carried out between 7th February and 3rd April of 2013, at the Department of Anatomy, University of Ilorin, Nigeria. Twenty-five Male Wistar rats with an average weight of 180g were bred in the animal holding of the department of Anatomy, university of Ilorin and sustained on rodent pellet from Bendel Feed (Ewu, Nigeria).

Throughout the experimental period, the environment had an equal light and dark hour daily with an average temperature of 24 °C while the rats were maintained by Institutional Animal Care and Use Committee (IACUC) standard.

**Preparation of Extract:** Fresh leaves of mango tree were plucked within the premises of the college of health sciences and authenticated at the department of Plant Biology of the University. The leaves were left in the dark under room temperature after washing carefully. After the leaves were dried, an electric machine was used to convert it to powder form. 100g of the powdered leaves were soften in 1Litre of distilled water for 24hours and then filtered to attain the water extract. The water extract was left to dry under vacuum using a rotary evaporator and the filtrate was stored at -20°C until used.

**Induction of Hyperglycemia:** 10 male Wistar rats were separated to be used for the diabetic control and extract control (Group A and E). They were neither fasted nor given alloxan. 15 male Wistar rats were fasted all night for 10 hours. Hyperglycemia was induced by injecting alloxan monohydrate (Sigma, USA) into each of the fasted rats at 150 mg/ Kg body weight intraperitoneally after dissolving the alloxan in normal saline.

The 10 male Wistar rats for control were administered the vehicle solution (normal saline) intraperitoneally. After 7 days of induction of alloxan, the glucose oxidase method was used to assay the blood glucose level of the treated group using an Accu Chek glucometer. Only animals whose level of blood glucose is higher than 300mg/dl were used for further treatment.

**Extract Administration:** *Mangifera indica* extract was orally given to the groups of hyperglycaemic rats at an hour between 7:00a.m – 9:00a.m for 28 days. Group A and B received only the vehicle (distilled water). Group C, D and E received 10mg/kg, 20mg/kg and 20mg/kg of *mangifera indica* respectively.

**Blood Glucose level Estimation:** In the control, untreated diabetic and *mangifera indica*-treated groups, weekly blood glucose level was checked using the glucose oxidase method.

### Novel object recognition test

The modified type of the Novel object recognition test<sup>10</sup> was used to access the short-term memory. The gadget consisted of a rectangular outline made of translucent plastic. The animal's behavior was observe and recorded for subsequent analysis by a video camera. Five days before the test, the rats were exposed to the translucent plastic in a test room for 10 min/session/d. On the 6th day of exposure which is also the day 28 of treatment, each rat was placed in the center of the translucent plastic and shown 2 matching objects for 5 min which is the trial phase before it was taken back to its cage. 1 hour retention interval was used, during which a novel object was used to replace 1 of the 2 objects. The novel object was placed at the same locations

as the earlier ones before the rats were allowed to explore them for 5 min which is the test phase. The exploration of an object by a rat means touching the object with its nose or forepaws at a distance of 2 cm. Revolving around the object without straight exploration was not accepted. Olfactory cues and dirt was avoided on the apparatus and objects during the trial period by cleaning with 20% ethanol. The Recognition index was calculated as follows:

Recognition index (%) =

$$\frac{\text{Time spent on new object}}{\text{Total time spent on rearing both old and new object}} \times 100$$

**Termination of Treatment:** After the last dose of the extract was given, 24 hours later the rats were anaesthetized with ether (Sigma, MO). Each rat head was removed and a brain forceps was used to remove the brain. The most anterior part of the brain immediately after the olfactory bulb was considered as the prefrontal cortex, this part of the brain was either fixed for histology or blended for biochemical assays in formol-calcium or ice-cold 0.2M sucrose solution (pH 7.4, 0.1 M) respectively.

**Biochemical and Histological Processing:** The blend from each rats were centrifuged at 500 xg for 10 minutes. Markers of oxidative stress and total protein were assayed in the supernatant. While Biuret Method was used to assay the total Protein<sup>12</sup>, the Ohkawa et al.<sup>13</sup> and Misra and Fridovich<sup>14</sup> methods were used to assay the tissue malondialdehyde (MDA) and superoxide dismutase (SOD) respectively. Furthermore, the prefrontal cortex which have been fixed in formol-calcium were dried out and implanted in paraffin. A rotary microtome was used to cut the embedded tissues into an eight micrometer thick sections before they were stained using the Hematoxylin and Eosin method, as described by Bancroft and Stephens<sup>15</sup>. MW1-HD2 digital microscope was used in getting the images.

**Statistical Analysis:** All data were expressed as mean ± SEM. The means were analyzed by one-way analysis of variance (ANOVA), followed by the Bonferroni post-hoc test. Statistical significance was set at P<0.05.

## RESULTS

**Blood Glucose Level:** The blood glucose responses in the experimental groups are shown in the table below. On the 28th day of treatment, there was no statistical difference in the blood glucose levels of all the treated groups when compared to the control except in the untreated diabetic rats, where there was a significant increase (P<0.05) in the blood glucose level.

Values represent mean ± SEM (mg/dl); \* represent P<0.05 significantly different from control group

**Biochemical observation:** There was no significant difference (P>0.05) between the activities of the MDA and SOD of the control and the *mangifera indica*-treated groups. The SOD level of the diabetes untreated group decreased but not statistically different from the control (P<0.05) (figure 1). While the MDA level in the untreated group increased significantly from the control (Fig-2)

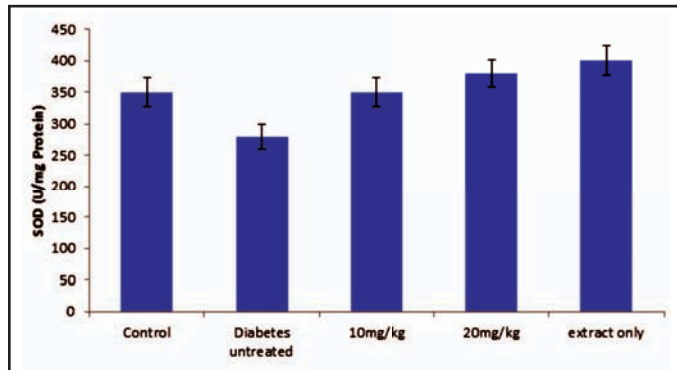
**Behavioral observation:** During the short term memory test, diabetes untreated rats did not show any liking toward the new

object. In opposite, mangifera indica-treated rats spent significantly more time exploring the novel object. The control

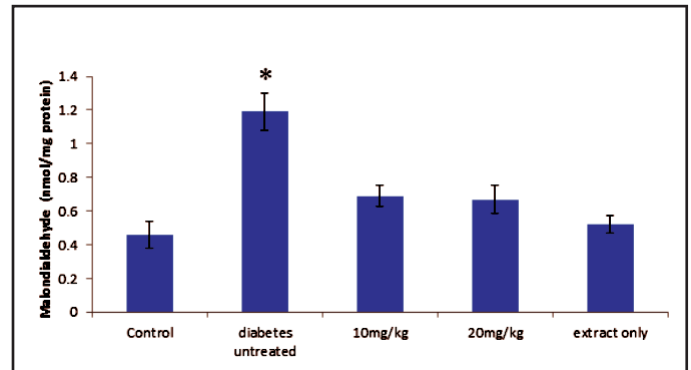
also spent a significant time exploring the novel object.

**TABLE-I: COMPARISON OF BLOOD GLUCOSE RESPONSES IN ALL GROUPS AT DIFFERENT INTERVALS OF INTERVENTION (n = 5)**

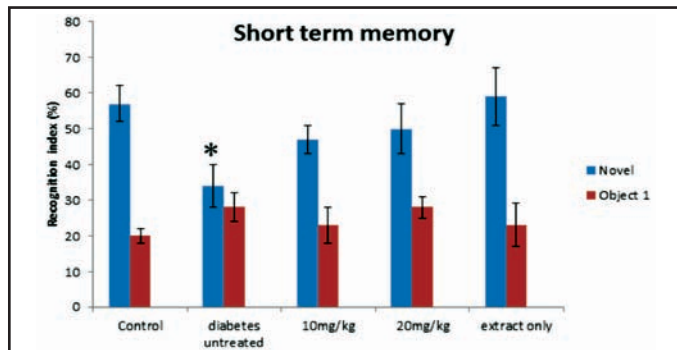
Group	Pre 1st Week	1st Week	2nd Week	3rd Week	4th Week
(A) Control	110±3	115±5	120±8	109±10	115±11
B (Diabetes untreated)	450±20*	470±23*	430±20*	410±19*	340±19*
C (10mg/kg )	390±17*	410±21*	350±20*	210±19*	130±16
D (20mg/kg)	400±16*	420±19*	340±20*	187±10*	125±6
E (Extract only)	110±6	115±7	102±6	98±4	106±4



**FIGURE 1: PREFRONTAL SUPEROXIDE DISMUTASE LEVELS. BAR REPRESENTS MEAN ± SEM \* REPRESENT P<0.05 (n = 5)**

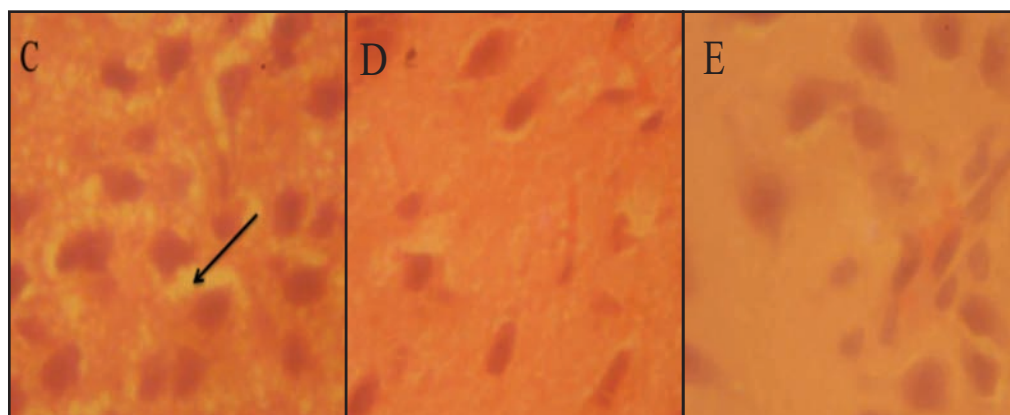
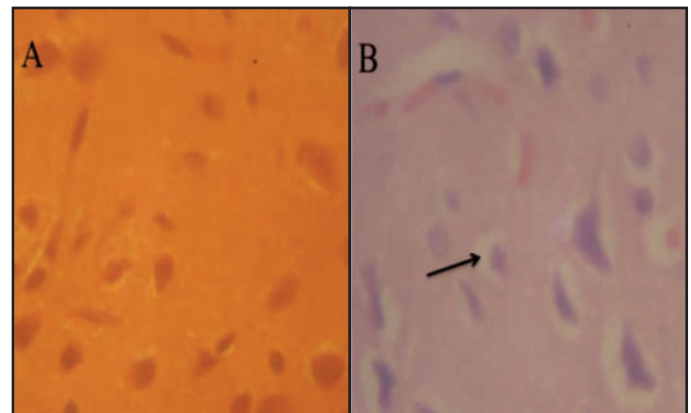


**FIGURE 2: PREFRONTAL MALONDIALDEHYDE LEVELS. BAR REPRESENTS MEAN ± SEM \* REPRESENT P<0.05 (n = 5)**



**FIGURE-3: SHORT TERM MEMORY USING NOVEL OBJECT RECOGNITION TEST PERFORMED ON THE RATS AT 28 DAYS OF TREATMENT (n = 3)**

**HISTOLOGICAL OBSERVATIONS**



(Fig-4: A-E:shows photomicrographs of the prefrontal cortex of rats in Group A to E and sacrificed after 28 days of treatment. Vacuolations is prominent in the untreated diabetic group

(black arrow), while well-stained nuclei of the neurons are seen in the control and mangifera indica-treated groups H&E x400).

## DISCUSSION

Both high and low glucose levels are threat for neurostructural and cognitive mutilation in diabetes mellitus. White and grey matter destruction of the brain in diabetic human and animals have been reported<sup>16</sup>. The sequential sequence of diabetes mellitus is connected with different levels of cognitive loss in human<sup>17</sup> and animals<sup>18</sup>. The morphological changes and mechanisms driving such diabetes-related neurocognitive mutilation are also becoming popular.

Type 2 diabetes patients are more prone to Vascular and Alzheimer's dementia<sup>19</sup>. It has been reported by Reaven et al.<sup>20</sup> that results from cognitive tests measuring learning, logic and compound psychomotor feat was contrarily proportional to glucose control in a small sample of people with type 2 diabetes, this is similar to the findings in the diabetic rat that show no preference toward the novel object unlike the mangifera indica treated rats that show preference toward the novel object. Alzheimer's dementia and diabetes mellitus has been suggested to share common abnormalities and disrupt common cellular and molecular pathways<sup>21</sup>. They also reported that Alzheimer's dementia potentiates the progression of diabetes mellitus and vice versa.

The level of the prefrontal antioxidant assayed was significantly different in the diabetes untreated rats compared to the control, this is in accordance with the findings of Erejuwa et al.<sup>22</sup> who reported that there is increased oxidative stress in diabetes; but with the treatment of the diabetes rats with mangifera indica, the level of superoxide dismutase was increased while the malondialdehyde level was decreased, the mechanism by which mangifera indica does this is still unclear but phytochemical analysis has reported the leaves of mangifera indica to contain viamag which showed a noteworthy inhibitory potential on the peroxidation of rat brain phospholipid and prohibited DNA loss caused by bleomycin or copper-phenanthroline systems<sup>23</sup>. Another reason for the antioxidant properties of mangifera indica could be due to the existence of mangiferin as its chief component<sup>24</sup>.

At the end of 28 days of treatment with aqueous extract of mangifera indica, normoglycaemia has been restored in the mangifera indica treated groups; this is similar to the finding of Aderibigbe et al.<sup>25</sup> and Morsi et al.<sup>26</sup>. It has been reported previously that mangifera indica mechanism of action is by increasing release of insulin from pancreatic beta cells thereby causing Intestinal reduction of absorption of glucose<sup>27</sup>.

In contrast to the findings in the alloxan-induced untreated diabetic rats, histological and biochemical studies from the mangifera indica treated groups suggest that chronic administration of mangifera indica to rats is not associated with neuronal injury. As shown above, neuronal microanatomy of mangifera indica-treated rats was comparable to the control. Besides, the anti-oxidant level of the mangifera indica-treated rats suggests that its oral administration to diabetic rats does not produce neurotoxicity.

## CONCLUSION

The result from the present experiment show that chronic

treatment with aqueous leaf extract of mangifera indica produces normoglycaemia in hyperglycemic rats, its possesses no adverse effects on neuronal histology and is not neurotoxic. Thus, alternative approach to the management of diabetes mellitus and Alzheimer's dementia in human may thus include the use of the leaf extract of mangifera indica as an anti-diabetic therapy pending further studies.

## REFERENCES

1. Kantarova D, Buc M, Stuchlikova M, Mokaň M, Vrlik M. Ethio-pathogenesis of autoimmune diabetes morphology of the pancreas in rats with experimental diabetes mellitus. *Bull Exp Biol Med* 2007; 143(3): 368-71.
2. Jamil M, Amin Z, Chaudhry TH, Shaheen J, Ali ZR. Management of diabetic foot infections. *J Coll. Phys & Surg. Pakistan* 2001; 11(10): 606-10.
3. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; 40(4): 405-12.
4. Bnouham M, Ziyat A, Mekhfi H, Tahri A, Legssyer A. "Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000)". *Int J Diabetes & Metabolism* 2006; 14: 1-25.
5. Okolie UV, Okeke CE, Oli JM, Ehiemere IO. Hypoglycaemic indices of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy humans. *Afr. Journal biotechnology*. 2008; 7(24): 4581-85.
6. Shah KA, Patel MB, Patel RJ, Parmar PK. *Mangifera Indica* (Mango). *Pharmacogn Rev*. 2010; 4(7): 42-8
7. Arab L, Sadeghi R, Walker DG, Lue LF, Sabbagh MN. Consequences of aberrant insulin regulation in the brain: Can treating diabetes be effective for Alzheimer's disease. *Curr. Neuropharmacol*. 2011; 9(4): 693-705.
8. Cardoso S, Correia S, Santos RX, Carvalho C, Santos MS, Oliveira CR et al. Insulin is a Two-Edged Knife on the Brain. *J. Alzheimers Dis.*, 2009, 18, 483-507.
9. Gerozissis K. Brain insulin, energy and glucose homeostasis; genes, environment and metabolic pathologies. *Eur. J. Pharmacol.*, 2008, 585, 38-49.
10. Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res*. 1988; 31:47-59.
11. Li W, Yu J, Liu Y, Huang X, Abumaria N, Zhu Y et al. Elevation of Brain Magnesium Prevents and Reverses Cognitive Deficits and Synaptic Loss in Alzheimer's Disease Mouse Model. *The J of Neuroscience*, 2013 33(19):8423-41
12. Weichselbaum WE. An accurate and rapid method for the determination of protein in small amounts of blood serum and plasma. *Am J Clin Path*. 1946; 10: 40-9.
13. Ohkawa H, Ohishi N Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979; 95 (2): 351-8.
14. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. 1972; 247 (10): 3170-5.
15. Bancroft J, Stevens A. *Theory and Practice of Histological Techniques*. 2nd Edn. New York, Churchill Livingstone. 1982; 131-5.

16. Akinola OB, Gabriel M, Suleiman A, Olorunsogbon F. Treatment of alloxan-induced diabetic rats with metformin or glitazones is associated with amelioration of hyperglycemia and neuroprotection. *The Open Diabetes Journal* 2012; 5: 8-12.
17. Arvanitakis Z, Bennett DA, Wilson RS, Barnes LL. Diabetes and cognitive system in older black and white persons. *Alzheimer Dis Assoc Disord* 2010; 24 (1): 37-42.
18. Kuhad A, Selthi R, Chopra K. Lycopene attenuates diabetes associated cognitive decline in rats. *Life Sci* 2008; 83 (3): 128-34
19. Ott A, Stolk RP, Hofman A, van Harskamp F, Grobbee DE, Breteler MM. Association of diabetes mellitus and dementia: the Rotterdam Study. *Diabetologia* 1996; 39:1392-97
20. Reaven GM, Thompson LW, Nahum D, Haskins E. Relationship between hyperglycaemia and cognitive function in older NIDDM patients. *Diabetes Care* 1990; 13:16-21
21. Sadeeq AA, Ibegbu AO, Rilwan HA, El-Ladan IS, Adamu LH. Diabetes of the brain. Abstracts, society of experimental and clinical anatomist of Nigeria; university of Ilorin, Nigeria, 2013 p. 23
22. Erejuwa OO, Gurtu S, Sulaiman SA, Ab Wahab MS, Sirajudeen, KNS, Salzihan MS. Hypoglycaemic and antioxidant effects of honey supplementation in streptozotocin-induced diabetic rats. *Int J Vitam Nutr Res* 2010; 80:74-82.
23. Martinez G, Delgado R, Perez G, Garrido G, Nunez Selles AJ, Leon OS. Evaluation of the in-vitro antioxidant activity of *Mangifera indica* L: Extract (Vimang) *Phytother Res.* 2000; 14:424-7
24. Gabino G, Deyarina G, Cheyla R, Nunez-Selles AJ, Rene D. Scavenger effect of a mango (*Mangifera indica* L.) food supplement's active ingredient on free radicals produced by human polymorphonuclear cells and hypoxanthine-xanthine oxidase chemiluminescence systems. *Food Chem.* 2008; 107:1008-14
25. Aderibigbe AO, Emudianughe TS, Lawal BA. Anti-hyperglycaemic effect of *Mangifera indica* in rat. *Phytother Res.* 1999; 13:504-7
26. Morsi RMY, EL-Tahan NR, El-Hadad AMA. Effect of Aqueous Extract *Mangifera Indica* Leaves as Functional Foods. *J. Applied Sci Res.* 2010; 6(6): 712-721
27. Akbar W, Miana GA, Ahmad SI. Clinical investigation of hypoglycaemic effect of leaves of *Mangifera indica* in type-2 (NIDDM) diabetes mellitus. *Pakistan J of Pharm* 2006; 23(2):13-18