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The Effect of Okra (*Abelmoschus esculentus*) on the Hepatic Metabolism of Carbohydrates and Lipids

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ABSTRACT

Background and Objective: Okra (*Abelmoschus esculentus*) is one of the most popular and commonly used members of the *Malvaceae* family. The study investigated the phytochemical content of okra and its attendant metabolic role on the liver in maintaining the optimal concentrations of glucose and lipids in the body. **Materials and Methods:** Thirty albino rats divided into 5 groups (A, B, C, D and E) constituted the sample size as validated by mead's equation. The control group was given sterile water, whereas okra extract of varying concentrations was given to the remaining four treatment groups. The administration was repeated daily for 4 weeks followed by anesthesia and then blood collection. **Results:** Data analysis was performed on SPSS version 21 software by using one-way ANOVA (*post hoc*-LSD) statistical tool. The biochemical parameters analysed included liver chemistries, lipid profile, glucose and glycated haemoglobin using, WHO approved methods. The study revealed a significant decrease in concentrations of Aspartate Aminotransferase (AST), albumin and triacylglycerol, whereas other parameters were stable. **Conclusion:** Based on the results above, it could be concluded that *A. esculentus* enhances the metabolic role of the liver in maintaining the optimal concentrations of lipids and glucose in the body.

KEYWORDS

Abelmoschus esculentus, lipids, liver enzymes, glucose, hepatic metabolism, glycated haemoglobin

INTRODUCTION

Okra (*Abelmoschus esculentus*) is one of the classical examples of the plant used not only as food but also for its unique therapeutic significance owing to its several beneficial effects like hypoglycaemic, hypocholesterolemic, antioxidant, antimicrobial, anti-inflammatory, anti-constipation, anti-cancer activities and host of other potentials[1]. It is in demand all over the world as a vegetable for its health and nutritional benefits[2]. Okra fruits are readily available and affordable in Nigerian communities and are a good source of various vitamins and minerals in addition to their high dietary fiber content[3].

The liver is a critical organ in the human body that is responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, vitamin storage and the synthesis of proteins and biochemicals necessary for digestion and growth among other functions[4]. It is also a prerequisite organ for the metabolism of lipids and carbohydrates. Glycogenesis, gluconeogenesis and glycogenolysis occur in the liver and are the basic reactions that optimally keep lipid and glucose balance.

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Metabolic processes in the body revolve around three major molecules which are lipids, carbohydrates and proteins. These molecules dictate the physiological and pathological state of humans and animals. Imbalances and balances of these molecules are regulated by an interplay of *in vivo* reactions in the liver. A huge deal of research has been conducted on the nutritional and toxicological status of okra as it affects humans and animals[5-9]. These have not taken cognizance of the molecular interplays in the liver as it affects the consumption of okra. A compromised liver has the preponderances of distorting the metabolism of lipids and carbohydrates resulting in its respective associated dysfunctions. This is presented as either excess or deficit concentrations of the lipids or glucose in the blood.

Parameters such as Aspartate Aminotransferases (AST), Alanine Aminotransferases (ALT), Alkaline Phosphatase (ALP), total proteins, albumin, globulins, total cholesterol (CT), Triacylglycerol (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), glucose, Glycated Haemogolobin (HbA1c) were estimated to critically profile the metabolism of carbohydrates and lipids in the liver. Therefore, the study was designed to interrogate the status of the liver as it affects lipid and carbohydrate metabolism upon the consumption of okra.

MATERIALS AND METHODS

Study area: The study was conducted at Otuoke in Ogbia Local Government Area of Bayelsa State and the okra was bought from Otuoke market. Similarly, the animal breeding and intoxication took place at the Biochemistry Laboratory of the Federal University Otuoke, Bayelsa State, whereas the biochemical analysis was carried out at Eni-Yimini Laboratory LTD, Yenezue-Gene Epie, Bayelsa State. The study took three months to complete (April-July, 2022). The presentation of the map of the study locations is sourced from the study of Udom *et al.*[10] as shown in Fig. 1.

Mead's resource equation was employed for the calculation of the sample size[11]. A total of 30 albino rats divided equally into 5 groups inclusive of controls were used for the study. The rats were acclimatized for two weeks and subsequently distributed randomly into 5 treatment groups of 6 rats each while the

Fig 1: Map of Bayelsa State showing Ogbia
Source: Udom *et al.*[10]

experiment lasted for 4 weeks. The groups were housed separately in different cages. Groups 2-5 were given different mL of the okra extract orally for four weeks, whereas the control group was deprived of the okra extract.

Experimental animals: The albino rats were obtained from the central animal house unit of the University of Port-Harcourt, Rivers State, Nigeria. The rats were allowed to acclimatize for two weeks with access to diet (grower pellet feed) and treated water. Exception of the control group, other were administered okra extract. The rats were housed in plastic cages under conditions at room temperature.

Collection and identification of plant material: The Okra (*Abelmoschus esculentus*) was bought from a local market in Otuoke, Ogbia Local Government Area, Bayelsa State. The crop was identified and authenticated at the Department of Biology, Federal University, Otuoke, Bayelsa.

Extraction method: The methodology used for the extraction of the okra extract was derived from Wu *et al*.[12] procedure. The fresh and mature okra pods were cut into a thickness of 2-3 cm and then sun-dried to obtain dried okra having a moisture content of 13%. One killogram of dried okra was added to 25 kg of distilled water. The okra mixture was heated to 100°C and then held for 2 hrs in the induction cooker (Phiima 2 Burner Induction Hob-Deluxe Nigeria). The extract was coarsely filtered with 2 layers of gauze and centrifuged at 3500 rpm for 10 min with a centrifuge (Africa Nigeria Centrifuge C-802 b and Wincom Company Limited). The supernatant was combined and concentrated to 1/3 volume by a rotary evaporator (Rotary Evaporator|RV131-4E's United States of America) at 60°C, 30 rpm. The concentrated liquid was freeze-dried by a freeze-dryer (Manifold Freeze Dryer LZ-MFD-A110-Nigeria).

Experimental design: Rats were randomly divided into 5 groups (A, B, C, D and E) after the weeks of acclimatization. Group A was considered control and was given sterile water (n = 6). Group B was administered 0.4 mL of okra extract (n = 6). Group C was administered 0.8 mL of okra extract (n = 6). Group D was administered 1.0 mL of okra extract (n = 6) and group E was administered 1.5 mL of okra extract (n = 6). The administration was repeated for four weeks. At the end of the experiment, the rats were given anaesthesia. The blood samples were collected into ethylenediaminetetraacetic acid (EDTA) and plain containers for the biochemical analysis. Below is a tabulated presentation of the experimental design and the treatment regimes of the various study groups (Table 1).

Selection criteria: The selection criteria were derived from a study by Zebedee *et al*.[13]. Rats used were healthy and active as confirmed and approved by the university veterinarian. Rats showing signs or symptoms of illness were excluded. The research utilized only male albino rats of the same age and weight. The age range was between six to eight months. The weight brackets were 1.0-1.2 kg.

Ethical approval: The study design and ethical compliance documents were approved by the Directorate of Research and Quality Assurance of the Federal University Otuoke, Bayelsa State, Nigeria. The ethical protocol followed that of the internationally recognized procedures of the Institutional Animal Care and Use Committee (IACUC) for handling the rats.

Table 1: Experimental design and administration of treatment

Group	Number of animals	Administration
A (control)	6	Distilled water
B	6	0.4 mL of okra extract
C	6	0.8 mL of okra extract
D	6	1.0 mL of okra extract
E	6	1.5 mL of okra extract

Laboratory methods: Serum total protein and albumin concentrations were estimated using biuret and bromocresol green (BCG) methods respectively. Randox Laboratories (United Kingdom) test kits were the choice reagents for total protein, albumin and glucose estimations. A mathematical formula used by Solomon and Martin[14] in the estimation of serum globulin concentration was employed. ELITech Clinical Systems kits were used for the assay of serum Aspartate Aminotransaminase (AST) and alanine aminotransferase activities. Agappe Diagnostics (Switzerland) kits were used for the estimation of serum total cholesterol, triacylglycerol and HDL concentrations. Plasma LDL concentration was obtained mathematically[15]. The glucose oxidase method was used for the estimation of plasma glucose concentration. Glycated Haemoglobin (HbA1c) was analyzed using a clover test kit (Codix Pharma-United Kingdom). The presence of alkaloids, saponins and flavonoids and tannins was estimated using the method described by Harborne[16].

Statistical analysis: Data obtained from the test carried out were analyzed using One-way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 21. The level of significance was considered at $p<0.05$.

RESULTS

The experimental design and the concentrations of okra extract administered to the various groups was shown in Table 1. The Table 2 represented the quantitative presentation of the phytochemical contents in *Abelmoschus esculentus*. This result revealed the presence of alkaloids, tannin, flavonoids and saponins. The percentage composition of alkaloids was (0.09%), tannins (0.154%), flavonoids (3.5%) and saponins (0.98%). The Table 3 showed the comparison of the mean concentration and standard deviation of the hepatic biochemical parameters. The result showed that Aspartate Aminotransaminase (AST) and albumin decreased significantly ($p<0.05$) across the groups thereby making them significant, whereas Alanine Aminotransferase (ALT), total protein (TP) and globulin were not significant ($p>0.05$). The results in Table 4 revealed that the mean plasma triglyceride TG was significantly reduced ($p<0.05$) on treatment

Table 2: A quantitative presentation of phytochemical content in *Abelmoschus esculentus*

Phytochemicals	Percentage
Alkaloids	0.09
Tannins	0.154
Flavonoids	3.5
Saponins	0.98

Table 3: A comparison of the mean concentration and activities of liver markers upon exposure to *Abelmoschus esculentus* extract

Liver parameter	Group A (control) n = 6	Group B n = 6	Group C n = 6	Group D n = 6	Group E n = 6	F-test	p-value
AST (U L^{-1})	19.54±9.07	1.86±1.23[a]	6.20±5.08[ab]	4.31±2.63[ab]	5.13±8.89[ab]	4.731	0.021
ALT (U L^{-1})	9.65±5.84	5.04±7.14	5.20±7.74	6.03±5.74	2.40±4.14	1.125	0.143
TP (g L^{-1})	79.77±16.09	86.67±9.61	87.05±24.39	82.13±5.09	76.41±0.98	0.282	0.882
ALB (g L^{-1})	34.18±1.00[a]	29.45±3.27[a]	30.15±1.18[a]	24.83±4.64[a]	30.50±0.39[a]	3.906	0.048
GLO (g L^{-1})	45.59±16.87	57.22±11.92	56.89±23.21	57.29±1.35	45.91±0.58	0.578	0.687

AST: Aspartate aminotransaminase, ALT: Alanine aminotransaminase, TP: Total protein, ALB: Albumin, GLO: Globulin, a: $p<0.05$ vs group B, C and D, b: $p<0.05$ vs group C and D, c: $p<0.05$ vs group D, data are expressed as Mean±SD and significant at 0.05 confidence interval

Table 4: A comparison of the mean concentration and activities of lipid parameters upon exposure to *Abelmoschus esculentus* extract

Parameter	Group A (control)	Group B	Group C	Group D	Group E	F-value	p-value
CHO (mmol L^{-1})	3.38±0.34	3.02±0.44	3.83±0.76	3.26±0.43	2.91±0.38	1.400	0.309
TG (mmol L^{-1})	2.05±0.22	1.10±0.09	1.86±0.34	1.81±0.32	1.93±0.10	8.522	0.004
HDL (mmol L^{-1})	0.79±0.76	0.91±0.45	1.04±0.35	0.47±0.28	0.05±0.07	1.693	0.235
LDL (mmol L^{-1})	2.11±0.69	1.61±0.13	1.95±0.36	1.95±0.10	1.98±0.35	0.663	0.633

CHO: Total cholesterol, TG: Triacylglycerol, HDL: High density lipoprotein, LDL: Low density lipoprotein, data are expressed as Mean±SD and significant at 0.05 confidence interval

Table 5: A comparison of the mean concentration and activities of some glycaemic parameters upon exposure to *Abelmoschus esculentus* extract

Parameter	Group A	Group B	Group C	Group D	Group E	F-value	p-value
Glucose (mmol L^{-1})	2.442±1.439	4.971±0.566	4.739±1.395	4.099±2.054	6.531±2.633	2.126	0.152
HbA1c (%)	7.000±0.600	9.600±1.709	3.600±0.529[ab]	3.100±0.265[ab]	3.300±1.127[ab]	25.338	0.000

HbA1c: Glycated haemoglobin, a: $p<0.05$ vs group B, C and D, b: $p<0.05$ vs group C and D, c: $p<0.05$ vs group D, data are expressed as Mean±SD and significant at 0.05 confidence interval

with an aqueous extract of *Abelmoschus esculentus* compared with the control group not induced. The Table 5 showed the ANOVA average concentration and standard deviation of glucose concentration and glycated hemoglobin, in the various test groups. A significant ($p<0.05$) decrease in HbA1c was observed in the test groups C, D and E when compared to the control group.

DISCUSSION

The study revealed the presence of phytochemicals; alkaloids, flavonoids, tannins and saponins in *Abelmoschus esculentus* (Table 2). This study shared similarities in findings with other studies on the rich content of phytochemicals in okra[17,18]. The nutritional and medicinal importance of okra is attributable to its rich content of phytochemicals. Okra's flavonoid content can reduce the activity of the nuclear receptor transcription factor PPARγ, a key regulator of lipid and glucose homeostasis[19]. Flavonoids such as isoquercitin and quercitin are known to play an important role in the reduction of blood glucose and triglyceride by improving glucose tolerance and increasing lipoprotein lipase activities[20-22].

Furthermore, the study also revealed a decrease in serum Aspartate Aminotransferase (AST) activity and albumin concentration, whereas other parameters were stable (Table 3). The presentation of the laboratory data as per the liver function test is indicative of the nutritional and medicinal values of okra extract to the efficient functioning of the liver. The result of the analysis agreed with the work of Wahyuningsih *et al.*[9] on the value of okra to health. Other studies have also posited the medical importance of okra[20,23-25].

However, the analytical comparison of lipid parameters revealed a significant decrease in the concentration of plasma TG, whereas other lipid parameters were stable (Table 4). This implied that *Abelmoschos esculentus* aqueous extract possesses an anti-hyperlipidemic activities. This study concurs with the findings of Elkhalif *et al.*[26]. The inherent capacity of okra on reducing the plasma concentration of TG could be attributed to the rich content of some phytochemicals. Okra is rich in flavonoid compounds (isoquercitin and quercitin) and can reduce triglyceride levels by increasing lipoprotein lipase LPL activity[18,20,21]. Similarly, the anti-oxidant activity can limit lipid peroxidation which is vital to curbing a lot of disease conditions[27].

This study revealed a significant decrease in the concentration of HbA1C, whereas glucose concentration was not affected (Table 5). The finding on the non-significance difference in glucose concentration agreed with that of Fan *et al.*[19] and Anjani *et al.*[20]. This result, to some extent, corresponds with the findings of Moradi *et al.*[28]. The non-enzymatic binding of circulating glucose to hemoglobin results in the formation of glycosylated hemoglobin. Higher blood glucose levels cause greater binding, which raises the amount of glycosylated hemoglobin[29]. HbA1c is a better measure of the average plasma glucose level than FPG[30]. This is indicative of okra as a good anti-hyperglycemic extract. An increase in HbA1c concentration has been shown to correlate with aging[31]. Hence, the decrease in the concentration of HbA1c could support the suggestion that okra contains an anti-aging agent.

Summarily, the study has clearly shown that the liver is the center for glycogenolysis and lipolysis is not in any way compromised by the consumption of okra. Rather, okra enhanced the functionality of the liver by ensuring that glucose and lipids are metabolized efficiently and their optimal concentrations are maintained.

CONCLUSION

The study revealed a significant decrease in some hepatic liver parameters and triacylglycerol. Other parameters were stable. This connotes that okra does not have deleterious effects on the liver, but rather enhances the metabolic role by maintaining the concentrations of glucose and lipids in the body.

SIGNIFICANCE STATEMENT

This study discovers the positive effect of okra on carbohydrate and lipid metabolism in the liver. This study will help researchers and nutritionists with the choice of diet compositions in managing diseases associated with the liver and ancillary organs. Similarly, biochemists can probe further into the molecular basis of the positive interactions of okra and the liver as it affects lipid and carbohydrate metabolism.

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REFERENCES

1. Viuda-Martos, M., A.E.N.G.S. El Gendy, E. Sendra, J. Fernandez-Lopez, K.A. Abd El Razik, E.A. Omer and J.A. Perez-Alvarez, 2010. Chemical composition and antioxidant and anti-*Listeria* activities of essential oils obtained from some Egyptian plants. J. Agric. Food Chem., 58: 9063-9070.
2. Pem, D. and R. Jeewon, 2015. Fruit and vegetable intake: Benefits and progress of nutrition education interventions-narrative review article. Iran. J. Public Health, 44: 1309-1321.
3. Gemede, H.F., N. Ratta, G.D. Haki, A.Z. Woldegiorgis and F. Beyene, 2015. Nutritional quality and health benefits of "Okra" (*Abelmoschus esculentus*): A review. Int. J. Nutr. Food Sci., 4: 208-215.
4. Trefts, E., M. Gannon and D.H. Wasserman, 2017. The liver. Curr. Biol., 27: R1147-R1151.
5. Sereno, A.B., C.D. Pinto, F.A. Andrade, M.A.B. da Silva and A.C. Garcia, C.C.H. Krüger and I.J.de Messias Reason, 2022. Effects of okra (*Abelmoschus esculentus* (L.) Moench) on glycemic markers in animal models of diabetes: A systematic review. J. Ethnopharmacol., Vol. 298. 10.1016/j.jep.2022.115544.
6. Adelakun, O.E., O.J. Oyelade, B.I. Ade-Omowaye, I.A. Adeyemi and M. van de Venter, 2009. Chemical composition and the antioxidative properties of Nigerian okra seed (*Abelmoschus esculentus Moench*) flour. Food Chem. Toxicol., 47: 1123-1126.
7. Fauza, A., A.N. Al-Baarri and K. Djamiatun, 2019. Potency of okra flour (*Abelmoschus esculentus*) in improving adiponectin level and total antioxidant capacity of high fat diet streptozotocin rat model. Potravinarstvo Slovak J. Food Sci., 13: 644-650.
8. Caluête, M.E.E., L.M.P. de Souza, E. dos Santos Ferreira, A.P. de França, C.A. de Almeida Gadelha, J. de Souza Aquino and T. Santi-Gadelha, 2015. Nutritional, antinutritional and phytochemical status of okra leaves (*Abelmoschus esculentus*) subjected to different processes. Afr. J. Biotechnol., 14: 683-687.
9. Wahyuningsih, S.P.A., A.A. Mwendolwa, D. Winarni, R.W. Anggreini and B.K.K. Mamuaya, 2021. Protective effect of red okra (*Abelmoschus esculentus* (L.) Moench) pods against sodium nitrite-induced liver injury in mice. Vet. Med. Int., Vol. 2021. 10.1155/2021/6647800.
10. Udom, G.J., H.O. Nwankwoala and T.E. Daniel, 2018. Physico-chemical evaluation of groundwater in Ogbia, Bayelsa State, Nigeria. Int. J. Weather Clim. Change Conserv. Res., 4: 19-32.
11. Hubrecht, R. and J.K. Kirkwood, 2010. The UFAW Handbook on the Care and Management of Laboratory and other Research Animals. 8th Edn., John Wiley and Sons Inc., Hoboken, New Jersey, ISBN-13: 9781405175234, Pages: 837.
12. Wu, L., M. Weng, H. Zheng, P. Lai, B. Tang, J. Chen and Y. Li, 2020. Hypoglycemic effect of okra aqueous extract on streptozotocin-induced diabetic rats. Food Sci. Technol., 40: 972-978.

13. Zebedee, L.U., O.N. Jeremiah, A.E. Soroh and E.Y.S. Agoro, 2020. Haematological presentations in acute and chronic tramadol intoxication. Toxicol. Forensic Med. Open J., 5: 26-36.
14. Solomon, A.E.Y. and W.M. Martin, 2018. The effects of chronic carbon monoxide intoxication on some liver biochemical parameters in rabbits. J. Indian Soc. Toxicol., 14: 12-16.
15. Agoro, E.S., G.C. Chinyere and E.I. Akubugwo, 2020. Emerging concept of vitreous concentrations of proteins and lipids as discriminant of fresh water drowning death. J. Forensic Legal Med., Vol. 73. 10.1016/j.jflm.2020.101994.
16. Harborne, J.B., 1973. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hall, London, UK, ISBN: 9780412105401, Pages: 278.
17. Saha, D., B. Jain and V.K. Jain, 2011. Phytochemical evaluation and characterization of hypoglycemic activity of various extracts of *Abelmoschus esculentus* Linn. fruit. Int. J. Pharm. Pharm. Sci., 3: 183-185.
18. Khomsug, P., W. Thongjaroenbuangam, N. Pakdeenarong, M. Suttajit and P. Chantiratikul, 2010. Antioxidative activities and phenolic content of extracts from okra (*Abelmoschus esculentus* L.). Res. J. Biol. Sci., 5: 310-313.
19. Fan, S., Y. Zhang, Q. Sun, L. Yu and M. Li *et al*., 2014. Extract of okra lowers blood glucose and serum lipids in high-fat diet-induced obese C57BL/6 mice. J. Nutr. Biochem., 25: 702-709.
20. Anjani, P.P., E. Damayanthi, Rimbawan and E. Handharyani, 2018. Antidiabetic potential of purple okra (*Abelmoschus esculentus* L.) extract in streptozotocin-induced diabetic rats. IOP Conf. Ser.: Earth Environ. Sci., Vol. 196. 10.1088/1755-1315/196/1/012038.
21. Herowati, R., L. Puradewa, J. Herdianty and G.P. Widodo, 2020. Antidiabetic activity of okra fruit (*Abelmoschus esculentus* (L) Moench) extract and fractions in two conditions of diabetic rats. Indonesian J. Pharm., 31: 27-34.
22. Zim, A.F.M.I.U., J. Khatun, M.F. Khan, M.A. Hossain and M.M. Haque, 2021. Evaluation of *in vitro* antioxidant activity of okra mucilage and its antidiabetic and antihyperlipidemic effect in alloxan-induced diabetic mice. Food Sci. Nutr., 9: 6854-6865.
23. Irshad, M., B. Debnath, S. Mitra, Y. Arafat, M. Li, Y. Sun and D. Qiu, 2018. Accumulation of anthocyanin in callus cultures of red-pod okra [*Abelmoschus esculentus* (L.) Hongjiao] in response to light and nitrogen levels. Plant Cell Tissue Organ Cult., 134: 29-39.
24. Alqasoumi, S.I., 2012. 'Okra' *Hibiscus esculentus* L.: A study of its hepatoprotective activity. Saudi Pharm. J., 20: 135-141.
25. Hu, L., W. Yu, Y. Li, N. Prasad and Z. Tang, 2014. Antioxidant activity of extract and its major constituents from okra seed on rat hepatocytes injured by carbon tetrachloride. BioMed Res. Int., Vol. 2014. 10.1155/2014/341291.
26. Elkhalifa, A.E.O., E. Alshammari, M. Adnan, J.C. Alcantara and A.M. Awadelkareem *et al*., 2021. Okra (*Abelmoschus esculentus*) as a potential dietary medicine with nutraceutical importance for sustainable health applications. Molecules, Vol. 26. 10.3390/molecules26030696.
27. Agoro, E.S., E.I. Akubugwo, G.C. Chinyere, I.E. Nwachuku and V.N. Agi, 2017. Vitreous humour lipid peroxidation as an emerging concept of acute carbon monoxide poisoning. Int. J. Forensic Sci. Pathol., 5: 392-399.
28. Moradi, A., M.J. Tarrahi, S. Ghasempour, M. Shafiepour, C.C.T. Clark and S.M. Safavi, 2020. The effect of okra (*Abelmoschus esculentus*) on lipid profiles and glycemic indices in Type 2 diabetic adults: Randomized double blinded trials. Phytother. Res., 34: 3325-3332.
29. Rohlfing, C.L., R.R. Little, H.M. Wiedmeyer, J.D. England and R. Madsen *et al*., 2000. Use of GHb (HbA_{1c}) in screening for undiagnosed diabetes in the US population. Diabetes Care, 23: 187-191.
30. Karnchanasorn, R., J. Huang, H.Y. Ou, W. Feng, L.M. Chuang, K.C. Chiu and R. Samoa, 2016. Comparison of the current diagnostic criterion of HbA1c with fasting and 2-hour plasma glucose concentration. J. Diabetes Res., Vol. 2016. 10.1155/2016/6195494.
31. Dubowitz, N., W. Xue, Q. Long, J.G. Ownby and D.E. Olson *et al*., 2014. Aging is associated with increased HbA_{1c} levels, independently of glucose levels and insulin resistance, and also with decreased HbA_{1c} diagnostic specificity. Diabetic Med., 31: 927-935.