

Original Research Article

MODULATORY ROLES OF CLOVE AND FERMENTED GINGER SUPPLEMENTS ON LIPID PROFILE AND THYROID FUNCTIONS IN HIGH FAT DIET INDUCED INSULIN RESISTANCE RABBITS

ABSTRACT

Aims: To evaluate the effects of clove buds and fermented ginger rhizome supplements on Lipid profile and thyroid functions in high fat diet induced hyperglycemic rabbits.

Study design: High fat diet was fed to rabbits for eleven weeks to ascertain diabetic animal model (DAM), thereafter, DAM were treated with supplements for six weeks.

Place and duration of study: Department of Human Physiology, Ahmadu Bello University Zaria, Kaduna Nigeria. Between May, 2015 to October, 2015.

Methodology: Thirty (30) male rabbits (8-10 weeks of age) divided into six groups of (n=5) were used, Group I (Normal control) was treated with standard animal feed (SAF) throughout the experimental period, Group II-VI were treated with high fat diet (SAF = 69% + Cholesterol = 1% + Ground nut meal = 20% + ground nut oil = 10%) for 11 weeks to induced hyperglycemia and ascertain DAM for the study. Thereafter, DAM groups (Group II-VI) were treated for six weeks of experimental protocol; Group II was treated with SAF only, Group III = DAM treated on SAF + cholestran (0.26 g/kg), Group IV = DAM treated on SAF + clove buds (12.5%) supplements, Group V = DAM treated on SAF + fermented ginger (12.5%) supplements, and Group VI = DAM treated on SAF + clove buds (12.5%) + fermented ginger (12.5%). After treatment, animals were sacrifice and sera from blood samples were used for laboratory assessments of lipid profile (total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c)), triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) using standard procedures, data obtained were statistically analyzed.

Results: A significantly ($P=0.5$) decrease in TC level in DAM group treated on fermented ginger (12.5%) when compared to DAM treated on SAF. T_3 and T_4 levels significantly decrease in all supplement treated groups compared to DAM treated on SAF group.

Conclusion: Clove (12.5%), fermented ginger (12.5%) and clove (12.5%) + fermented ginger (12.5%) supplements reveal a pronounced ameliorative effects on altered physiological parameters in rabbits fed on high fat diet. Hence, further work may validate for reliability and medical use.

Keywords: clove and fermented ginger supplements, lipid profile, thyroid hormones.

Introduction

High Fat Diet (HFD) is a nutritional condition that accounts for the largest incidence of metabolic syndrome in the world [1]. Defective insulin secretion leads to various metabolic aberrations in T2DM. The impairments include hyperglycaemia due to insulin-stimulated glucose uptake, up-regulated hepatic glucose production and dyslipidaemia[2]. The excessive consumption of high fat diet has been associated with an increased incidence of enhanced formation of oxidative stress, consequently, leads to high levels of circulating free fatty acids (FFA) and glucose are potent inducers of reactive oxygen species (ROS) in cells [3]. Lipotoxicity impairs cell function and viability due to chronic exposure to FFA, leading to the induction of β -cell endoplasmic reticulum stress, and glucose-induced β -cell dysfunction and apoptosis [4].

Hyperthyroidism have been proved to worsening of metabolic disorder such as diabetes. It was shown that surgical removal of parts of thyroid gland had an ameliorative effect on the restoration of glucose tolerance in hyperthyroid patients, suffering from co-existing diabetes [5]. There is a deep underlying relation between diabetes mellitus and thyroid dysfunction [6]. Thyroid hormones directly control insulin secretion. Hypothyroidism, result into a reduction in glucose-induced insulin secretion by beta cells, and the response of beta cells to glucose or catecholamine is increased in hyperthyroidism due to increase beta cells mass. Moreover, insulin clearance is increased in thyrotoxicosis [7]. Thyroid hormones are insulin antagonists, both insulin and thyroid hormones are involved in cellular metabolism. Excess or deficit of the hormones can result in functional derangement of the other[8]. In hypothyroidism, glucose-induced insulin secretion by the β -cell is reduced while in hyperthyroidism, β -cell response to glucose or catecholamine stimulation appears to be increased and is accompanied by an increased β -cell mass[9,10]. The rates of glucose oxidation and glycogen synthesis are decreased in hypothyroidism due to down-regulation of glucose transporter GLUT5 in humans and impaired GLUT4 transporter in animal

model[11]. Hyperthyroidism is associated with increased GLUT2 expression, as compared to the hypothyroid state [12]. Alterations in lipid metabolism further link thyroid hormone to insulin resistance[13].Thyroid hormones-dependent fatty acid uptake is tissue specific and may be increased in both thyrotoxicosis and hypothyroidism [14]. Thyroid hormone stimulates catecholamine action, which in turn increase lipolysis in adipose tissue, hence increasing circulating FFas[15]. The excessive consumption of high fat diet has been associated with an increased incidence of diabetes mellitus. They are enhanced by formation of oxidative stress form as a result of accumulation and adipose tissue expansion and consequently resulted into high levels of glucose which are potent inducers of cellular reactive oxygen species (ROS) [16, 17,18]. Diabetes mellitus is considered as a common, growing, serious and costly, but potentially preventable public health problem[19]. Continuous consumption of calories-rich meals, junk food and sedentary lifestyle has culminated into an epidemic of diabetes worldwide.The existing management of diabetes is indeed costly and associated with many side effects ranging from flatulence, constipation and hypoglycemia, hence, the need for an effective approach with fewer or non-side effects is needed for diabetes mellitus. *Syzygiumaromaticum* (clove) and *Zingiberofficinale* (ginger) bud are important spices used in the preparation of several delicacies and in folklore for diabetes management (Adefeghaet *al.*, 2014), the effects of their extracts has been studied in animal model with paucity in the results. This study is aimed to determine the effect of clove and fermented ginger supplements on some physiological parameters in high fat diet induced hyperglycemia in rabbits.

MATERIALS AND METHODS:

Materials

Digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany). Glucose- test strips for assessment of plasma glucose levels, manufacture by accu-check Advantage II, Roche Diagnostics GmbH Germany.Animal feed (pelletised growers feed, of vital feed, km 17, Zawan Roundabout P.O. box 13462, jos, Plateu state Nigeria.), (Crude protein 13%, fat 8%, crude fibre 15 %, calcium 0.9%,). Clove buds and fresh Ginger rhizome, Dissecting kits, Digital weighing scale, Test tubes/rack, syringe

and needle, Microplate reader RT-2100C, Rayto, 800D Table Toplow Speed centrifuge, and Micropipette automated and graded

Drugs and Reagents

All chemicals used were of analytical grades; these included:

- i. Cholesterol (powder) was purchased from KEM LIGHT Laboratory PVT. LTD, Mumbai India. (CAS: 57-88-5, m.w: 386.67, lot no.: 100814).
- ii. Cholestran powder (made in Egypt PHARCO pharmaceuticals Alexandria) was purchased from Amira Pharmacy, tudunwadazaria.
- iii. Methylated spirit, cotton wool,
- iv. Rabbit T₃, T₄ and TSH ELISA Kits, (GenAsia Biotech Co., Ltd., 7th floor, Wujiaochang Technology Building, No. 1675, Huangxing Road, Yangpu District, Shanghai, China).

Animals

A total of thirty (30) male rabbits aged 5-8 weeks were used for the study, animals were housed in the animal house of Department of Human Physiology, Faculty of Medicine Ahmadu Bello University Zaria, under standard laboratory conditions and had access to feed (Pelletised Growers feed) and water ad libitum. Animal care and use was in accordance with the guide for the care and use of laboratory animals, institute for laboratory animal research, national institute of health (NIH Publication No.80-23; 1996).

Methods:

Authentication of Supplements

Clove (*Syngiziumaromaticum*) buds and ginger (*Zingiberofficinale*) rhizome after purchased were presented to the herbarium unit of the department of biological sciences, faculty of science, Ahmadu Bello University, Zaria, where they were identified and authenticated with a deposited voucher number of 900127 and 2261 respectively.

Preparation of high fat diet

The high fat diet was constituted in the Animal house of the Human Physiology Department as follows;

- 30 % (7.5kg) per weight out of 25kg of standard animal feed, was removed,

109 Cholesterol powder weighing 0.25kg, Groundnut mill 5kg, and ground nut oil 2.25kg were accordingly
 110 added into the feed in a large bowl each at a time and mix thoroughly,
 111 The final constituted diet composed of: about 40% fat, Crude protein 14%, crude fibre 15 %, calcium
 112 0.9%, metabolised energy 2550 Kcal/Kg min.

113 **Preparation of Supplement Composed Diets**

114 The supplements containing diet were reconstituted in the Animal house of the Human Physiology
 115 Department, ABU, Zaria.

116 **Fermented Ginger Supplemented Diet**

117 Raw fresh ginger rhizome were slice and air dried, then macerated in water for 48hrs and then air dried
 118 again, This was then reduced using grinding engine to a powder state. 3.125kg of the fermented ginger
 119 powder was then weight to give 12.5% of 25kg bag of normal feed and then, 3.125kg was weight and
 120 removed out of 25kg bag of standard feed. The fermented ginger powder was then added into the feed in
 121 a big bowl and mixed thoroughly (fermented ginger 12.5 % supplemented diet).

123 **Clove Supplemented Diet**

124 Clove was reduced using grinding engine to a powder state, 3.125kg of the powder was then weight to
 125 give 12.5% of the total weight (25kg) of standard feed and the same quantity of the feed was removed
 126 and then the clove powder was then added in a big bowl and mixed thoroughly (clove 12.5 %
 127 supplemented diet).

128 **Fermented Ginger and Clove Supplemented Diet**

129 Out of a bag weighing 25kg of standard feed, 6.25kg was removed, then 3.125kg powder of fermented
 130 ginger and clove each was measured and added at a time into the feed in a big bowl and mix thoroughly
 131 to give a mixture of supplemented diet (fermented ginger 12.5 % plus clove 12.5% supplemented diet).

132 **Induction of Hyperglycemia**

133 Hyperglycemia was induced by feeding rabbits with high fat diet for about eleven (11) weeks, after which
 134 each animal was bled from marginal vein on the ear lobe, with a drop of blood, using strip and a digital
 135 glucometer, glycemic level were measured and recorded for each animal. Rabbits having glucose levels
 136 greater than 140mg/dl were considered Hyperglycemic (diabetic model for the study), [20].

Experimental design

Rabbits with glucose level of 140mg/dl and above were considered diabetic model and randomly grouped (N=5) as follows;

Group i, Normal rabbits fed on animal standard feed

Group ii, Diabetic rabbits fed on standard feed for six weeks

Group iii, Diabetic rabbits fed on standard feed and administered cholestran (0.26g/kg body weight) for six weeks.

Group iv, Diabetic rabbits fed on 12.5% clove supplement for six weeks.

Group iv, Diabetic rabbits fed on 12.5% fermented ginger supplement for six weeks.

Group v, Diabetic rabbits fed on 12.5 % clove and fermented ginger 12.5% supplements co-administered for six weeks.

Determination of Blood Glucose Levels

During the experimental period blood glucose levels were determined at weekly intervals, with a digital glucometer Using the glucose oxidase principle [22] (Beach and Turner, 1958) (Accu-Chek Advantage, Roche Diagnostic, Germany), Rabbit were bled through marginal vein of the ear lobe by the use of disposableniddle, the result were recorded in mg/dl [23](Rheney and Kirk, 2000).

Termination of the Study

Animals were sacrifice at the end of six weeks study period by light anesthesia using chloroform vapour in an air-tight chamber, blood samples were collected via cardiac puncture using syringe and released into blood tubes (Plain tubes). Blood sample collected were centrifuge at 2000g for 5- 10min using a bench centrifuge to get serum for laboratory analysis and assay, which include;

Determination of lipid profile

Total serum cholesterol level was estimated by the enzymatic method of Watson (1960).Determination of High density lipoprotein cholesterol (HDL-C).andSerum high density lipoprotein cholesterol (HDL-C) was estimated by the method reported by [24],Serum triglycerol (TAG) was estimated by the method of [25].While serum low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated according to the Friedewald's formula:

- $LDL-C = TC - (HDL-C + TAG/5)$

- and VLDL-C=TAG/5 (Friedewald *et al.*, 1972)
- LDL-C = Low density lipoprotein cholesterol
- VLDL-C = very low density lipoprotein cholesterol
- HDL-C = high density lipoprotein cholesterol.
- TC = total cholesterol
- TAG = serum triglycerol

Estimation of Thyroid hormones (T_3 , T_4), and thyroid stimulating hormone (TSH).

Thyroid profile was estimated by using the enzyme-linked immunosorbent assay (ELISA) kits, using rabbit T_3 , T_4 and TSH ELISA kits from Gen Asia Biotech Co., Ltd., Shanghai, China) in accordance to the manufacturers guide.

Ethical Approval

The rabbits were handled in accordance with the principles guiding the use and handling of experimental animals Ahmadu Bello University, Zaria, Nigeria.

Blood Glucose Level Determination

Blood samples were collected from the marginal vein ear lobe of the rabbits at an interval of eight weeks on weekly bases. Determination of blood glucose level was done by the glucose-oxidase principle [26], using the digital glucometer (Accu-Check Advantage, Roche Diagnostic, Germany), and results were obtained as mg/dL.

Collection and Preparation of Serum Samples for Analysis

Six weeks after the treatment period, all rabbits were subjected to light anaesthesia by exposing them to chloroform soaked in cotton wool placed in anaesthetic box covered with lid. Blood samples of about 5 ml were drawn from the heart of each sacrificed animal from all groups by cardiac puncture. The blood sample was put in EDTA bottle to prevent clotting, after with serum was extracted for biochemical assay.

Statistical Analysis

Blood glucose levels were expressed in mg/dL as mean $\pm$ SEM. The data were analyzed using ANOVA followed by Tukey's post-hoc test to show multiple comparisons across experimental groups. Values of $P = 0.05$ were considered as significant [27]

RESULTS

Table 1, Lipid Profile of high fat diet induced- Hyperglycemic Rabbits treated with clove 12.5%, fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, and control groups for six weeks

GROUPS	TC (mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL(mg/dL)
Group 1 (NC)	144.4±3.63	88.6±11.5	20.33±2.70	39.4±14.25
Group 2 (DC)	180.7±14.74	111.8±7.1	26.90±2.72	47.31±9.81
Group3(D+Chl 0.26g/kg)	184.3±11.32	79.8±10.9	23.11±2.10	18.6±7.52
Group 4 (D+CLV 12.5%)	180.15±15.17	117.5±16.24	20.81±1.4*	61.37±13.07
Group 5 (D+Fgg 12.5%)	143.06±23.47*	80.45±5.8*	17.20±3.72*	39.5±6.02
Group 6 (D+CLV 12.5% and Fgg 12.5%)	178.80±28.10	125.91±21.42	14.95±1.06*	69.71±10.55

Significant at P= 0.05 value: n=5 ; *=significant difference

(NC= normal control); (DC= diabetic control); (Chl.=cholestran); (Fgg= Fermented ginger supplemented diet); (CLV= clove supplements).

Table 1, shows the results of lipid profile in control, and high fat diet induced-insulin resistance rabbits treated with clove, fermented ginger and a mix of clove and fermented ginger supplemented diet. Total cholesterol and high density lipoprotein cholesterol level were seen to be significantly lower in group 5 than that of group 2, triglyceride levels were seen to be significantly lower also in group 5 and 6, when compared to group 2 (diabetic rabbits fed on standard feed group).

Table 2, Serum thyroids hormones and TSH level in control, and high fat diet induced- Hyperglycemic Rabbits treated with clove 12.5%, fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, for six weeks

GROUPS	T3 (ng/ml)	T4 (ng/ml)	TSH (ng/ml)
Group 1	1.8±0.12	74.33±3.5	1.3±0.12
Group 2	2.1±0.071	79.4±1.5	0.8±0.093
Group 3	1.84±0.93	61.2±0.6	1.00±0.071
Group 4	1.72±0.06	47.4±1.36*	0.94±0.07
Group 5	1.6±0.14*	49.8±2.9*	1.64±0.093*
Group 6	1.5±0.93*	58.00±2.02*	1.1±0.071

Significant at P= 0.05 value: n=5; *=statistically significant difference

Table 2, shows the results of serum thyroid hormones and thyroid stimulating hormone levels in control, and high fat diet induced insulin resistance rabbits treated with clove, fermented ginger and a mix of clove and fermented ginger supplemented diet. The results reveal a statistically significant decrease in serum T3 in groups 4 and 5 levels, when compared to that of diabetic group on normal feed (group 2). T4 levels level were seen to be statistically significantly lowered in all supplemented diet treated groups (clove 12.5%, ginger 12.5% and combined clove 12.5% + ginger 12.5%), groups 4, 5, and 6, when compared to that of the diabetic rabbits group on normal feed (group 2). while the results on serum TSH shows a statistically significant increase in groups 5 and 6, when compared to diabetic rabbits on normal feed (group 2).

DISCUSSION

Effect of Clove and Fermented Ginger on Lipid profile

In the present study, the results of lipid profile shows significant decrease in total cholesterol level in diabetic rabbits fed on 12.5% fermented ginger supplement diet (group 5) when compared to that of group 2 (diabetic rabbits fed on standard feed), the level of cholesterol in group 5 was noted to have no significant difference with that of normal control group rabbits, furthermore other diabetic treated groups (group 2, 3, 4, and 6) were seen to have a significant increase in total cholesterol level. These results is

attributed to the effects of high fat diet in the experimental animals. triglycerides shows a decrease in value that is statistically significant different in diabetic rabbits fed on 12.5% fermented ginger supplement diet (group 5) when compared to that of group 2 (diabetic rabbits fed on standard feed). Results of high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol shows a statistically significant decrease in the group treated with fermented ginger, this result is in accordance with the work of [28] and. Both work shows the significant decrease effect of clove and ginger peel in total serum cholesterol, triglycerides, and low density lipoprotein. It has been established that chronic intake of high fat diet leads excessive adipose tissue fat accumulation, excessive fat is an attributing factor to insulin resistance, therefore the abnormalities seen in this study on lipid profile of high fat diet induced insulin resistance rabbits on normal feed may as a result of the effects of high fat diet over time that leads to uncontrol hyperglycemia in the animals or insulin resistance [30], while in groups 5 treatment with 12.5% fermented ginger supplement indicate an improvement in lipid profile when compared to that of group 2 (diabetic rabbits on normal feed).

Based on clinically recommendations, Lipid profile associated changes characterized by high plasma triglyceride concentration, low HDL cholesterol concentration and increased LDL-cholesterol particles concentrations is attributed to increased free fatty acid flux (lipogenesis) which leads to insulin deficiency or insulin resistance, hyperlipidemia or elevated lipid levels leads to beta-cells failure and contributes to insulin dysfunction as seen to occur in diabetic dyslipidemia. Therefore, distorted lipid profile is related to increased risk of cardiovascular related diseases (CVDs) and high mortality rate associated with CVDs in diabetes mellitus.

The results of the present study shows an improvement in lipid profile especially in the diabetic rabbits group fed on 12.5% fermented ginger when compared with lipid profile of diabetic rabbits fed on standard feed, this result agree with the work of [31], and is in line with a related study aimed to investigate the potential effect of fresh ginger extracts *Zingiber officinale* roscoe (Family: Zingiberaceae) on serum lipid profile in alloxan-induced diabetes and propylthiouracil-induced hypothyroidism in rats, and revealed a decrease in the levels of total cholesterol (TC), and low density lipoprotein (LDL) in the serum of rats that were treated by ginger extracts, compared with the control groups [32].

Effect of Clove and Ginger on Serum Thyroid Stimulating Hormone, T₃, and T₄

Diabetes and thyroid disorders have been shown to mutually influence each other and associations between both conditions have long been reported [33, 34]. Thyroid hormones contribute to the regulation of carbohydrate metabolism and pancreatic function, and on the other hand, diabetes affects thyroid function tests to variable extents. The result of this study reveal a statistically significant increase in serum T₃, and T₄ levels in diabetic rabbits group on normal feed, when compared to that in supplement treated groups (clove 12.5%, fermented ginger 12.5% and combined clove 12.5% + fermented ginger 12.5%), this could be one among the attributing factors that causes no significant decrease on blood glucose level of the diabetic animals on normal diet group. Thyroid hormones affect glucose metabolism via several mechanisms. Higher levels of thyroid hormones has long been recognized to promote hyperglycemia [35]. During hyperthyroidism, the half-life of insulin is reduced most likely secondary to an increased rate of degradation and an enhanced release of biologically inactive insulin precursors [36, 37]. As well, another mechanism explaining the relationship between higher levels of thyroid hormones and hyperglycemia is the increase in glucose gut absorption mediated by the excess thyroid hormones [38, 39].

Endogenous production of glucose is also enhanced in higher levels of thyroid hormones via several mechanisms. Thyroid hormones produce an increase in the hepatocyte plasma membrane concentrations of GLUT2 which is the main glucose transporter in the liver, and consequently, contribute to the increased hepatic glucose output and abnormal glucose metabolism [40, 41]. Additionally, increased lipolysis is observed in higher levels of thyroid hormones resulting in an increase in FFA that stimulates hepatic gluconeogenesis. The increased release of FFAs could partially be explained by an enhanced catecholamine-stimulated lipolysis induced by the excess thyroid hormones [42], this may explain the distorted lipid profile observed in the group of diabetic animals on normal feed.

It is well known that diabetic subjects with Higher levels of thyroid hormones experience worsening of their glycemic control and thyrotoxicosis has been shown to precipitate diabetic ketoacidosis in subjects with diabetes [43, 44].

CONCLUSION

Dyslipidemia was seen in high fat diet-induced insulin resistance rabbits, ameliorative effects of 12.5% fermented ginger supplemented diet on total cholesterol and triglycerides was pronounce in this study to restore the dyslipidemia. Clove, fermented ginger and their co-supplementations restored thyroid hormones levels in diabetic model animals almost to that of normal rabbits on standard feed.

REFERENCES

1. Buettner R, Scholmerich J, Bollheimer LC. High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring)*. 2007;15: 798-808.
2. Baxter JD, Webb P. Thyroid hormones mimetics: potential applications in atherosclerosis, obesity and type 2 diabetes. *Nature Reviews Drug Discovery*, 2009; 8(4): 308-320.
3. Supale S, Li N, Brun T, Maechler P. Mitochondrial dysfunction in pancreatic β - cells. *Trends in Endocrinol and Metab*. 2012;23(9):477-478. 5.
4. Tang C, Naassan AE, Chamson- Reig A, Konlajian K, Goh TT, et al. Susceptibility to fatty acid-induced β -cell dysfunction is enhanced in prediabetic diabetes-prone biobreeding rats: A potential link between β -cell lipotoxicity and islet inflammation. *Endocrine*. 2013;154:89-101.
5. Collier FA, Huggins CB. Effect of hyperthyroidism upon diabetes mellitus: striking improvement in diabetes mellitus from thyroidectomy. *Annals of Surgery*, 1927; 86(6): 877-884.
6. Brenta G, Danzi S, Klein I. Potential therapeutic applications of thyroid hormones analogs. *Nature Clinical Practice of Endocrinology and Metabolism*, 2010; 3(9): 632-640.
7. Stanicka S, Vondra K, Polikanova T, Vlcek P, Hill M, Zamrazil V. Insulin sensitivity and counter-regulatory hormones in hyperthyroidism and during thyroid hormones replacement therapy. *Clinical Chemistry and Laboratory Medicine*, 2005; 43(7): 715-720.
8. Singh G, Gupta V, Sharma AK, Gupta N. Frequency of thyroid dysfunction among diabetes in punjabi population. *Advance in Bioresearch*, 2011; 2: 3-9.

- 310 9. Stanicka S, Vondra K, Pclikanova T, Vlcek P, Hill M,Zamrazil V. Insulin sensitivity and counter-
311 regulatory hormones in hyperthyroidism and during thyroid hormones replacement therapy.
312 Clinical Chemistry and Laboratory Medicine, 2005; 43(7): 715-720.
- 313 10. Mitrou P, Raptis SA, Dimitriadis G. Insulin action in hyperthyroidism: a focus on muscle and
314 adipose tissue. Endocrine Reviews, 2010; 31(5): 663-679.
- 315 11. Klieverik LP, Coomans CP,Endert E. Thyroid hormone effects on whole-body energy homeostasis
316 and tissue specific fatty acid uptake in vivo. Endocrinology, 2009; 150: 56339-5648.
- 317 12. Stanicka S, Vondra K, Pclikanova T, Vlcek P, Hill M,Zamrazil V. Insulin sensitivity and counter-
318 regulatory hormones in hyperthyroidism and during thyroid hormones replacement therapy. Clinical
319 Chemistry and Laboratory Medicine, 2005; 43(7): 715-720.
- 320 13. Mitrou P, Raptis SA, Dimitriadis G. Insulin action in hyperthyroidism: a focus on muscle and adipose
321 tissue. Endocrine Reviews, 2010; 31(5): 663-679.
- 322 14. Peppas M, Varela L, Vazquez MJ. Skeletal muscle insulin resistance in endocrine disease. Journal of
323 Biomedicine and Biotechnology, 2010; 52(7): 8-15.
- 324 15. Stanicka S, Vondra K, Pclikanova T, Vlcek P, Hill M,Zamrazil V. Insulin sensitivity and counter-
325 regulatory hormones in hyperthyroidism and during thyroid hormones replacement therapy. Clinical
326 Chemistry and Laboratory Medicine, 2005; 43(7): 715-720.
- 327 16. Keaney JF, Larson MG,Vasan RS. Obesity and systemic oxidative stress: clinical correlates of
328 oxidative stress in the Framingham study. Arteriosclerosis Thrombosis Vascular Biology,2003; 23: 434-
329 439.
- 330 17. DeMarco VG, Johnson MS, Whaley-Connell AT, Sowers JR. Cytokines abnormalities in the etiology
331 of the cardiometabolic syndrome. Current Hypertens Rep,2010; 12: 93-98
332
- 333 18. Otani H. Oxidative stress as pathogenesis of cardiovascular risk associated with metabolic syndrome.
334 Antioxidant Redox Signal,2011; 15: 1911-1926.
- 335 19. Adams SS, Imran S, Wang S, Mohammed A, Kok S. The hypoglycemic effect of Pumpkins as Anti-
336 Diabetic and functional medicines. Food research international,2011; 44(4): 862-867.

20. Jimoh A, Tanko Y, Ahmed A, Mohammed A, Ayo JO. Protective Effect of Resveratrol Co-Administered with High Fat Diet on Blood Glucose Homeostasis and Thyroid Function in Rabbits. *Cell Biology*. 2015; 3, 1, 19-24.
22. Beach EF, Turner JJ. An enzymatic method for glucose determination in the body fluids . *Clinical Chemistry*,1958; 4: 468.
23. Rheney CC,Kurk KK. Performance of three blood glucose meters. *Annal of Pharmacotherapy*,2000; 34(3): 317-321.
27. Duncan RC, KnappRG, Miller MC. Test of hypothesis in population means. In: *Introductory Biostatistics for the health sciences*, John Wiley and Sons Inc. NY:1977; 71-96
28. AshadeO, Adelusi OE,Ligalinusirat A. Histopathological effects of untreated ginger peel (zingiberofficinale) fish meal on the intestinal tissue profiling of African Cat fish (*Clariasgrariepinus*), *International journal of fisheries and aquatic studies*, 2013; 2 (2): 95-98.
30. Madkor HR, Mansour SW, Ramadan G. Modulatory effects of garlic, ginger, turmeric and their mixture on hyperglycemia, dyslipidemia and oxidative stress in streptozotocin-nicotinamide diabetic rats. *British Journal of Nutrition*, 2011;105:1210–1217.
32. Al-Noory AS, Amreen AN, Hymoor S. Antihyperlipidemic effects of ginger extracts in alloxan-induced diabetes and propylthiouracil-induced hypothyroidism in (rats). *Journal of pharmacognosy Research*, 2013; 5:157-61
35. Maxon HR, Kreines KW, Goldsmith RE, Knowles HC, “Long-term observations of glucose tolerance in thyrotoxic patients,” *Archives of Internal Medicine*, 1975;135; 11: 1477–1480,
36. O'Meara NM, Blackman JD, Sturis J, PolonskyKS. “Alterations in the kinetics of C-peptide and insulin secretion in hyperthyroidism,” *Journal of Clinical Endocrinology and Metabolism*, 1993; 76; 1: 79–84.
37. Dimitriadis G, Baker B, Marsh H. “Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans,” *The American journal of physiology*,1985; 248 (5) E593–E601,
39. Matty AJ, Seshadri B. “Effect of thyroxine on the isolated rat intestine,” *Gut*, 1965;(6) 200–202,
40. Dimitriadis G, Mitrou P, Lambadiari V. “Insulin action in adipose tissue and muscle in hypothyroidism,” *Journal of Clinical Endocrinology and Metabolism*, 2006; 91, (12) 4930–4937,
41. Cettour-Rose P, Theander-Carrillo C, Asensio C. “Hypothyroidism in rats decreases peripheral glucose utilisation, a defect partially corrected by central leptin infusion,” *Diabetologia*, 2005;48, (4) 624–633,
42. Maratou E, Hadjidakis DJ, Kollias A. “Studies of insulin resistance in patients with clinical and subclinical hypothyroidism,” *European Journal of Endocrinology*, 2009;160 (5), 785–790.
43. Solá E, Morillas C, Garzón S, Gómez-Balaguer M, Hernández-Mijares A. “Association between diabetic ketoacidosis and thyrotoxicosis,” *ActaDiabetologica*,2002;39; 4: 235–237.

374

375 44. Bhattacharyya A, Wiles PG. "Diabetic ketoacidosis precipitated by thyrotoxicosis," *Postgraduate*
376 *Medical Journal*, 1999; 75; 883: 291–292.

377

378