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Antidiabetic and Antioxidative Activity of *Physalis* Powder or Extract with Chromium in Rats

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Abstract: The present study was designed to investigate the effect of Physalis either powder or extract only or with chromium on diabetic rats. Forty-nine adult male albino rats Sprague Dawley strain were injected with a single intraperitoneal dose of streptozotocin to induce diabetes. Diabetic rats were divided into 7 groups (7 for each group) that were positive control (C+) and 6 treated groups that were amaryl drug (AD), chromium (Cr), Physalis extract (PE), Physalis powder (PP), Physalis extract+ chromium (PE Cr) and Physalis powder + chromium (PP Cr). In comparing with AD group, results revealed non significant difference in final weight, weight gain, food intake and FER; serum hemoglobin (HG), packed cell volume (PCV), glucosalated heamoglobin (Hb A1c %), alanine amino transferase and γ-glutamyl transferase enzyme activities (ALTand γGT), glutathione-peroxidase (GPX), nitric oxide (NO) and liver glycogen in Cr, PE, PP, PECr and PPCr groups. Cr group showed a significant increase in serum glucose and significant decrease in insulin while PE group showed a significant increase in serum glucose and superoxide dismutase (SOD) and liver GPX but showed significant decrease in insulin, aspartate aminotransferase (AST), creatinine, urea, uric acid and malondialdehyde (MDA). PP group showed a significant increase in serum glucose and SOD and liver SOD but a significant decrease in insulin, total bilirubin, creatinine, urea and uric acid. PECr group showed a significant increase in serum and liver SOD beside significant decrease in serum AST, alkaline phosphatase (ALP), total bilirubin, creatinine, urea and uric acid and liver cholesterol, total lipid and MDA. PPCr group showed significant decrease in serum insulin, ALP, total bilirubin, creatinine, urea and uric acid and liver total lipid and MDA. Our study clearly demonstrated that administration of a combination of chromium and *Physalis* extract or powder could ameliorate the healthy status of diabetes in rats.

Key words: Chromium · *Physalis* · Diabetes · Rats

INTRODUCTION

Diabetes refers to a disorder of metabolism of carbohydrate, fat and protein metabolism. This is due to problems with the secretion or action of insulin or both and leads to high blood sugar content [1]. Certain studies reveal that many traditional plant treatments exist as a hidden wealth of potentially useful natural products for diabetes control. Searching for new antidiabetic drugs from natural plants is still attractive around the world because they contain substances that take alternative and safe effect on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having antidiabetic effect [2, 3]. In folk medicine, *Physalis* alkekengi belongs to the family Solanaceae and has been used as a medicinal herb to treat cancer, leukemia, malaria, asthma, hepatitis, dermatitis and rheumatism [4]. Physalis contain of physalin, citric acid, beta-carotene, iron, calcium and vitamin C as the major components of P. alkekengi extract. Physalin is the most chemical compound with various pharmacological characteristics [5]. Chromium is a mineral that found in food items such as fish, poultry, meat and whole grain breads. However, processed foods are very low in chromium, as the food gets stripped of the natural chromium during processing. Chromium is an essential trace mineral for the metabolism of carbohydrates, helps to regulate the metabolism of blood sugar and thus helps deter diabetes and controls fat and cholesterol levels in the blood. The role of chromium in weight loss is to

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correct insulin resistance [6]. Therefore, the present study has been carried out to explore the antidiabetic and antioxidative activity of physalis powder or extract with chromium in streptozotocin induced diabetic in rats.

MATERIALS AND METHODS

Materials: Streptozotocin (STZ) was obtained from SIGMA Company for Pharmaceutical Industries Cairo, Egypt. Amaryl drug is antidiabetic produced by Saofi - Avents Egypt under license of Saofi-Avents Germany. Chromium drug is produced by Arab Company for Pharmaceuticals and Medicinal Plants (MEPACO) Egypt. The rat dose of amaryl drug (AD) and chromium were 0.36 mg/kg body weight daily and 36 mcg/100 g body weights, respectively which dissolved in distilled water and given to rats by oral intubation. These doses were based on a human therapeutic dose which converted to rat dose according to Paget and Barnes [7]. BioMeriuex Kits were purchased from Alkan Co. for Chemicals and Biodignostics (Dokki, Egypt). Fresh husk tomato fruits "*Physalis spp* was obtained from local markets in Egypt. The basal diet was prepared according to NRC [8]. Forty-nine healthy Sprague-Dawley albino rats were purchased from Helwan Farm for Laboratory Animals, Cairo, Egypt. Rats were kept under observation for 5 days before experiment and fed on the standard diet and water *ad libitum*.

Methods

Preparation of *Physalis* Powder and Methanol Extract: *Physalis* fruit was washed with water, crushed and dried in air oven at 50ºc then grinded in blender to powder Physalis powder was added in basal diet as 10 % in substitution of fiber. To prepare the methanol extract, 100 gram of physalis powder was added to 1000 ml of 70% methanol (v/v) at room temperature for 20 hours with slowly rotated during this time. After filtration, ethanol was evaporated at low pressure at 30 centigrade degree [9]. *Physalis* extract was dissolved in normal saline and given to rats at dose 500 mg/kg body weight by oral intubation.

Grouping of Rats: After incubation period, the rats were injected with a single intraperitoneal dose of streptozotocin (55 mg/kg body weight) in 0.1 M citrate buffer of pH 4.5 then supplied with 5% glucose solution for 48 h after injection to induce diabetes according to Peschke *et al.* [10]. The rats were divided into 7 groups (7 for each group) that were positive control (C+) group and 6 treated groups amaryl drug (AD), chromium (Cr), *Physalis* extract (PE), *Physalis* powder (PP), *Physalis* extract+ chromium (PE Cr) and *Physalis* powder + chromium (PP Cr). Feeding and growth performance were monitored by recording daily food intake, body weight gain and feed efficiency ratio (FER) according to Chapman *et al.* [11] as mean daily body weight gain divided by mean daily food intake. The rats were sacrificed after 60 days. Blood and livers were collected for biochemical analysis.

Analyses: Blood glucose, hemoglobin (HG) and packed cell volume (PCV) were estimated according to Sasaki *et al.* [12], Drabkin [13] and Mc Inory [14], respectively. Serum glucosalated heamoglobin (Hb A1c %) and insulin were estimated according to Abraham *et al.* [15] and Wilson and Miles [16], respectively. Moreover, Serum alanine, aspartate amino transferase, alkaline phosphatase and γ-glutamyl transferase enzyme activities (AST, ALT, AP, γGT respectively) were estimated according to Reitman and Frankel [17], Kind and King [18] and Draper and Hadley [19], respectively. Serum total bilirubin, creatinine, urea and uric acid were determined according to Jendrassik [20], Bonsens and Taussky [21], Patton and Crouch [22] and Fossati *et al.* [23], respectively. In addition, serum glutathione-peroxidase (GPX), superoxide dismutase (SOD) and nitric oxide (NO) were determined by enzymatic colorimetric procedures according to Habig *et al.* [24], Dechatelet *et al.* [25] and Green *et al.* [26], respectively. Livers were immediately perfused with 50 to 100 of ice cold 0.9% NaCl solution. Liver glycogen, cholesterol, total lipids, glutathione peroxidase (GPX), superoxide dismutase (SOD) and malondialdehyde (MDA) were determined as described by the method of Rerup and Lundquist [27], Richmond [28], Folch *et al.* [29], Weiss *et al.* [30], Beuchamp and Fridovich [31] and Uchiyama and Mihara [32], respectively.

Statistical Analysis: Collected data were presented as mean ±SD and statistically analyzed using one way analysis of variance (ANOVA). Student "t" test was used for significance. Differences were considered significant at $p<0.05$ according to Artimage and Berry [33].

RESULTS

The average beginning weight of the rats was 201 ± 10 g. There was no significant difference in food intake among groups but there was significant increase in final

Table 1: Mean values ± SD of body weight gain, food intake and feed efficiency ratio (FER) of the experimental rat groups

	Variables				
Groups	Initial weight (g)	Final weight (g)	Weight gain (g)	Food intake (g/d)	FER
C+	203.11±0.33^{a}	264.77±22.11^{b}	61.66±8.46^{b}	18.33±1.44^{a}	0.056±0.001^{b}
AD	205.14±4.22^{a}	319.47±23.03^{a*}	114.33±10.01^{a**}	18.66±1.36^{a}	0.102±0.003^{a***}
Cr	203.21±6.14^{a}	313.54±24.44^{a*}	108.33±12.05^{a**}	17.25±1.11^{a}	0.104±0.001^{a***}
PE	204.21±5.11^{a}	314.43±25.25^{a*}	110.22±11.24^{a**}	17.86±1.21^{a}	0.102±0.002^{a***}
PP	201.11±5.18^{a}	306.22±21.11^{a**}	105.11±11.22^{a**}	17.33±1.14^{a}	0.101±0.002^{a***}
PECr	201.33±4.13^{a}	318.66±26.10^{a*}	117.33±11.03^{a**}	18.11±1.08^{a}	0.107±0.003^{a***}
PPCr	200.31±5.16^{a}	313.52±22.20^{a*}	113.21±10.11^{a**}	18.20±1.39^{a}	0.103±0.003^{a***}

^{abcd}Mean values in each column having similar letters were not significantly different

Table 2: Mean values ± SD of glucose, (HbA_{IC}), insulin, HG and PCV of the experimental rat groups

	Variables				
Groups	Glucose (mg/dl)	HbA_{IC} %	Insulin (µ/l)	HG (g/dl)	PCV %
C(+ve)	317.31±38.21^{a}	8.14±0.88^{a}	8.60±1.16^{c}	10.16±1.33^{b}	25.14±4.33^{b}
Drug	120.11±6.21^{c***}	5.11±0.67^{b**}	17.25±2.14^{a***}	12.30±1.26^{a**}	34.17±3.21^{a**}
Cr	142.16±8.41^{b**}	4.60±0.33^{b**}	14.01±2.20^{b**}	12.22±1.80^{a**}	35.67±4.67^{a**}
P E	131.12±7.27^{b**}	4.67±0.51^{b**}	14.33±2.11^{b**}	13.44±2.01^{a**}	37.10±3.16^{a**}
P P	130.21±9.33^{b**}	4.17±0.44^{b**}	15.16±1.99^{b**}	12.69±1.03^{a**}	34.91±4.25^{a**}
P E Cr	117.31±5.16^{c***}	4.01±0.36^{b**}	16.21±2.36^{ab***}	13.77±1.31^{a**}	36.66±3.67^{a**}
P P Cr	122.16±6.42^{c***}	5.21±0.60^{b**}	14.33±1.89^{b**}	13.36±1.51^{a**}	37.32±3.51^{a**}

^{abcd}Mean values in each column having similar letters were not significantly different

Table 3: The Mean values ± SD of serum ALT, AST, ALP and γGT of the experimental rat groups

	Variables			
Groups	ALT (µ /ml))	AST (µ /ml)	ALP (µ /ml)	γGT (µ /ml)
C+	63.14±8.11^{a}	71.41±9.91^{a}	103.21±13.65^{a}	9.98±1.40^{a}
AD	42.36±7.14^{b**}	50.16±6.36^{b**}	72.33±8.24^{b**}	7.16±1.26^{b*}
Cr	48.33±5.21^{b**}	54.72±6.33^{b**}	75.16±8.36^{b**}	7.11±1.20^{b*}
PE	39.41±5.61^{bc**}	44.20±5.21^{c***}	52.67±5.44^{c***}	6.11±0.88^{b*}
PP	41.18±4.33^{b**}	52.35±6.11^{b**}	70.01±8.11^{b**}	7.35±1.11^{b*}
PECr	41.33±3.99^{b**}	45.21±4.11^{c***}	50.11±5.11^{c***}	6.25±1.01^{b*}
PPCr	49.36±3.69^{b**}	53.33±5.01^{b**}	54.36±5.16^{c***}	7.14±1.31^{b*}

^{abcd}Mean values in each column having similar letters were not significantly different

weight ($p<0.05$), weight gain ($p<0.01$) and FER ($p<0.001$) in all groups compared with C+ group. There was no significant difference in final weight, weight gain and FER in Cr, PE, PP, PECr and PPCr groups compared with AD group as illustrated in Table 1.

The values of serum glucose ($p<0.01$and0.001) and HbA1c ($p<0.01$) were significantly decreased while insulin ($p<0.01$and0.001), HG and PCV ($p<0.01$) were significantly increased in all diabetic treated rat groups compared with C+ group. On the other side, the values of HbA1c, HG and PCV were insignificantly difference compared with AD group. The value of serum glucose increased in Cr, PE and PP while insulin decreased in Cr, PE, PP and PPCr compared with AD group as illustrated in Table 2.

The values of serum ALT, AST, ALP ($p<0.01$ and 0.001) and γGT ($p<0.05$) were significantly decreased in all treated rat groups compared with C+ group. On the other side, the values of serum ALT and γGT were insignificantly difference compared with AD group. The value of serum AST was significantly decreased in PE and PECr while the value of serum ALP was significantly decreased in PE, PECr and PPCr compared with AD group as illustrated in Table 3.

The values of total bilirubin, creatinine and urea were significantly decreased ($p<0.01$ and 0.001) in all diabetic treated rat groups while the value of uric acid was significantly decreased ($p<0.05$, 0.01 and 0.001) in PE, PP, PECr and PPCr groups compared with C+ group.

Table 4: Mean values ± SD of total bilirubin, creatinine, urea and uric acid of the experimental rat groups

	Variables			
Groups	Total bilirubin (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
C+	1.96±0.25^{a}	1.78±0.33^{a}	52.14±7.33^{a}	5.11±1.10^{a}
AD	1.23±0.22^{b**}	1.51±0.38^{b**}	48.31±6.20^{b**}	4.11±1.21^{a}
Cr	1.32±0.33^{b**}	1.01±0.14^{bc**}	42.14±7.11^{bc**}	4.21±1.11^{a}
PE	1.11±0.24^{b**}	0.87±0.11^{cd***}	35.91±5.14^{c***}	3.11±0.88^{bc*}
PP	0.99±0.05^{c***}	0.72±0.22^{d***}	38.16±6.14^{c***}	2.61±0.33^{cd**}
PECr	0.55±0.06^{d***}	0.65±0.10^{d***}	34.34±5.13^{c***}	2.03±0.57^{d**}
PPCr	0.59±0.05^{d***}	0.71±0.18^{d***}	35.81±3.20^{c***}	2.11±0.43^{d***}

^{abcd} Mean values in each column having similar letters were not significantly different

Table 5: The Mean values ± SD of serum, GPX, SOD and NO of the experimental rat groups

	Variables		
Groups	GPX (µ /ml)	SOD (µ /ml)	NO (µ mol/l)
C+	65.14±9.11^{C}	20.11±3.69^{C}	10.89±2.14^{a}
AD	88.33±11.17^{ab**}	26.14±5.27^{b*}	7.33±1.13^{b*}
Cr	89.81±9.91^{a**}	28.31±6.11^{ab**}	6.21±1.11^{b**}
PE	95.34±12.10^{a***}	30.38±6.27^{a**}	6.11±1.21^{b**}
PP	91.41±10.22^{a***}	29.36±5.51^{a**}	6.35±1.14^{b**}
PECr	99.36±10.41^{a***}	31.14±4.21^{a**}	5.21±0.88^{bc**}
PPCr	95.31±9.03^{a***}	27.21±3.21^{ab**}	6.01±1.03^{b**}

^{abcd} Mean values in each column having similar letters were not significantly different

Table 6: Mean values ± SD of liver glycogen, cholesterol and total lipid of the experimental rats groups

	Variables		
Groups	Glycogen (mg/100g)	Cholesterol (mg/100g)	Total lipid (mg/100g)
C+	5.33±0.67^{c}	7.11±1.03^{a}	50.11±6.77^{a}
AD	10.14±1.55^{a***}	5.03±0.77^{b**}	41.15±5.11^{b**}
Cr	8.31±1.28^{ab**}	5.11±0.66^{b**}	40.21±5.22^{b**}
PE	9.96±1.35^{a**}	4.81±0.51^{bc**}	38.47±4.31^{bc**}
PP	9.55±1.49^{a**}	4.36±0.33^{bc**}	37.11±5.21^{bc**}
PECr	11.14±1.89^{a***}	3.21±0.23^{c***}	35.21±4.10^{c***}
PPCr	10.81±1.88^{a***}	4.17±0.60^{bc**}	36.67±3.30^{c***}

^{abcd} Mean values in each column having similar letters were not significantly different

Table 7: Mean values ± SD of liver GPX, SOD and MDA of the experimental rats groups

	Variables		
Groups	GPX (µg/g)	SOD (µ/mg)	MDA (nmol/g)
C+	55.14±7.36^{c}	43.16±5.18^{c}	22.43±4.41^{a}
AD	90.11±10.12^{b**}	55.81±5.17^{b**}	11.30±2.14^{b***}
Cr	111.12±11.14^{a***}	58.17±6.11^{b**}	10.69±1.81^{b***}
PE	117.33±13.12^{a***}	62.14±7.14^{ab***}	8.66±1.10^{c***}
PP	115.14±12.03^{a***}	63.14±6.60^{a***}	9.14±1.25^{bc***}
PECr	123.24±15.51^{a***}	71.21±8.25^{a***}	8.11±1.14^{c***}
PPCr	120.21±14.31^{a***}	68.60±7.34^{ab***}	8.96±1.30^{c***}

^{abcd} Mean values in each column having similar letters were not significantly different

The value of total bilirubin significantly decreased in PP, PECr and PPCr groups while the values of creatinine, urea and uric acid significantly decreased in PE, PP, PECr and PPCr groups compared with AD group. The values of total bilirubin, creatinine, urea and uric acid were insignificantly difference in Cr group showed compared with AD group as illustrated in Table 4.

All treated diabetic rat groups showed a significant increase in the value of serum GPX ($p<0.01$ and 0.001) and SOD ($p<0.05$ and 0.01) but showed a significant decrease NO ($p<0.05$ and 0.01) compared with C+ group. The values of serum GPX and NO were in non significant difference in Cr, PE, PP, PECr and PPCr groups while the value of serum SOD was significantly increased in PE, PP and PECr groups compared with AD group as shown in Table 5.

All treated diabetic rat groups showed a significant increase in the value of liver glycogen and a significant decrease in the values of liver cholesterol and total lipids ($p<0.01$ and 0.001) compared with C+ group. Cr, PE and PP, groups showed non significant difference in liver glycogen, cholesterol and total lipids compared with AD group. P E Cr group showed a significant decrease in the values of liver cholesterol and total lipids but PPCr showed a significant decrease in the value of liver total lipids compared with AD group as shown in Table 6.

All treated diabetic rat groups showed a significant increase in the value of liver GPX and SOD but showed a significant decrease in the value of liver MDA ($p<0.01$ and 0.001) compared with C+ group. The value of liver GPX was significantly increased in Cr, PE, PP, PECr and PPCr groups while the value of liver SOD was significantly increased in PP and PECr groups but the value of liver MDA was significantly decreased in PE, PECr and PPCr groups compared with AD group as shown in Table 7.

DISSCUSION

It is known that, the single high-dose streptozotocin induced diabetes mellitus in rats that arises from irreversible destruction of the β-islet cells of the pancreas, causing degranulation or reduction of insulin secretion resulted in hyperglycemia and decreased body weight [34]. The main cause of STZ-induced β-cell death is alkylation of DNA by the nitrosourea moiety of this compound and production of NO and reactive oxygen species may be involved in DNA fragmentation [35]. Oral antidiabetic sulfonylurea drug (amryel) exerts their effects by stimulation of beta cells in the pancreas to produce more insulin [36]. The obtained results could be explained in many previous researches. Insulin is a hormone secreted by the pancreas that metabolizes and stores carbohydrates, proteins and fats. Insulin transport glucose from blood into different cells of the body. When the pancreas produce low level of insulin or improperly work of insulin, the glucose stays in the blood cells, which makes the blood sugar level high [37]. The obtained results of the chromium are referring to the benefits effect on improving insulin sensitivity by enhancing intracellular insulin receptor. Chromium works with insulin to regulate glucose and also lowered free fatty acid levels by 24%, blood urea by 33% and creatinine level by 25% and reduced the severity of glomerular sclerosis and confirmed by normal renal tubular appearance in a rat model of type 2 diabetes mellitus group treated with chromium [38]. The obtained results of *Physalis* were agreed with Estakhr and Javdan [39] who recorded that oral administration of ethanolic extract of *physalis* normalized the levels of blood glucose. The presence of potent antidiabetic active principles as physalin, citric acid and vitamin C in the extract which inhibit of glycogen phosphorylase enzyme that catalyzes the process of glycogenolysis thereby inhibiting glucagon which on feedback inhibition the production of insulin.

The addition of chromium with physalis in diabetic rats showed improved final weight and weight gain. That results may be related to the effect of ethanolic extract of *Physalis* which showed inhibitory activity on the amylase, lipase and alpha glucosidase, thus suggesting that extract might be useful in the treatment to limit dietary fat and glucose absorption and the accumulation of fat in adipose tissue [40]. The effect of chromium on body weight noted in this study were not expected based on published reports, suggesting that chromium is important in the burning of carbohydrates and fats in the body. Chromium supplement is believed to reduce food cravings and appetite, thereby assisting weight loss [41]. But these obtained results may be due to chromium helps maintained the efficiency of insulin, which then helps maintain the blood glucose level. These nutritional supplements permit the transmission of glucose to muscle cells instead of fat cells. Chromium supplement increases protein synthesis Thus, it is beneficial in building muscles. Moreover, Chromium supplementation has at best modest effects on en body weight or composition in individuals with diabetes [42]. It is believed that the extract of phasylis administration increased the superoxide dismutase and catalase activities. The lipid peroxidation and protein oxidation levels were reduced

[43]. Chromium supplementation was an effective treatment strategy to minimize increased oxidative stress in type 2 diabetes mellitus patients whose HbA(1C) level was >8.5% [44]. The results suggested that the *Physalis* powder *or* extract exhibit hypoglycaemic and antioxidant activity in streptozotocin diabetic rats. The mechanism of combination of active components *Physalis* with chromium as antidiabetic effect remains to be elucidated by further examinations.

REFERENCES

1. Gillies, C.L., K.R. Abrams, P.C. Lambert, N.J. Cooper, A.J. Sutton and R.T. Hsu, 2007. Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: systematic review and meta-analysis. BMJ, 10: 334(7588): 299.
2. Abd EI-Ghany, M.A., 2002. Study on the protective effect of onion (juice or powder) on diabetic rats. Egypt. J. Nutr., XVII(3): 227-244.
3. Huang, T.H., B.P. Kota, V. Razmovski and B.D. Roufogalis, 2005. Herbal or natural medicines as modulators of peroxisome proliferator-activated receptors and related nuclear receptors for therapy of metabolic syndrome. Basic Clin. Pharmacol. Toxicol., 96: 3-14.
4. Wu, S., L. Ng, Y. Huang, D. Lin, S. Wang, S. Huang and C.Lin, 2005. Antioxidant activities of *Physalis peruviana*. Biological and Pharmaceutical Bulletin, 28(6): 963-966.
5. Masao, K., O. Toichi and N. Masaki, 1988. Structure of physalin M isolated from Physalis alkekengi Var. fracheti. BCSJ, 61(7): 2696-2698.
6. Patrick, H., 2004. The New Optimum Nutrition Bible. Berkeley: Crossing Press. National Institute of Health Office of Dietary Supplements.
7. Paget, G.E. and J.M. Barnes, 1964. Inter Species Dosages Conversion Scheme in Evaluation of Results and Quantitative Application in Different Species Toxicity Test, Academic Press London and NY., pp: 135-165.
8. NRC, 1995. National Research Council: Nutrient Requirements of Laboratory Animals. Fourth Revised Edition, National Academy Press. Washington, DC., pp: 29-30.
9. WHO, 1983. Protocol CG-04: Preparation of alcoholic extract for bioassay and phytochemical studies (APJF/IP, 1001A). Geneva, World Health Organization.
10. Peschke, E., H. Ebelt, H.J. Bromme and D. Peschke, 2000. Classical and new diabetogens: Comparison of their effects on isolated rat pancreatic islets *in vitro*. Cell Mol. Life Sci., 57(1): 158-164.
11. Chapman, D.G., R. Gastilla and T.A. Campbell, 1950. Evaluation of protein in food. I. A. Method for the determination of protein efficiency ratio. Can. J. Biochem. Physio., 1(37): 679-686.
12. Sasaki, T., S. Matsy and A. Sonae, 1972. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsh. Kagaku., 1: 346-353.
13. Drabkin, D.I., 1949. The standardization of hemoglobin measurements. Am. J. Med. Sci., 21(7): 710.
14. Mc Inory, R.A., 1954. A micro heamatocrit for determining the packed cell and hemoglobin concentration on capillary blood. J. Clin. Path., 7(1): 32-36.
15. Abraham, E.C., T.A. Huff, N.D. Cope, J.B. Wilson, E.D. Bransome and T.H. Huisman, 1978. Determination of the glycosalated heamoglobin (Hb_{A1}) with a new microcolumn procedure. Suitability of the technique for assessing the clinical management of diabetes mellitus. Diabetes, 27(9): 931-7.
16. Wilson, M.A. and L.E. Miles, 1977. Radioimmunoassay of Insulin in Handbook of Radio Immunoassay G.E. Abraham, Ed., M. Inc. New York, pp: 275.
17. Reitman, S. and S. Frankel, 1957. Determination of glutamate pyruvat transaminase and glutamate oxaloacetate transaminase. Amer. J. Clin. Path., 28: 56-63.
18. Kind, P.R and E.J. King, 1954. Estimation of alkaline phosphatase activity by determination of hydrolyzed phenol with aminoantipyrene. J. Clin. Path., 7: 322-326.
19. Draper, H.H., E.J. Squires, H. Mahmoodi, J. Wu, S. Agarwal and M. Hadley, 1993. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological material. Free Radic. Biol. Med., 15: 353-363.
20. Jendrassik, L., 1938. Colorimetric determination of bilirubin. Biochem., 97: 72-81.
21. Bonsens, K.E. and D.H. Taussky, 1984. Determination of serum creatinine. J. Ch. Inv., 27: 648-660.
22. Patton, C.J. and S.R. Crouch, 1977. Enzymatic colorimetric method to determination urea in serum. Anal. Chem., 49: 464.

23. Fossati, P., L. Prencipe and G. Berti, 1980. Use of 3,5 dichloro-2-hydroxybenzene sulfonic acid /4-amlnophenazon chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clin. Chem., 26: 227-231.
24. Habig, W., M. Pabst and W. Jakoby, 1974. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-9.
25. Dechatelet, L.R., C. Mc Call, L.C. Mc Phial and R.B. Johnston, 1974. Superoxide dismutase activity in leukocytes. J. Clin. Invest., 53: 1197-1201.
26. Green, L., D. Wagner, J. Glokowski, P. Skipper, J. Wishnok and S. Tannenbaum, 1981. Analysis of nitrite, nitrate and [15N] nitrite in biological fluids. Anal. Biochem., 126: 131-138.
27. Rerup, E. and S. Lundquist, 1967. Precipitation and purification of liver glycogen in rats. Acta Pharmmic. Tox., 25: 47-51.
28. Richmond, N., 1973. Colorimetric method of determination of total cholesterol and high density lipoprotein cholesterol (HDLc). Clin. Chem., 19: 1350-1356.
29. Folch, J., M. Lees and G.H. Stanley, 1957. A simple method for isolation and purification of total lipid from animal tissue. J. Biol. Chem., 266: 497-509.
30. Weiss, C., H.S. Marker and G.M. Lehrer, 1980. Sensitive fluorometric assays for glutathione peroxidase and reductase. Anal. Biochem., 106: 512-516.
31. Beuchamp, C. and J. Fridovich, 1971. Superoxide dismutase. Improved an assay applicable to acrylamide gels. Anal. Biochem., 44: 276-287.
32. Uchiyama, M. and M. Mihara, 1978. Determination of malondialdhyde precursor in tissues by thiobarbituric acid test. Anal. Biochem., 86(1): 271-278.
33. Artimage, G.Y. and W.G. Berry, 1987. Statistical Methods. 7th Ed. Ames, Iowa State University Press, pp: 39-63.
34. Pushparaj, N.P., H.K. Tan and H.C. Tan, 2001. The mechanism of hypoglycemic action of the semipurified fractions of *Averrhoa bilimbi* in streptozotocin diabetic rats. Life Sci., 70: 535-47.
35. Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. Physiol. Res., 50: 536-46.
36. Derek, R., 2001. Current therapeutics algorithms for type 2 diabetes. Diabetes, 4: 38-49.
37. Lokesh, D. and S.D. Amit, 2006. Diabetes mellitus- its possible pharmacological evaluation techniques and naturotherapy. Int. J. Green Pharm., 1: 15-28.
38. Sahin, K., M. Onderci, M. Tuzcu, B. Ustundag, G. Cikim, I. Ozercan, V. Sriramoju, V. Juturu and J. Komorowski, 2007. Effect of chromium on carbohydrate and lipid metabolism in a rat model of type 2 diabetes mellitus: the fat-fed, streptozotocin-treated rat. Metabolism, 56(9): 1233-40.
39. Estakhr, J. and N. Javdan, 2011. The effects of hydro alcoholic extract of physalis alkekegi in alloxan induced diabetic rats. Pharmacologyonline, 2: 874-878.
40. Raju, S.N., D. Sathis Kumar, B. Otilia, B. David, P. Yogeswaran, D. Narender -Prasad and A. Harani, 2010. Inhibitory Effects of Ethanolic Extract of Physalis minima on Amylase, Lipase and Alpha Glucosidase. Research J. Pharmacognosy and Phytochemistry, 2(2): 159-162.
41. Lukaski, H.C., W.A. Siders and J.G. Penland, 2007. Chromium picolinate supplementation in women: effects on body weight, composition and iron status. Nutrition, 23: 187-195.
42. Hallmark, M.A., T.H. Reynolds, C.A. DeSouza, C.O. Dotson, R.A. Anderson and M.A. Rogers, 1996. Effects of chromium and resistive training on muscle strength and body composition. Med. Sci. Sports Exerc., 28: 139-144.
43. Mora, C., M. Diana, N. Arag and F. Luis, 2010. Effects of *Physalis peruviana* fruit extract on stress oxidative parameters in streptozotocin-diabetic rats. Lat. Am. J. Phar., 29(7): 1132-6.
44. Levina, A. and P.A. Lay, 2008. Chemical properties and toxicity of chromium (III) nutritional supplements. Chemical Research in Toxicol., 21: 563-571.