International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Potential Protective Effect of Fortified Camel Milk Products with Chromium on Alloxan Induced Hyperglycemia in Rats

Ayman M. Badawi[1], Mahmoud M. Motawee[2]

[1] Biochemistry Division and [2] Nutrition Division, National Organization for Drug Control and Research, Giza, Egypt

Abstract: ***Oxidative stress and dyslipidemia enhancement are associated with diabetes mellitus (DM). Camel milk (CM), Yogurt-Camel milk (YCM) and Probiotic–Camel milk (PCM) alone or in supplementation with other drug are gaining increasing recognition due to their beneficial effects in prevention many health problems. This study was designed to investigate the protective effect of CM, YCM and PCM alone or in combination with chromium picolinate (CM-CrPi, YCM-CrPi and PCM-CrPi) on alloxan-induced diabetic rats. Animals were randomly divided into eight groups: Group I served as normal control, while animals of group 2 were rendered to diabetes by alloxan injection (150 mg/kg bw, i.p), meanwhile groups 3-8 fed daily with CM products by oral gavage (2 ml/rat) for 8 weeks then diabetes was induced by alloxan injection. CM, YCM and PCM pre-treatment reduced ($p < 0.05$) glycaemia, HbA1C with improved lipids profile, lipid peroxidation, total antioxidant capacity and elevated serum insulin in diabetic rats. A histopathological examination of pancreases tissue was observed. Camel's milk products supplemented with CrPi has potential ameliorated effect in diabetic rats and can protect against hyperglycemia with improve lipid pattern, antioxidant activity and healthy status of diabetic rats***

Keywords: Camel's milk, Yogurt, Probiotic, Chromium, alloxan, Lipid profile, lipid peroxidation

1. Introduction

Diabetes mellitus (DM) represents a major public health concern and is associated with marked increase in morbidity and mortality rate**[1]** . DM is an endocrine metabolic disorders of multiple etiology characterized by chronic hyperglycemia that leads via several mechanism i.e. glucose auto oxidation, stimulation of poly-pathway, activation of reduced nicotinamide adenine dinucleotide phosphate oxidase and production of advanced glycation end products with increased production of reactive species. The resulting oxidative stress can play a key role in diabetes pathogenesis **[2]**.In addition, hyperglycemia in diabetic patient is associated with alteration in lipid metabolism whereas DM is recognized as a major risk factor for cardio-vascular disease**[3]**.Management of DM can involve a number of options, including synthetic drugs , insulin, control of blood glucose with diet and exercise,though some may require medication for hyperglycemia or concomitant cardiovascular disease Camel milk is known of its medical properties which are widely exploited for human health**[4]**.Camel milk is considered to have anti-cancer**[5]**, hypo-allergic **[6],** hepatoprotective **[7]** and anti-diabetic properties**[8,9,10]**, whereas Agrawal *et al*.**[9]**reported that, there is a zero prevalence of diabetes in camel milk consuming due to CM has reach with insulin and insulin like protein**[9]**.There is a growing interest in probiotic interventions for the management and treatment of diabetes**[11]**. Probiotics are defined as living microorganisms in food and dietary supplements that up on ingestion in sufficient amount scan improve the health of the host beyond their basic nutritional content**[12].** In animal studies, it has been confirmed that probiotics treatments inhibits β cells destruction in the islets of Langerhans in diabetic mice**[13, 14, 15].**It was proposed that dairy product are more effective for administration probiotics**[16]** and the probiotic may be useful in therapy and in reducing serum total cholesterol(TC) ,triglycerides (TG), low density lipoprotein cholesterol (LDL-C), lipid peroxidation with increases High-density lipoprotein cholesterol levels(HDL-C)**[17]**. Zhang *et al.***[18]** reported that the probiotic may improve glucose metabolism with potential greater effect when the duration of intervention is ≥ 8 weeks. Chromium is thought to play a key role in carbohydrate metabolism by potentiating the action of insulin**[19, 20]**. It has also in several animal and human studies, chromium complexes of picolinic acid most commonly use as dietary supplement, and has been shown to modulate intracellular pathways of glucose and improve lipids profile in diabetic patients**[21**,**22].**

Therefore, the present study was designed to throw some light on utilization of fortified camel milk yogurt-probiotic bacteria with chromium as protective agent against hyperglycemia, lipid peroxidation and associated hyperlipidemia in alloxan treated rats

2. Materials and Methods

2.1 Materials

- Alloxan monohydratewas purchased from Sigma chemical Company (St Louis Mo, USA) and Chromium picolinate (CHROMIUM®capsules) was obtained from EPACO Co., Cairo, Egypt.
- Skim milk powderand MRS (Agar and broth medium) from OXIOD Co., England.
- Camel milk samples werecollected early in the morning from herdof camels by hand milking

fromElareesh,Sini governrate-Egypt insummer 2014. The samples were collected in sterile screw bottles, kept in cool box until transported to the laboratory, and stored at5±1^{O}Cfor subsequent processing.

- Starter bacteria:

1-Commercial starter cultures: (YOFLEX- YC-X11), which contained *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, were provided by Chr. Hansen (Milwaukee , WIS., U.S.A.) and stored in deep freezer until used.

2-Probiotic bacteria Cultures: *Lactobacillus acidophilus* 142, *Bifidobacterium* SPP 420, *Streptococcus thermophiles* R, were obtained fromDanisco culture Neibull , GmbH- starter culture and Media , D-25899 Niebull, Germany.

- Animals

Male albino rats weighting 150-160 g were obtained from the central animal housing, NODCAR, Egypt. The animals were maintained in standard plastic cages at temperature of 22 ± 1^{O}C and light–dark cycle of 12/12 h in the Animal Housing of Biochemistry Division to acclimatize two weeks before experiment. The animals were fed with commercial pellets and given free access to fresh water *ad libitum*. The experimental protocols were approved according to the Guide for the care and use laboratory animals of local Ethics Committee, NODCAR, Egypt.

2.2. Methods

Milk composition:
The method of A.O.A.C.**[23]** was used to determine total protein, fat, ash and total solid contents in raw camel milk and also the carbohydrates content was determined by difference as described by Ihekoronyand Ngoody **[24]**. The pH of samples was measured using a digital pH meter equipped with a temperature sensor (PH meter model: 3510 Jenway, UK).

Maintenance of probiotic strains:
Probiotic bacteria were adopted according to Taranto *et al*. **[25],** Kimoto et *al*.**[26]**andPigeon *et al*. **[27].**

Preparation of starter culture:
Theday prior to yogurt manufacture, starter culture was made by addition of 2% of*Lactobacillus delbrueckii* ssp. *Bulgaricus* and *streptococcus thermophilu* mother culture into camel milk in different separated container with or without chromium picolonte (360 µg/100ml). Then, stirred well and incubated at 43^{o}C for 5 hours at the end of clotting.

Yogurt making according to Tamime [28] and the manufacture of camel milk products:
Camel milk products were prepared by heating or pasteurizedCM at 75 ^{O}C for 30 minutes and subsequently cooled until 43^{O}C then divided into four separated parts as follow:

1) YCM group:CM was inoculated with 2% w/w of starter culture only.
2) YCM-CrPi group: CM was inoculated with 2%w/w of commercial starter culture plus chromium picolinate (360ug/100 ml).
3) PCM group: CM was inoculated with 2% w/w of starter culture of probiotic culture only.
4) PCM-CrPi group: CM was inoculated with 2% w/w of starter culture of probiotic culture with chromium picolinate (360ug/100 ml).

Camel milk products and CrPi administration:
Each rat was daily administrated camel milk products (2.0ml/rat/day)using oral gavages**[29]** and /or supplemented with chromium picolinate at 36 µg (4.45 µg of Cr)/ kg.bwt. as a recommended rats dose that calculated according to Paget and Barnes **[30]**for eight weeks before alloxan injection.

Induction of hyperglycemia
The animals were fast overnight, and received a single intraperitoneal injection (i.p.) dose of alloxan monohydrates (150 mg /kg body weight), freshly prepared in citrate buffer 0.1M (pH = 4.5) as vehicle**[31]**. Rats were kept on glucose 5% to avoid hypoglycemia that might be occurred. Control rats were received citrate buffer alone. The animals were considered as diabetic on third day after alloxan injection

Experimental Protocol
A total of eighty male rats were divided randomly into equal eight groups (ten rats each). Rats were daily received orally Camel's milk and / or fortified milk products using oral gavages for eight weeks before alloxan injection as follows: Group (G1)negative control,group (G2) positive control (diabetic rats),group (G3) rats fed with raw camel milk (CM),group (G4) rats fed with CM supplemented with CrPi, group (G5) Rats fed with YCM, group (G6) rats fed with YCM supplemented withCrPi, group (G7) rats fed with PCM and group (G8) rats fed with PCMsupplemented with CrPi.After treatment period for eight weeks, rats in groups 2-6 injected with alloxan to induced hyperglycemia, on third day after alloxan injection, the animals were fasted over night before being anesthesia with ethyl ether and the blood samples were collected. The serum and whole blood with EDTA were used for various biochemical analyses.

Biochemical analysis
Samples were analyzed for the following biochemical parameter: blood glucose **[32]**, insulin **[33]** and HbA1C **[34]**. Lipids profile including total lipids **[35],**total cholesterol **[36]**, triglycerides **[37]** and high-density lipoprotein cholesterol [38] and alsolow-density lipoprotein cholesterol was calculated according toFriedewald *et al*.**[39]**.Lipidperoxidation in the term of malondialdehyde (MDA) level was determined according to Wills**[40]** using 1, 1, 3, 3, tetraethoxypropane as standard and the total antioxidant capacity (TAC)was measured according to Koracevic *et al.***[41]**.

Histopathological studies
The pancreas of each rat were examined grossly for histological study. The tissues were washed with normal saline and immersion fixed immediately upon removal in 10% formal saline for twenty four hours, proceeds to paraffin sections, stained with hematoxyline and eosin and examined under light microscope **[42].**

Statistics Analysis

All the values in the test are presented as means ± standard error,SE (n= 6). Statistical differences between the various groups were evaluated using one-way analysis of variance (ANOVA) and followed by Duncan' multiple comparison test. Values at $P < 0.05$ were considered significant.The data were analyzed using statistical package for social science (SPSS version 21.0).

3. Results

Gross composition of camel milk:

Camel's milk is opaque white and normal odour and salty taste. The compositions of examined chemical characteristics of fresh collected camel milk were depicted in Table 1

Table 1: Chemical composition of fresh camel milk (± SE)

Sample	pH	Chemical composition (%)				
		Ash	Protein	Fat	Total carbohydrates	Total Solid
Bulk Camel milk	6.6 ± 0.1	0.80 ± 0.02	3.8±0.1	3.2 ± 0.1	5.4 ± 0.1	13.2 ± 0.1

The obtained data in Figures 1-3 revealed that, alloxan injection significantly increased ($P<0.05$) serum glucose level, HbA1C , TG, TC, LDL-C and plasma lipid peroxidative index in the term of malondialdehyde (MDA) . Alloxan injection led to a significant decrease serum insulin, HDL-C levels and total antioxidant capacity.

The protective effect of raw CM and CM products with or without CrPi supplementation on glucose, HbA1C and insulin levels:

The protective effect of CM, YCM and PCM administration with or without CrPi supplementation on glucose, HbA1c and insulin levels are presented in Figure 1 data depicted in Figure 1a revealed that, the Pre-administration of the examined products caused a significant reduction in glucose level. The obtained results also revealed that YCM, PCM and their fortified products with CrPi pre-administration induced more pronounced decreasing effect by about 53-68% respect to diabetic group. This reduction in glucose level is associated with a significant reduction in glycosylated hemoglobin (Figure 1b).The obtained data revealed that CM supplemented with CrPi elicited a more pronounced decreasing effect (36%) while YCM and YCM-CrPi pre-administration reduced HbA1C by about 22-26% with respect to diabetic group. Meanwhile CM, PCM and PCM-CrPi administration before alloxan injection elicited 16, 15 and 18% respectively, decreasing effect with regard to diabetic group (Figure 1b).Figure 1c revealed the efficiency of the examined materials in protecting β cells against the deleterious effect of alloxan through improving the insulin level when compared with alloxan injected rats. More protective effect was induced by the pretreatment with PCM-CrPi and YCM-CrPi before alloxan injection the induction of diabetes. The obtained data revealed the efficiency of CM or CM products in controlling the blood glucose level against the harmful effect of alloxan.

The protective effect of raw CM and CM products with or without CrPi supplementation on serum lipid profile:

Figure 2shows that Alloxan injection markedly increased serum lipid profile in association with HDL-C reduction ($p<0.05$). CM products supplemented with or without CrPi caused a marked protection on S.TC level by about 34 % for YCM-CPi and PCM pre-administration (Figure 2a), while the other camel's milk products pre-administration induced a marked protection by about 21-29 % with respect to diabetic rats.

As regard to Figure 2b, CM, YCM-CrPi and PCM pre-administration significantly increased HDL-C level compared to diabetic control. Meanwhile, rats fed with CM-CrPi elicited a more protection by about 58% with respect to serum TG compared to diabetic control (Figure 2c), while rats pre-administered with CM, YCM-CrPi, YCM and PCM- CrPi for eight weeks before alloxan injection showed a marked reduction in serum TG by about 36-39% compared to diabetic rats.PCM-CrPi and YCM pre-administration for eight weeks before alloxan injection induced a reduction in LDL-C by about 36 and 20% (Fig. 2d), meanwhile, YCM-CrPi, PCM and CM pre-administration caused a more pronouncedreduction in LDL-C levels.Figure 2e shows that CM and CM milk products pre administration with or without CrPi on serum total lipid significantlyreduce total lipids levels when compared with diabetic control. The obtained results revealed that rats pre-administered of fortified milk products with CrPi induceda more pronounced effect in reducing total lipid (40-52%) compared diabetic control.

The protective effect of raw CM and CM products with or without CrPi supplementation on malondialdehyde and total antioxidant capacity levels:

Figure 3 shows that alloxan injection caused a significant elevation on MDA with markedly reduction in TCA .The protective effect of raw CM and YCM, PCM (camel milk products) with or without CrPi pre administration before the induction of diabetes on lipid peroxidation (MDA) is shown in Figure3a. The obtained results revealed that CM and YCM alone or in combination with CrPi caused a well-marked protective effect against the generation of oxidative stress as detected by lowering MDA levels. The obtained data revealed that CM or YCM in combination with CrPi showed a more pronounced decrease in MDA levels (39.6 and 52.8%, respectively) while the pre-administrate of CM or YCM alone induced a 19.8 and 34.9%, decrease in MDA level with respect to alloxan treated rats. Meanwhile, the pre-administer of camel milk products with or without CrPi before the induction of diabetes elicited a marked improvement($p<0.05$) in TAC level (Figure 3b) and the data reveled that YCM-CrPi induced the most pronounced effect (60%) while other dairy treatments caused elevation in TAC by about (33-47%) compared with diabetic control.

4. Histological Observations

The histological examination of pancreatic tissue revealed that, healthy rats showing normal histological structure of the islands of Langerhans cells as endocrine portion and acini as exocrine one (Fig. 4A). As regard to Figure 4B Alloxan injection caused a sever atrophy in island of Langerhans cells with degenerative change and reduction in number and size with focal inflammation cells infiltration in interlobular connective tissue. Meanwhile, the pre-administration of CM showed mild degenerative change in pancreatic islets with nearly intact histological and inflammatory cells infiltration was detected in the islands Langerhans cells with CM-CrPi pretreated rats (Fig. 4C and 4D).The pre-administration with YCM or YCM-CrPi for eight week before alloxan injection showed mild atrophy, moderated size in island of Langerhans cells with focal inflammatory cells infiltration surrounding the blood vessel associated with fibrosis surrounding the pancreatic islets and fatty degenerative changes in rats with YCM-CrPi pre-administration and displayed mild atrophy in Langerhans cells (Fig. 4E and 4F). The pre-administration of PCM and PCM-CrPi showed intact of Langerhans cells with focal inflammation and hemorrhage (Fig. 4G and 4H).

5. Discussion

This study was performed to evaluate the efficacy of raw Camel milk, CM-products (YCM and PCM) and in supplementation with chromium picolinate (CM-CrPi, YCM-CrPi and PCM-CrPi) on protection against hyperglycemia, dyslipidemia and oxidative stress induced by alloxan injection.The obtained results revealed that, the single i.p injection of alloxan was able to induce a producible model of diabetic rats that had elevated glucose, HbA1C in association with insulin deficiency. It has been reported that, alloxan action in the pancreas is preceded by its rapid uptake by pancreatic β-cells that have been proposed to be one of the important features determining alloxan diabetogenicity **[43]**.The histopathological examination of the pancreas three days after alloxan injection shows degenerative change in pancreatic islets with reduction in their size compared with non-treated rats (Figure 4 A and B). These findings are in the harmony with the results ofAlam *et al.* **[44]** and Shajeela *et al*. **[45]**who assessed the role of alloxan in the induction of chemical diabetes by damaging the insulin secreting pancreatic β cells resulting in a decrease in the endogenous insulin release.

This work demonstrates that, administration of raw CM alone before alloxan injection markedly lowered glucose levels and protected β cells from the deleterious effect of alloxan as evidenced by alleviating insulin deficiency. These findings are in accordance with that obtained by Sboui *et al*.**[46]**who reported the effectiveness of raw or pasteurized CM in treatment of diabetic dogs.

Indeed, the potent protective role of CM against alloxan induced hyperglycemia is not of our expectation with respect to the high insulin and insulin content in camel milk as previously reported **[9,47]**. In addition, it has been reported that CM does not form coagulum in acidic environment of the stomach, which may in turn provides a rapid CM with its specific insulin like protein / insulin through stomach that remains available for absorption in intestine **[10]**. Moreover, it has been reported that, unlike human immunoglobin, which has a more complex structure, with two light chains contains bound to the heavier Y-shaped main chain, Camel immunoglobin has only the main Y- shaped heavy chain without these additional parts **[48]**. The Camel's antibodies find it easier to penetrate to the enzyme active sites; this action of camel immunoglobin present in milk might offer a better action of other protein like insulin**[9, 10, 49]**.

In the present work pre administration of CM supplemented with Chromium successfully decreased blood glucose level with respect to alloxan injected rats, **[50]**.Stifflerr*et al*.**[50]** reported that, a biological active form of chromium enhance the effect of insulin on glucose metabolism through the enhancement of insulin receptors activity towered glucose utilization. According to the studies of Jain*et al.* **[51]** and Seluck *et al*. **[52]** CrPi bears an anti-inflammatory activity that could be contributed to the effectiveness of CrPi in reversing some abnormalities caused by alloxan.

More importantly CM was not pre-administered alone in the current study but rather it was supplemented as camel milk's products (YCM and PCM) with or without CrPi which potentiated the protective effect of CM. The obtained data revealed the efficacy of CM contained probiotic bacteria in protecting against alloxan-induced hyperglycemia. It has been reported that, probiotic inhibit the depletion of insulin secretion with the protection of pancreatic β cells from damage, probiotic may delay the alterations in glucose hemostasis by maintaining insulin level as described by Yadav *et al*. **[53]** and it has been also suggested that, the probiotic displayed compositional changes of the intestinal microflora in diabetes that leads to improve blood glucose and improves insulin-binding through the inhibition of β cells destruction **[54]**. The addition of CrPi to yogurt or probiotic significantly potentiates the protective effect of yogurt and probiotic pre-administration. Our data underscore the complementary benefit effect of combining of raw CM or yogurt made and probiotic addition with chromium picolinate in partially protecting β cells.

Glycosylated hemoglobin (HbA1C) determination is self-monitoring of blood glucose therefore, play an important complementary role for the management of diabetes **[55]**. The recorded datarevealed that, the increase in the level of glycosylated hemoglobin in alloxan-injected rats was due to the presence of excessive concentration of blood glucose.The pre-administration of CM, YCM and PCM either alone or in combination with CrPi significantly decreased HbA1C levels. These results indicate the beneficial protective effects of the examined materials incontrolling blood glucose level.

DM is associated with profound alterations in the blood lipid and lipoprotein profiles **[56,57]** in the current study, alloxan injection caused a significant increase in the levels of plasma total lipid, TC, TG and LDL-C levels in association with decrease in HDL-C level. This disturbance in lipid profile of diabetic rats could predict risk factor for cardiovascular disease and could be attributed to increased mobilization of free fatty acids from adipose tissue [**58,59]**. The obtained increase in triglycerides in diabetic rats could be attributed to increase in the activity of hormone sensitive lipase, which catalysis the mobility of free fatty acids from triacylglycerol stored in adipocyte as described by Almeide *et al.* **[60]**. Meanwhile the obtained increase in LDL-C could be resulted from glycosylation of lysyl residues of apoprotein B, which leads to the reduction in LDL-C metabolism due to a decrease in the affinity of LDL-C for its receptors **[59].**

Pretreatment of normal rats with CM, YCM and PCM either alone or in combination with CrPi produced profound protection against the alterations in serum lipid profiles that resulted from alloxan injection. These results are in agreement with the work of Hellal *et al*. **[61]**who found that CM exhibited a protective effect and lowered total lipids, TC, TG and LDL-C.Strains of the genera *Lactobacillus acidophilus*, *Bifidobacterium* SPP are the most widely used probiotic bacteria **[62]**. In the present study, the pre- administration of probiotic withfermented CM produced a markedreduction in lipid profile that reflects the role of probiotic in increasing the lipid lowering effect of CM. This finding confirmed that concluded by Yadav *et al.* **[63]** who found that *Lactobacillus acidophilus* and *Lactobacillus casie* significantly delayed hyperglycemia and dyslipidemia**.** This potentiating role could be attributed to the production of short chain fatty acid by probiotic upon fermentation that decrease the cholesterol synthesis**[64]**. It has been proposed that some strains of *Lactobnacilalus* assimilate or incorporate some of the cholesterol removal from medium into the cellular membrane during growth **[66]**. As a result cholesterol incorporated into or adhered to the bacterial cells would be less accessible for absorption from the intestine into the blood **[66]**.The obtained results of the current study demonstrates that, both CM-yogurt (YCM) and probiotic (PCM) had positive effect on lipid profile. It may be pointed out that the observed effect on HDL-C could be the result of sphingolipids in yogurt and in cell membranes of probiotic bacteria. Sphingolipids can be found in lipid–rich structure and have effects on cholesterol metabolism and transport **[67**]. Previous studies **[21, 68, 69]** have demonstrated that, the treatment with chromium may reduce serum lipid levels in diabetic conditions. Chromium trivalent has also been reported to elevate adiponectin level and to inhibit the inflammatory pathways in diabetic rats **[51]**, that could be play a role in modulating lipase sensitive hormone. The obtained results revealed that the pre-administration of CM with CrPi reduced glucose levels and increased insulin content, decrease TC and TG levels this clearly proved the effectiveness of this trace element on abnormal blood lipids together with lowering of glucose levels **[70]**.

In the present work, elevated level of MDA was recorded in alloxan injected rats which indicates the formation and propagation of free radicals that lead to the generation of oxidative stress. This increase in plasma MDA level is associated with a decrease in TAC. These results reflect the destruction of β calls and the mobilization of free fat depots that caused the destabilization, disintegration and alteration in membrane fluidity which leads to cell lysis **[59, 71]**. The obtained decrease in TAC of diabetic rats reflects the cumulative damaging effects of alloxan against blood antioxidant. Based on the present work the ability of CM products either alone or in combination with CrPi to protect against the destructive effect of diabetes is a clear indication of the antioxidant properties of the examined materials. CM was found to contain high concentration of vitamins A, B2, C and E and is very rich in magnesium and other trace elements **[7, 72]**.The combination of CM with probiotic and CrPi exhibited the most pronounced protective effect against alloxan–induced MDA rise which reflects the efficacy of probiotic and CrPi in regulating the redox status of diabetic rats. It has been reported that, the overall protective effect of probiotic could be attributed to their ability to produce antioxidant enzyme like SOD, metal chelating activities and promote the production of antioxidant bio-molecules such as exopolysacchrides that showing *invitro* antioxidant and free radical scavenging activities **[73]**. Several authors hypothesized that, probiotics exert their protective effects against oxidative stress by restoring gut microbiota **[74].**

The obtained results revealed that , the pre-administration of CM , fermented CM (yogurt, YCM) and probiotic (PCM) with or without CrPi supplementation before alloxan – injection exhibited a significant protective effect through reduction of hyperglycemia in association with marked elevation of insulin level, dyslipidemia as well as attenuation of oxidative damage resulted from alloxan-induced debates.

However, the pre-administration of fermented CM supplemented with probiotic and / or CrPi elicited a more pronounced effect with respect to glucose and insulin level, the histological examination of pancreatic tissue in the different examined groups revealed the efficiency of raw CM milk or fortification with CrPi (Figure 4C and D) in modulating the pancreatic tissue.

In conclusion, the fortification of the pre-administered camel milk, yogurt and probiotic with Chromium picolinate plays an efficient protective role against hyperglycema, associated hyperlipidemia and oxidative stress with restoring ROS-antioxidant balance

6. Acknowledgment

The authors thank Professor Adel Bakeer, Histopathology department, Faculty of Veterinary Medicine, Cairo University for his kind help.

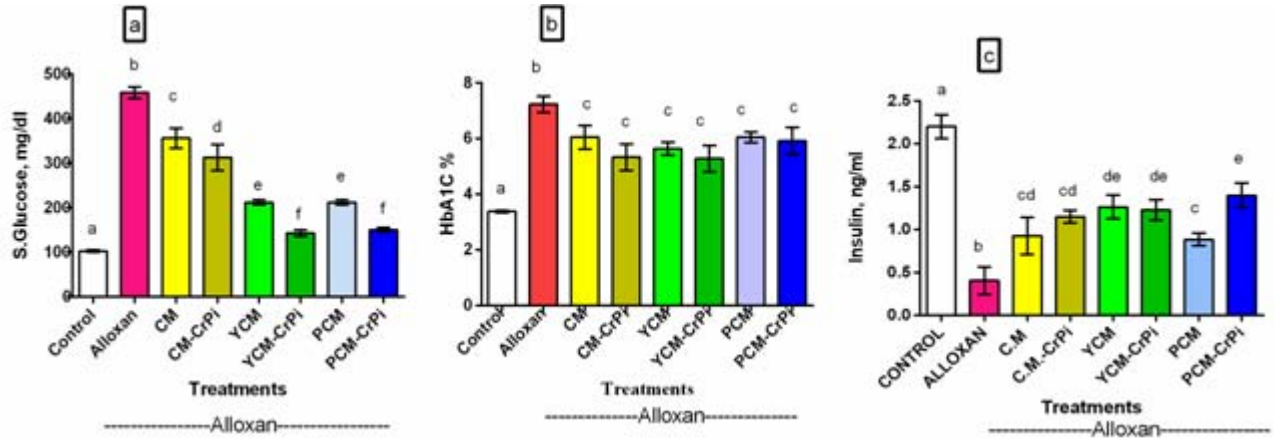

Figure 1: The prophylactic effect of fortified camel milk products with chromium picolonate on blood glucose, HbA1C% and insulin levels against alloxan induced diabetes. Values are expressed as mean ± SE (n=6). Mean values with different superscripts are significant (P<0.05).

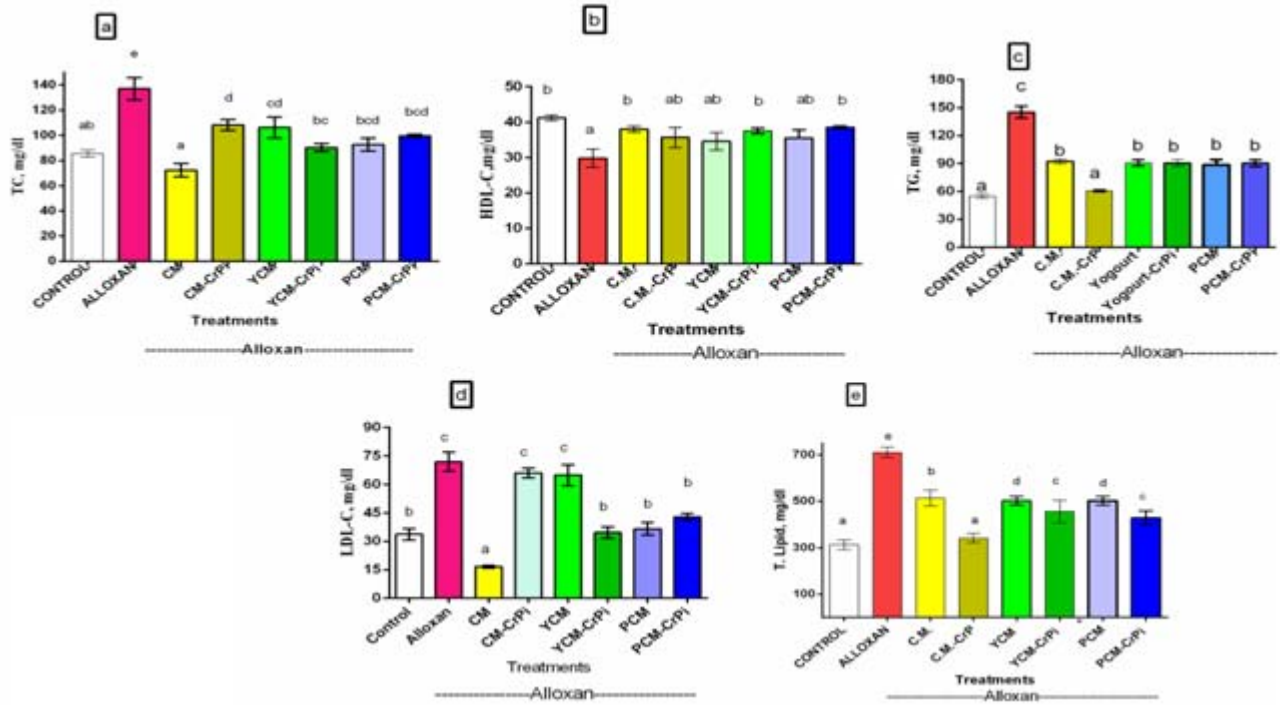

Figure 2: The prophylactic effect of fortified camel milk products with chromium picolonate on blood lipids profile level against alloxan induced diabetes. Values are expressed as mean ± SE (n=6).Mean values with different superscripts are significant (P<0.05).

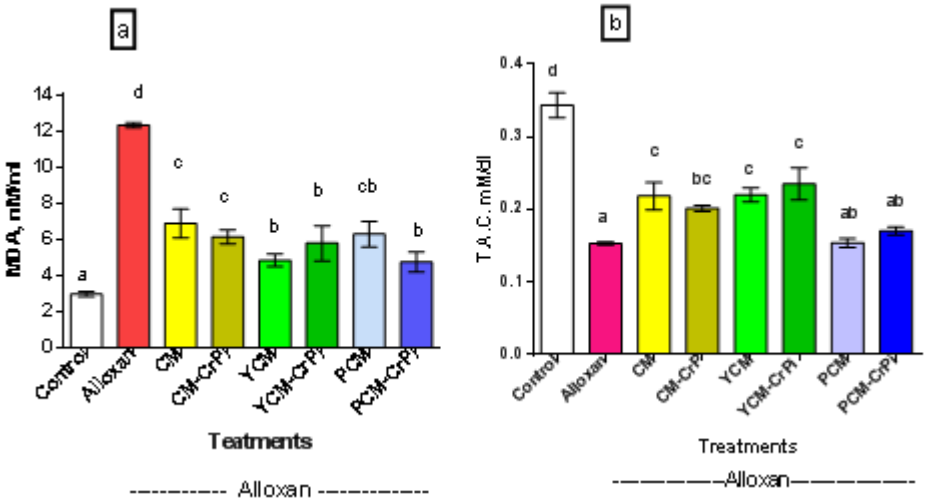

Figure 3: The prophylactic effect of fortified camel milk products with chromium picolonate on blood MDA and TAC levels against alloxan induced diabetes. Values are expressed as mean ± SE (n=6). Mean values with different superscripts are significant (P<0.05).

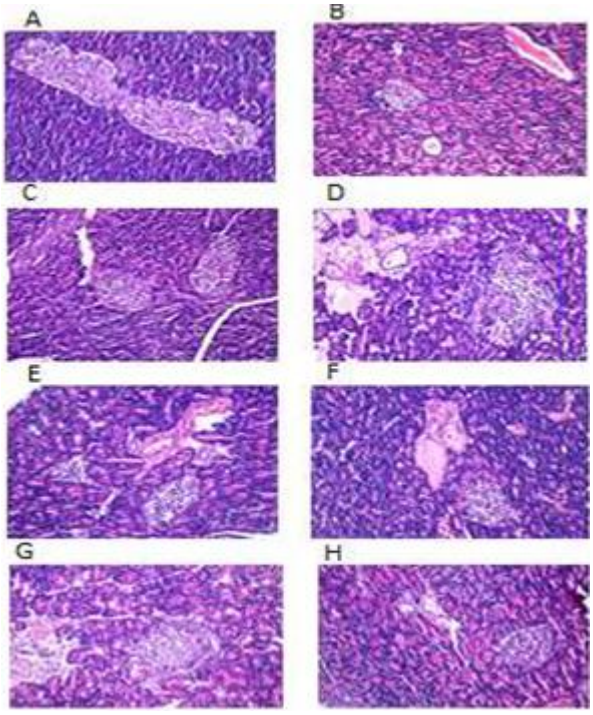

Figure 4: HE of pancreas.(A) Normal control showing nearly normal intact histological of Langerhans island cells; (B) diabetic control showing sever atrophy with dilated sinusoids and fatty hepatocytes;(C) CM pre-administration showing nearly normal intact histological of Langerhans island cells; (D)CM-CrPi pre-treatment showing inflammation cells infiltration, in stroma with adema in the Langerhans cells and normal intact structural.(E)YCM-pre-treated moderate size in island of Langerhans cells. (F) YCM-CrPi showing moderate atrophy in island of Langerhans cells (G) PCM pre-administration showing nearly normal intact histological structure in island of Langerhans cells. (H) PCM-CrPi, showing nearly normal intact histological structure in island of Langerhans cells (**X**40).

References

[1] Taheri E, DjalaliM, Saedisomeolia A, MoghadamA, Djazayeri A, Qorbani M. The relationship between theactivates of antioxidant enzymes in red blood cells and body mass index in Iranian type 2 diabetes and healthy subjects. J Diabetes Metab Disord 2012; 11, 3:1-5.

[2] Tiwari BK, Pandey KB, Abidi AB and Rizvi SI. Markers of Oxidative Stress during Diabetes Mellitus. J Biomarkers 2013;2013 Article ID 378790, 8 pages.

[3] Martín-Timón I, Sevillano-Collantes C, Segura-Galindo A, del Cañizo-Gómez FJ.Type 2 diabetes and cardiovascular disease have all risk factors the same strength?.World J Diabetes 2014; 5(4): 444-470.

[4] Yadav AK, Kumar R, Priyadarshini L, Singh J. Composition and medicinal properties of camel milk: A Review. Asian J Dairy & Food Res 2014; 34(2): 83-91.

[5] Magjeed, NA. Corrective effect of milk camel on some cancer biomarkers in blood of rats intoxicated with aflatoxin B1. J Saudi Chem Soc 2005;9(2): 253–263.

[6] Shabo Y, Barzel R, Margoulis M, Yagil R. Camel milk for food allergies in children. Immunology and Allergy 2005; 7:796–798.

[7] Al-Fartosi KG, Majid A, Mohammed A. Auda MA and Hussein MH.The Role of Camel's Milk against Some Oxidant-AntioxidantMarkers of Male Rats Treated With CCl4. Int J Res Pharm Biomed Sci 2012;3(1):385-389.

[8] Agrawal RP, Sahani MS, Tuteja FC, Ghouri SK, Sena DS, Gupta R and Kochar DK. Hypoglycemic activity of camel milk inchemically pancreatectomized rats-An experimental study; Int J Dia Dev Countries 2005a; 25:75-79.

[9] Agrawal RP, Budania S, Sharma P, Rajeev Gupta, Kochar DK, Panwar RB and Sahani MS . Zero prevalence of diabetes in camel milk consuming Raico community of north-west Rajasthan,India. Diabetes Res Clin. Pract. 2007;76:290-296.

[10] Khan AA, Alzohairy MA, Mohieldein AH. Antidiabetic effects of camel milk in streptozotocin-induced diabetic rats. Am J Bioch and Mol Biolo 2013;3(1):151-158.
[11] Yadav H, Jain S, Yadav M. Probiotics and diabetes / obesity. Bioactive Food as Dietary Interventions for Diabetes. Bioactive Foods in Chronic Disease States 2012;307–317
[12] FAO/WHO (2001):"Regulatory and clinical aspect of dairy probiotic."
[13] Yadav H, Jain S, Sinha PR. Effect of Skim Milk and Dahi (Yogurt) on Blood Glucose, Insulin and Lipid Profile in Rats Fed with High Fructose Diet. J Med Food 2006; 9(3): 328–335.
[14] Andersson U, Branning C, Ahrné S, Molin G, Alenfall J, Onning G, Nyman M, Holm C. Probiotics lower plasma glucose in the high fat fed C57bl/6j mouse. Benefic. Microbes 2010; 1(2): 189–196.
[15] Huang HY, Korivi M, Tsai CH, Yang JH, Tsai YC.Supplementation of *Lactobacillus plantarum* K68 and Fruit-Vegetable Ferment along with High Fat-Fructose Diet Attenuates Metabolic Syndrome in Rats with Insulin Resistance.Evid Based Complement Alternat Med. 2013; 943020. doi: 10.1155/2013/943020. Epub 2013 Apr 16.
[16] Lewis, SJ, Burmeister S. A double-blind placebo-controlled study of the effects of Lactobacillus acidophilus on plasma lipids. Eur J Clin Nutr 2005;59:776–780.
[17] Tsai CC , Chou LC, Lai SE, Huang CC. Effect of cholesterol lowering multiplex lactic acid bacteria on lipid metabolism in a hamster model. Afr J Microbiol Res 2016;10(20):709-716.
[18] Zhang Q, Wu Y, Fei X. Effect of probiotic on glucose metabolism in patients with type 2 diabetes mellitus: Ameta-analysis of randomized controlled trials. Medicina (Kaunas) 2016;52(1):28-34. doi: 10.1016/j.medici.2015.11.008.
[19] Sreejayan N, Dong F, Kandadi MR, Yang X, Ren J. Chromium Alleviates Glucose Intolerance, Insulin Resistance, and Hepatic ER Stress in Obese Mice. Obesity 2008; 16(6):1331–1337.
[20] Ghadieh HE, Smiley ZN, Kopfman MW, Najjar MG, Hake MJ, Najjar SM. Chlorogenic acid/chromium supplement rescues diet-induced insulin resistance andobesity in mice. Nutr Metab (Lond) 2015;22; 12:19.
[21] Cefalu WT, Wang ZQ, Zhang XH, Baldor LC and Russell JC. Oral chromium picolinate improves carbohydrate and lipid metabolism and enhances skeletal muscle Glut-4 translocation in obese, hyperinsulinemic (JCR-LA corpulent) rats. J Nutr (2002) ;32:1107–1114.
[22] Cefalu WT, Hu FB. Role of chromium in human health and in diabetes. Diabetes Care 2004; 27:2741–2751.
[23] AOAC (2005) "Official Methods of Analysis" Association of Official and Analytical Chemists Washington DC (12th ed)
[24] Ihekoronye AI, Ngoddy PO. Integrated Food Science and Technology. Macmilian Publishers, New York. 1985; Pg. 296-301.
[25] Taranto MP, Medici M, Perdigon G, Ruiz Holgado AP, Valdez GF. Evidence for hypocholesterolemic effect of Lactobacillus reuteri in hypercholesterolemic mice. J Dairy Sci 1998;81(9):2336-2340.
[26] Kimoto H, Ohmomo S, Okamoto T. Cholesterol Removel from Media by Lactococci. J Dairy Sci 2002;85:3182-3188.
[27] Pigon RM, Cuesta EP, Gilliland SE. Binding of free Bile Acids by cells of Yoghurt Starter Culture Bacteria J Dairy Sci 2002;85:2705-0710.
[28] Tamime AY. Starter Cultures. In: Tamime AY, editor. Fermented Milk. Blackwell Science Ltd; Oxford, UK. 2006; Pp.11-47.
[29] Isa SA, Ibrahim FG, Abubakar I. Effect of Camel Milk's Supplementation on Serum Glucose Levels, Lipid Profile and Body Weight of Alloxan-Induced Diabetic Rats. Nigerian J Basic Appl Sci 2013;21(3): 187-191.
[30] Paget GE, Barnes JM. In: "toxicity tests" volume (1) chapter (6) pp 135. Editor Laurance, D.R. and Bacharach A.L. Academic press, London, New York 1964.
[31] Tripathi UN, Chandra D. The plant extracts of Momordica charantia and Trigonella foenum graecum have antioxidant and anti-hyperglycemic properties for cardiac tissue during diabetes mellitus. Oxid Med and Cell. Longevity 2009;2:290–296.
[32] Trinder P. Determination of blood glucose using an oxidase-peroxidasesystem with a non-carcinogenic chromogen. J Clin Pathol 1969;22(2):158-61.
[33] Temple RC, Clark PM, Hales, C.N. Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. Diabetic Med 1992;9: 503-512.
[34] Geiger M, Binder BR. Nonenzymatic glycosylation as a contributing factor to defective fibrinolysis in diabetes mellitus. Homeostasis 1986; 16:439-446.
[35] Knight JA, Anderson S, Rawele JM. Chemical base of the sulfo-phosphate vaniline reaction for estimating total lipid. Clin. Chem. 1972; 18(3):723.
[36] Richmond W. Preparation and properties of a cholesterol oxidase from Nocardis sp., and its application to the enzymatic assay of total cholesterol in serum.Clin. Chem. 1973;19:1350-1356
[37] Fossati, P, Prencipe L. Serum trigycerides determined colorimetrically with an enzyme that produces hydrogen peroxide, Clin. Chem. 1982; 28:2077-2080.
[38] Burstein M, Scholnick HR, Haarfin R. Rapid method for isolation of lipoprotein from human serum by precipitation with polyamine. J Lipid Res. 1970; 11(6):583-95.
[39] Friedewald WT, Levy RT, Frederickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge Clin. Chem. 1972;18: 499-502
[40] Wills ED. Evaluation of lipid peroxidation in lipids and biological membranes. In: Biochemical toxicology: A practical approach. Edited by Snell K, Mullock B. Oxford: IRL Press. 1987; Pp.127-152.
[41] Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin. Path. 2001;54: 356-361.

[42] Banchroft JD, Steven A, Turner DR. Theory and practice of histological technique. Fourth Ed. Livingstone, New York, London, San Francisco, Tokyo 2001.
[43] Rohilla A, Ali S. Alloxan Induced Diabetes: Mechanisms and Effects. Int J Res Pharm and Biomed. Sci. 2012; (2):319-321.
[44] Alam MA, Subhan N, Chowdhury SA, Awal MA, Mostofa M, Rashid MA, Hasan CM, Nahar L and Sarker SD. Anthocephalus cadambaextract shows Hypoglycemic effect and eases oxidative stress in alloxan-induced diabetic rats. Braz J Pharmacogn 2012; 21(1):155-164.
[45] Shajeela. PS, Kalpanadevi V, Mohan VR. Potential antidiabetic, hypolipideamic and antioxidant effects of Xanthosoma Sagittifolium extract in alloxan induced diabetic rats. Int J Pharm Sci 2012;5 (Suppl 1):27-31.
[46] Sboui A, Khorchan T, Djegham M, Agrebi A, Dalleli A, Belhad O. Camel Milk as Adjuvant to Treat Alloxan Diabetes: Effect of Heat Treatment on this Property. J Diabetes Metab 2012; 3:190. doi:10.4172/2155-6156.1000190
[47] HamadEM, Abdel-Rahim EA, Romeih EA.Beneficial Effect of Camel Milk on Liver and Kidneys Function in Diabetic Sprague-Dawley Rats. Int J dairy sci 2012; 6(3):190-197.
[48] Hamers-Casterman C, Atarhouch T, Muyldermans S, Robiinson G, Hamers C, Songa EB. Naturally occurring antibodies devoid of light chains. Nature 1993; 363:446 – 448.
[49] Agrawal, RP. Beniwal, S. Sharma, Kochar DK, Tuteja FC, Ghorui SK, Sahani MS. Effect of raw camel milk in type 1 diabetic patient: 1 year randomized , J Camel Pract and Res 2005b;12(1):27-35
[50] Striffler JS, Polanesky MM, Anderson RA. Chromium improves insulin response to glucose in rats. Metabolism 2001; 44: 1314-1320.
[51] Jain SK, Croad JL, Velusamy T, Rains JL, Bull R. Chromium dinicocysteinate supplementation can lower blood glucose, CRP, MCP-1, ICAM-1, creatinine, apparently mediated by elevated blood vitamin C and adiponectin and inhibition of NF kappa B, Akt, and Glut-2 in livers of zucker diabetic fatty rats. Mol Nutr Food Res 2010; 54(9):1371-80.doi: 10.1002/mnfr.200900177
[52] Selcuk MY, Aygen B, Dogukan A, Tuzcu Z, Akdemir F, Komorowski JR, Atalay M,Sahin K. Chromium picolinate and chromium histidinate protects against renaldysfunction by modulation of NF-kappaB pathway in high-fat diet fed andStreptozotocin-induced diabetic rats. Nutr Metab (Lond) 2012; 9:30.
[53] Yadav H, Jain S, Sinha PR. Oral administration of dahi containing probiotic Lactobacillus acidophilus and Lactobacillus casei delayed the progression of streptozotocin-induced diabetes in rats. J Dairy Res., 2008; 75:189–195.
[54] Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Abu Al-Soud W, Sørensen SJ, Hansen LH, Jakobsenl M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLOS One 2010;5(2): e9085
[55] Thai AC, Yeo PPB, Chan L, Wang KW, Tan BY, Jocobs E. Glycosylated hemoglobin and diabetic control. Singapore Med J 1983; 24(4): 210-212.
[56] Silva M, Lima WG, Silva ME, Pedrosa ML. Effect of streptozotocin on the glycemic and lipid profiles and oxidative stress in hamsters. Arq Bras Enocrinol Metab 2011; 55:46-53.
[57] Chinonyel AS, Ikechukwu OA, Ijioma IS. Glucose level hematological parameters and lipid profile in *Ficus Sur* treated diabetic rats. J Agric Biol Sci 20114; 2(1):5-11.
[58] Elberry AA, Harraz FM, Ghareib SA, Gabr SA, Nagy AA, Abdel-Sattar E. Methanolic extract of *Marrubium vulgare* ameliorates hyperglycemia and dyslipidemia in streptozotocin-induced diabeticrats. Int J Diabetes Mellitus 2011;3 (1):37-44
[59] Rajeswari G, Rajagopalan V. Evaluation of anti-diabetic effects of *Chrysopogon zizanioides* Linn root extracts in streptozotocin induced diabetic wistar rats. Journal of Scientific and Innovative Research 2013; 2 (3): 555-574.
[60] Almeida DA, Braga CP, Novelli EL, Fernandes AA. Evaluation of lipid profile and oxidative stress in STZ-induced rats treated with antioxidant vitamin. Braz Arch Biol Tech 2012; 55(4):527-536.
[61] Helal EG, Abd-Elwahab SM, Mohammed AA. Effect of camel milk on allxan-induced diabetic rats. Egypt J Hos Med 2012; 49:539-554.
[62] Tannock GW. New perceptions of the gut microbiota: Implications for future research. Gastroenterol Clin North Am 2005; 34:361–382.
[63] Yadav H, Jain S, Sinha PR. Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats.Nutrition, 2007; 23(1): 62–68.
[64] El-Khamisy AS. Effect of *Bifidobacterium* and *Lactobacillus acidophilus* in diabetic rats. The 5th Arab and 2nd International Annual Scientific Conference; Mansoura University, Egypt. 2010.
[65] Noh DO, Kim SH, Gilliland SE. Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus*ATCC43121 J Dairy Sci 1997; 80:3107-3113.
[66] Amina Z, Noureddine S, Venkatesh A, Perumal V, Hichem B, Asma Z, Yamina M, Miloud H, Mebrouk K. Characterizatiin and potential Probitic attributes of lactobacillus plantarum DU10 isolated from Algeriam Camel Mik. Biotechnology 2014; 13(6):282-288.
[67] Vesper H, Schmelz EM, Nikolova-Karakashian MN, Dillehay DL, Lynch DV,Merrill AH Jr. . Sphingolipids in Food and the Emerging Importance of Sphingolipids to Nutrition. J Nutr 1999; 129(7): 1239–1250.
[68] Clodfelder BJ, Gullick BM, Lukaski HC, Neggers Y, Vincent JB. Oral administration of the biomimetic [Cr3O (O2CCH2CH3)6(H2O)3] increases insulin sensitivity and improves blood plasma variables in healthy and type 2diabetic rats. J Biol Inorg Chem 2005; 10:119–130.
[69] Yang X, Li S-Y, Dong F, Ren J, Sreejayan N. Insulin-sensitizing and cholesterol-lowering effects of chromium (D-Phenylalanine). J Inorg Biochem 2006; 100:1187–1193.

[70] Joseph E, DiSilvestro R, Carcache de Blanco EJ. Triglyceride lowering by chromium picolinate in type 2diabetic people. Int J Nutr and Metab. 2015; 7(2):24-28.
[71] Okoro, I.O.; Umar, I.A.; Atawodi, S.E. and Anigo, K.M. Antidiabetic effect of *Cleome rutidosperma* DC and *Senecio biafrae* (oliv. & hiern) extracts in streptozotocin induced diabetic rats. IJPSR 2014; 5(6): 2490-2507
[72] Yousef MI. Aluminum-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid.Toxicology 2004;199(1):47-57
[73] Davis CD, Milner JA. microflora, food componentsand colon cancer prevention. J Nutr Biochem 2009; 20:743–752.
[74] Amaretti A, di Nunzio M, Pompei A, Raimondi S, Rossi S, Maddalena M, Bordoni A. Antioxidant properties of potentially probiotic bacteria: *in vitro* and *in vivo* activities Appl Microbiol Biotechnol 2013; 97:809–817.