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

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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 monohydratet was 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.
Animals

- 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 thermophilus R, were obtained from Danisco culture Neibull, GmbH- starter culture and Media, D-25899 Nieboll, 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°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 Ihekoroanyd 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] and Pigeon et al. [27].

Preparation of starter culture:
The day prior to yogurt manufacture, starter culture was made by addition of 2% of Lactobacillus delbrueckii ssp. Bulgaricus and streptococcus thermophilus mother culture into camel milk in different separated container with or without chromium picolinate (360 μg/100ml). Then, stirred well and incubated at 43°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 pasteurized CM at 75°C for 30 minutes and subsequently cooled until 43°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 (360 μg/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 (360 μg/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 with CrPi, group (G7) rats fed with PCM and group (G8) rats fed with PCM supplemented 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 overnight 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 also low-density lipoprotein cholesterol was calculated according to Friedewald et al.[39]. Lipid peroxidation 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].
Figure 2 shows that Alloxan injection markedly increased without CrPi supplementation on serum lipid profile: The protective effect of raw CM and CM products with or without CrPi supplementation on serum lipid profile against the harmful effect of alloxan. The obtained data revealed that 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 2 shows 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-CrPi 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 pronounced decrease in LDL-C levels. Figure 2e shows that CM and CM milk products pre-administration with or without CrPi on serum total lipid significantly reduce total lipids levels when compared with diabetic control. The obtained results revealed that rats pre-administered of fortified milk products with CrPi induced 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 TAC . 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 Figure 3a. 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 revealed 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.

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).

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Ash</th>
<th>Protein</th>
<th>Fat</th>
<th>Total carbohydrates</th>
<th>Total Solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk Camel milk</td>
<td>6.6 ± 0.1</td>
<td>0.80 ± 0.02</td>
<td>3.8 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>13.2 ± 0.1</td>
</tr>
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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.

### 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.
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 severe 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 diabetic rats that had elevated glucose, HbA1C in association injection of alloxan was able to induce a producible model of dyslipidemia and oxidative stress induced by alloxan on 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).

In the present work pre administration of CM supplemented with Chromium successfully decreased blood glucose level with respect to alloxan injected rats, [50]. Stiffleret 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 Jainet 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 data revealed 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 [58,59]. The obtained increase in the activity of hormone sensitive lipase, which could be play a role in modulating lipase sensitive hormone.

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 marked reduction 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 casei 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 Lactobacilus acidophilus assimilate or incorporate some of the cholesterol 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 cells 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 efficiency 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 exopolysaccharides 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 hyperglycemia, associated hyperlipidemia and oxidative stress with restoring ROS-antioxidant balance

6. Acknowledgment

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Figure 1: The prophylactic effect of fortified camel milk products with chromium picolinate 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 picolinate 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 picolinate 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 (X40).

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