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Effect of Melatonin and Chromium Picolinate Administration to High Carbohydrate Diet-Fed Male Wistar Rats

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Abstract

Based on the hypothesis that consistent high carbohydrate diet (HCD) can induce insulin resistance (IR), the present study therefore investigated the induction of IR by feeding HCD. Novelty of the study is to evaluate the effects on pancreas, liver, and kidney tissues histology after eight weeks administration of melatonin and chromium picolinate (CrPic) either in single or in combination (melatonin+CrPic) to HCD rats' respectively. The male Wistar rats were divided into five groups of six rats each. Group I served as control to which normal diet was given, while unlimited HCD was given to group II Group III, IV, and V were also given melatonin, CrPic, and melatonin+CrPic along with HCD in that order. Histopathologic findings of HCD rat pancreatic tissue suggested that the diet altered the normal structure of islets with concomitant lesser cytoplasmic granularity in acinar cells. HCD rat liver showed enlarged hepatocytes with narrowing of sinusoidal spaces. Results revealed after administration of melatonin and CrPic either in single or in combination (melatonin+CrPic) to HCD rats for 8 weeks normalized pancreas and liver alterations when compared with HCD rat pancreas and liver tissues respectively. The present study clearly shows that HCD-fed male Wistar rats that are destined to attain IR and T2DM through diet can be prevented by giving melatonin and CrPic administration in alone or in combination. Promoting this research area through *in vitro* and in *vivo* studies will help us to understand melatonin+CrPic mechanistic insights that may ultimately assist clinicians in controlling hyperglycaemia by supplementing as an add-on therapy.

Keywords: Type 2 Diabetes mellitus; Melatonin; Chromium picolinate; Insulin resistance

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder in which there is less production or utilization of insulin regardless of hyperglycemia in the body. It is estimated that T2DM affected would rise from 30 million to probable 450 million in the world by year 2030 [1]. Genetic and/or certain contributing factors play a vital role in development of insulin resistance (IR) that further proceed towards T2DM [2]. Alarmingly, urbanization has diverged dietary intake [3], which accompanied with lack of exercise and improper sleep would alter the evolutionary concept of robustness with cell being exploited to experience IR [4,5]. However, there have been reports linking the fact that consumption of high carbohydrate diet (HCD) alone induces IR in humans and experimental rodents [1,3,6,7].

Both beta cell (β-cell) dysfunction and IR leads to subsequent unbalance in the bio-molecules metabolism resulting in persistent hyperglycemia. Studies observed increased production of nuclear factor-kappa-beta (NF-kB), tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) in hyperglycemia [2,4,5,8]. TNF-α, NF-kB, and IL-1β are potent inducers of IR [8]. In addition, hyperglycemia in diabetes mellitus exposes every cell to abnormally high glucose concentrations which selectively damages some cell types [8]. For example, in diabetic state, β-cells increase in size [9] due to overworking, trying to compensate the prevailing hyperglycemia. It has been documented that acute exposure of glucose concentrations induces β-cells proliferation [10]. It has been also documented that elevated glucose concentrations cause apoptosis, suppression of β-cells proliferation, reduction in the number of islets, vacuolar degeneration, interlobular, and interacinar fibrosis in cultured β-cells, humans, and T2DM animal models respectively [9-14]. Further, the factors which are commonly present in diabetes include hyperglycemia and oxidative stress. These have been shown to implicate alteration in the robust design of liver and kidney tissues in diabetes mellitus [6,15]. Limited morphological studies have been carried out on the pancreatic islets,

liver, and kidney in HCD-fed models [6]; therefore, the histological characteristics of HCD induced T2DM in the rats are still unclear.

Anti-hyperglycemic drugs though they are successful in reducing high blood glucose levels, are not able to stop the pathogenesis of diabetes and its complications. In the UK, Prospective Diabetes Study [16] deterioration of insulin secretion was seen in patients treated with sulfonylureas. Some studies have shown that certain hyperglycemic drugs may induce apoptosis in rodents pancreatic tissue [17,18]. Given the possible effect of some anti-hyperglycemic drugs, thus, there is an urgent need for alternatives which can delay or halt the progression of diabetes and its complications. To overcome this lacuna, nutraceuticals are being prescribed as an add-on supplement to the existing anti-diabetes therapy.

Melatonin, which is synthesized in the pineal gland and other tissues, influences several endocrine and biological functions. Some studies after supplementation of melatonin observed improvement in fasting blood sugar, total cholesterol (TC), and malondialdehyde (MDA, which is an index of lipid peroxidation) in metabolic syndrome individuals, at baseline these variables were altered [19]. Melatonin is an antioxidant hormone as it directly scavenges reactive oxygen species (ROS) [20,21]. Further, melatonin is also known to stimulate

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and expresses the activity of antioxidative enzymes such as glutathione peroxidase, superoxide dismutase (SOD), catalase (CAT), and nitric oxide (NO) synthase, in mammalian cells [22-24]. Trace element Chromium has been known for optimum insulin sensitive activity; normal carbohydrate and lipid metabolism [25]. In experimental diabetic models, chromium picolinate (CrPic) has been reported to reduce IR and restore pancreatic architecture [25].

Based on the hypothesis that IR is a modifying stage at which the progression of the disease can be halted or normalized if proper considerate measures are taken. The present study is the extension of previously published work [7]. The novel effect of melatonin and its synergistic action with CrPic on HCD rats with respect to pancreas, liver, and kidney morphological findings. The authors have taken the combination of melatonin+CrPic assuming that melatonin decreases oxidative stress and CrPic reduces hyperglycemia.

Materials and Methods

The study was performed after approval from institutional ethics committee of Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh. The experiment consisted of male Wistar rats of 16-22 weeks of age, weighed around 140- 150 grams and divided into five groups of six rats each. The control group was provided with 20 g/day normal diet to each rat. To the rest of the groups HCD was given unlimited access and sugar water at a concentration of 1g/mL to induce diabetes [6]. The diet was obtained from Provimi Healthcare Pvt Ltd. The HCD contained white bread, which consisted of 28% protein, 60% carbohydrate, 12% fats and sugar water at a concentration of 1g/mL [6]. The rest of the group III, IV, and V were given melatonin, CrPic, and melatonin+CrPic administration along-with HCD accordingly. Melatonin (200 µg/animal/day) was given according to the dosage administered in studies in studies [22,23]. The CrPic concentration (approximately 8.8 µg/kg/day for six rats) was given daily to respective group rats in the morning in the morning which is equivalent to a dose of 600 µg/ day for 70 kg adult human. The same dosage has been carried till the end of the experiment. Melatonin+CrPic (200 µg/animal/day +1.4 µg/ animal/day) were given to the rats (Group V) with a 30-minute time interval between administrations of each compound. CrPic and Mel were purchased from Health Aid Ltd, Middlesex and Aristo pharmaceuticals, India respectively. The experiment was carried for eight weeks. Accucheck-glucometer was used to check the blood glucose levels with the whole blood drop obtained from tail vein, every week till the end of experiment. Male Wistar rats that sustained the induction procedures and had fasting blood glucose above 160 mg/dl were included into the T2DM category [6,23]. At the end of experimental period, rats were fasted overnight. The next day rats were anesthesized by inducing chloroform inhalation, and then cervical dislocations performed and were sacrificed between 9 a.m. and 11 a.m. Blood was collected through cardiac puncture into respective vacutainers and special metal free tubes. Allowed it to clot for 30-min and centrifuged (Model: Sigma 3-30 K, Ser. No. 747052) at 3000 rpm in order to collect serum. Separated serum was made into aliquots and stored frozen at -80˚C for further determination of our study variables in a metal-free Eppendorf test tubes. For tissues, the peritoneum was cut open and pancreas, liver, and kidney of each rat were removed, washed with normal saline, and immersion-fixed in 10% formal saline immediately upon removal. These tissues were dehydrated, cleared, and embedded in paraffin to obtain sections. The sections were stained with hemotoxylin and eosin for histological examination following standard protocol [26].

TC, Triacylglycerols (TAGs) and High density lipoproteins (HDL) cholesterol were quantified by cholesterol oxidase-4-aminoantipyrine (CHOD-PAP), Glycerol phosphate oxidase-Peroxidase (GPO-POD), and polyethylene glycol-4-aminoantipyrine (PEG-PAP) methods respectively. Kits were obtained from Avantor Performance Materials India Limited, Dehradun, Uttarakhand, India. The lipid variables were measured with Lablife chem-master semi-autoanalyser. Serum insulin was measured by ELISA kit method obtained from Invitrogen Technologies, USA, using BioRad microplate reader. The homoeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated, as proposed by Muniyappa et al. [27], using the following equation:

HOMA-IR = (fasting glucose (mmol/L) X fasting insulin (µIU/mL)/22.5.

Serum nitric oxide (NO) was estimated by using Griess reagent method [28]. NO level was expressed in µmol/L.

Statistical Analysis

IBM SPSS version 20 was used to perform statistical analysis. The authors compared the means between two or more groups using one way analysis of variance (ANOVA). Then followed by Bonferroni multiple comparison test to compare means between the different supplemented groups. Pearson correlation was applied to know the association between two variables. Percentages were also calculated. $P < 0.05$ was considered significant.

Results

As shown in the Table 1, we observed increased HOMA-IR, blood glucose, serum TC and TAG levels. These variables were inversely proportional between group II rats (HCD without supplementation)

mn	Group I (control, n=6)	Group II (HCD, n=6)	Group III (HCD+CrPic, n=6)	Group IV (HCD+Mel, n=6)	Group V (HCD+CrPic+Mel, n=6)	ANOVA (P<0.05)
Insulin (µIU/mL)	34.2 ± 3.2	26.67 ± 3.18 [a]	28.9 ± 2.8 [a]	29.4 ± 2.98 [a]	24.78 ± 3.8 [a,c]	>0.05
HOMA-IR	12.3 ± 0.8	20.4 ± 3.7 [a]	15.8 ± 1.7 [b]	15.1 ± 1.12 [b]	14.21 ± 1.2 [b]	<0.001
TC (mg/dL)	131.3 ± 5.4	215.8 ± 16.8 [a]	190 ± 22.3 [a, b]	193.6 ± 19.7 [a, b]	170.1 ± 11.2 [a, b, d]	<0.001
TAG (mg/dL)	141.3 ± 2.1	214.1 ± 20.4 [a]	197.3 ± 26.2 [a, b]	188.6 ± 18.9 [a, b]	174.5 ± 14.5 [a, b, d]	<0.001
HDL (mg/dL)	48.16 ± 3.4	36.1 ± 3.93 [a]	41.1 ± 3.76 [b]	43.3 ± 2.31 [b]	43.1 ± 2.5 [b]	<0.001
NO (µmol/L)	15.6 ± 1.6	16.8 ± 1.2	15.2 ± 1.8	16.5 ± 1.9	15.6 ± 1.7	>0.05

[a] Compared with group I
[b] Compared with group II
[c] Compared with group III
[d] Compared with group IV
P <0.05

Table 1: Effects of high carbohydrate diet (HCD), chromium picolinate (CrPic) and melatonin (Mel) in separate or in combination treatments on insulin resistance (HOMA-IR), serum levels of insulin, total cholesterol (TC), triacylglycerols (TAGs), high density lipoproteins (HDLc), and nitric oxide (NO) variables in male Wistar rats.
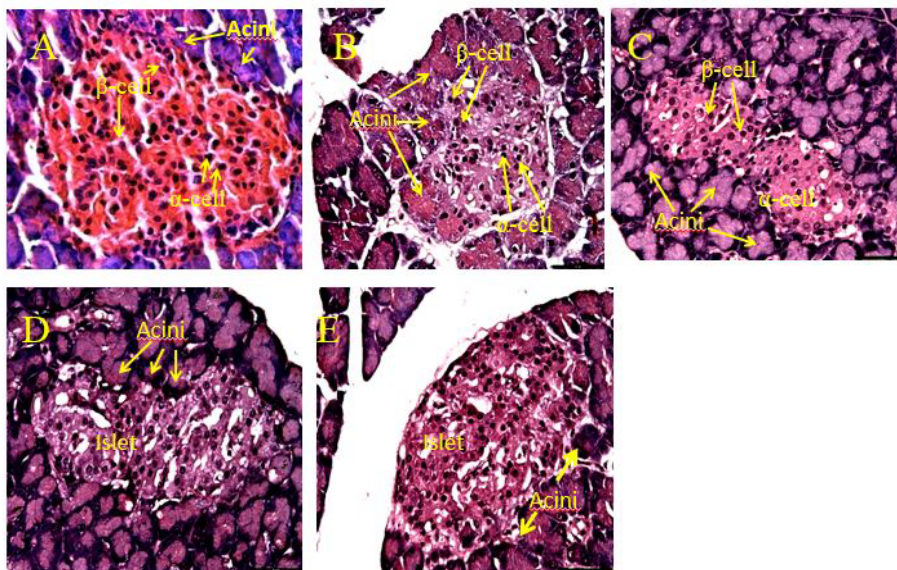
Figure 1: The histology of pancreas of rat stained with Hematoxylin and Eosin. Pancreas of Control rat (A) with 400x magnification showing Islet of Langerhans and typical exocrine acini. High carbohydrate diet- fed rats (HCD) (B) showing disorganized islet and capsule architecture. HCD rats supplemented with melatonin (C), chromium picolinate (CrPic) (D), and combination of melatonin+CrPic (E) are shown in the respective figures. The approximate number of Beta cells are given below adjacent to the figure alphabet: A=52; B=21; C=41; D=39; E=46.
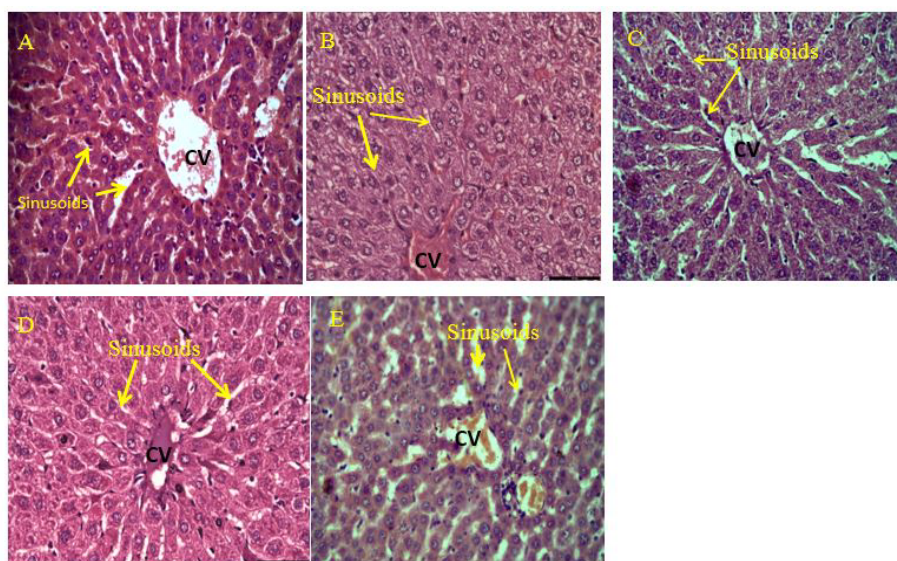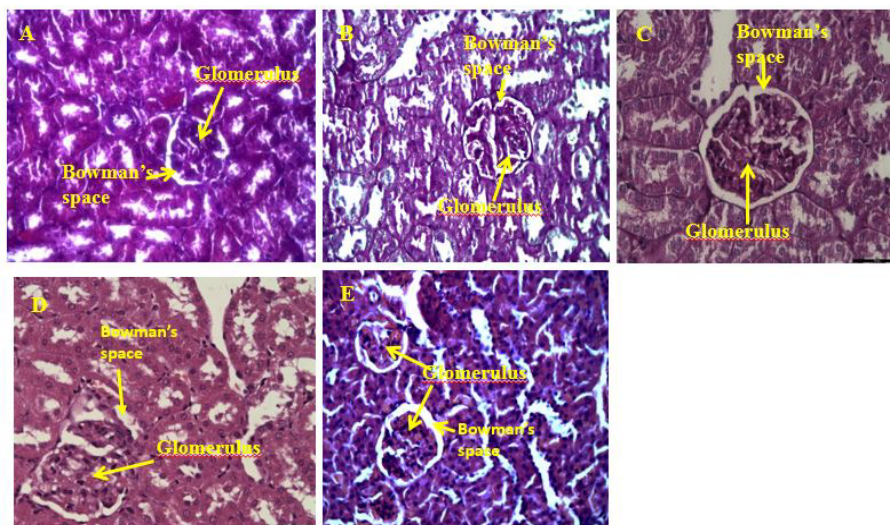

Figure 2: Histological of the liver of rat stained with Hematoxylin and Eosin. Control rat (2A) showing lobules with hepatocytes, central vein, and sinusoids are seen at high magnification (400x). High carbohydrate diet-fed (HCD) (2B) HCD rats supplemented with melatonin (2C), chromium picolinate (CrPic) (2D) and combination of melatonin+CrPic (2E) showing respective changes in the architecture.

and group I rats (control). We also observed significant differences in levels of glucose, TC, TAG, and HDLc compared between group II and with each supplemented HCD group i.e., group III (HCD+melatonin), IV (HCD+CrPic), and V (HCD+merlatonin+CrPic) respectively. No marked difference was observed in serum insulin and NO levels (P>0.05) but observed significant difference in HOMA-IR (P<0.05) when group II rats were compared with group III, group IV, and group V rats respectively. During the experiment period, the rats fed on HCD alone (group II) and also HCD rats with supplementation did not show aversion to consume diet (HCD) provided. The combination of melatonin+CrPic was more effective in reducing hyperglycemia.

In addition, CrPic was more effective compared with melatonin in alleviating blood glucose levels in HCD rats.

The histology of control rat pancreas (Figure 1A) revealed normal endocrine tissue with compact arrangement of β-cells, non β-cells and typical exocrine acini. In HCD (group II) rat pancreas (Figure 1B), we observed relatively decreased volume of β-cells and several non β-cells and moreover these cells were disorderly arranged. In addition, we observed lower cytoplasmic granularity in the acini. After administration of supplements melatonin, CrPic, and melatonin+CrPic to HCD rats, we observed relatively normal exocrine and endocrine cells (Figures 1C-1E).

Figure 3: Histology of kidney of rat stained with Hematoxylin and Eosin. Control rat (3A) showing Bowman's capsule, and glomerulus. High carbohydrate diet -fed (HCD) rat (3B) showing dilated glomerulus with marked constriction of Bowman's space. HCD rats treated with melatonin (3C), Chromium picolinate (CrPic) (3D), melatonin+CrPic (3E) showing varying degree of reversal in histological derangements in glomeruli and tubular structures.

Histology of control rat liver revealed lobules with hepatocytes with defined borders, central vein, and normal sinusoidal spaces at high magnification (400x) (Figure 2A). On contrary, in HCD (group II) rat liver (Figure 2B) we observed enlarged hepatocytes with vacuolations and narrowing of sinusoids. After administration of melatonin, CrPic, and melatonin+CrPic showed relatively normal histology of liver with clear sinusoidal spaces between hepatocytes (Figures 2C-2E).

The histology of control, HCD, HCD+melatonin, HCD+CrPic, and HCD+melatonin+CrPic rat kidney (Figures 3A-3E) showed normal Bowman's space and normal glomeruli.

Discussion

The questions posed in this study were to explore the effect of HCD on pancreas, liver, and kidney tissues histology. The present study was also to understand whether the effect of melatonin and CrPic either in single or in combination when given to HCD rats have any preventive property on β-cell, liver and kidney histo-pathological changes that arise from hyperglycemia and if so, their use as add on therapy over the existing therapy available in T2DM. This study has clearly brought out the alleviating action of melatonin and CrPic when administered singly or in combination to HCD rats. The results of the study are elaborately discussed below for easy comprehension of the significance of the present study.

In the literature survey, a study has been shown to induce cytokines production in polymorphonucleocytes and mononuclear cells with 300 calories of glucose meal [29]. This increase was compared to protein and lipid diets which produced significant lower amount of NF-kB than glucose meal [29]. TNF-α and NF-kB are considered to induce IR [8]. Earlier works demonstrated that persistent consumption of high carbohydrate foods induce IR [3,29,30]. Further, studies have also revealed a possible IR association with hyperglycemia and hyperinsulinemia [30,31]. Reports which observed IR, hyperglycemia, and hyperinsulinemia in T2DM subjects did not include histological insight in their studies [3,24,31]. Furthermore, it is also reported that IR and β-cell dysfunction place a major stress on the β-cell to augment their secretion of insulin to offset the defect in insulin action [32].

The data of control rat pancreas in the present study showed normal insulin production is directly relative to the normal histological architecture. This finding infers the consumption of normal diet directs β-cells to secrete insulin sufficiently to maintain the glucose level normal in the body. In group II rats, we observed continuous consumption of HCD induced IR which led to hyperglycemia in the present study. Subsequently, with time the β-cells begin to fail and subsequently glucose concentration begins to rise, leading to T2DM [32]. This happens if no necessary measures have taken to normalize elevated glucose levels or to defy the effects of HCD [2]. Similarly, the altered architecture of islets in the present study is probably due to the continuous consumption of unlimited amount of HCD. The altered histological change of endocrine part is the cause for decreased insulin level in the present study. Related observations of lower insulin level and architectural changes of endocrine part have also been reported by Sahin et al. [25] on streptozotocin and high fat diet–fed rats. Lower insulin and increased HOMA-IR has been observed in T2DM human subjects with minimum five years of known duration of the disease [33]. On the contrary, Bonora et al. [31] observed hyperinsulinemia and inferred hyperinsulinemia to decreased insulin extraction by liver in mild glucose intolerance and obese human individuals. Hyperinsulinemia has also been reported in individuals with non-alcoholic steatohepatosis, IR, and metabolic syndrome [34].

The present report observed an interesting finding in the exocrine part of HCD rat pancreas (group II) which showed lower cytoplasmic granularity. Physiologically, insulin receptors on the surface of acinar cells mediate the hormone's effects on cellular growth and enzyme synthesis when normal insulin flow is present [35]. To our understanding, the observed altered changes in exocrine acini of HCD rats in the present study is not clear, but evidences may point to portal system between endocrine and exocrine tissue [35] and to altered architecture of endocrine part of pancreas. The relative deficiency of insulin in the HCD rats may have hampered enzyme granules formation. The other reasons that affected enzyme granules formation are i) the diet given was protein deficient and ii) due to hypotrophic action of insulin. Thus, exocrine tissue around islet will be affected by substances (including excess glucose and lower insulin) that enter/exit

into/from the endocrine tissue. Entry of excess glucose places a demand on the β-cells to secrete more insulin to compensate the glucose which subsequently lead to progressive failure of β-cell [2,29] and as a result low insulin exits from pancreas. Several theories have been postulated to explain changes in exocrine tissue which may be due to lack of trophic insulin action and pancreatic fibrosis that could lead to impaired exocrine regulation as a result of angiopathy and neuropathy [36-39]. The present report differs from the studies conducted on T2DM models by Adeyi et al. [6] and Sahin et al. [25]. Moreover, this is the first study to report change in the exocrine tissue due to HCD rats. Literature survey reveals that there is virtual paucity of HCD studies and the available limited data is also often contradictory.

Melatonin and CrPic individually and in a combination administration for eight weeks prevented the histological changes and also reduced glucose levels in HCD-fed rats. However, it is important to note that the decreased levels of insulin following HCD did not change significantly after melatonin and CrPic supplementations. This finding could likely be due to increased extraction of insulin by the liver due to lowered IR which is evident from the significant change in HOMA-IR in melatonin and CrPic supplemented rats. Guan et al. [40] observed supplementation of Cr in the form of yeast improved the extraction of insulin by liver in pigs. Moreover, Ivy [41] confirmed that insulin will enhance glucose extraction from the blood. Hence, reduced ability of insulin to stimulate muscle blood flow is a characteristic of insulin-resistant obese individuals and T2DM individuals [41]. Furthermore, exercise has been found to alleviate IR and improve blood glucose [41]. Hence, reduced glucose, improved IR and extraction of insulin may be the likely factors to improve the acinar cytoplasmic granularity in HCD-fed supplemented rats. However, in rodents, treatment with GLP-1 receptor agonist Exendin which is drug of choice for T2DM, resulted in acinar cell death and shown inflammation [17,18].

The insulin action defect (IR) initially in T2DM history is exhibited primarily by liver and secondarily by skeletal muscle [2,29]. Studies have demonstrated that IR activates lipogenic enzymes which are coordinately regulated at the transcription level by sterol regulatory binding proteins (SREBP) [42-45]. SREBP are responsible for lipids synthesis. Physiological importance of HDL is to carry extra-hepatic cholesterol to liver for metabolism [46]. The exchange of TAGs with cholesterol takes place between HDL and very low density lipoproteins through a process called as reverse cholesterol transport [46]. In the present study, we observed obliteration of sinusoidal spaces with concomitant enlargement of hepatocytes in histology of HCD rat (group II) livers. We also observed increased TC and TAG and lower HDL levels in HCD rats serum (group II). The increased synthesis of cholesterol and TAGs in the present study is due to the continuous availability of precursor i.e. excess glucose in HCD rats (group II). Dyslipidemia in the present study also suggest improper transportation of lipids between lipoproteins and increased lipids (TC and TAGs) synthesis in liver. Therefore, the enlargement of hepatocytes is probably due to accumulation of lipid molecules. It has been shown that glucose is required for lipogenic enzymes where insulin plays only a permissive role [44,45]. One study demonstrated in insulin-depleted, STZ-administered mice that SREBP-1c induction does not require insulin [47]. Thus, we observed disruption in the architecture and is in consonance with other studies [6,25]. Studies have shown that the liver may also be affected by hyperglycemia and IR in the long term [14,15]. Previous studies that observed hyeprglycemia also observed dyslipidemia in animal models and as well in hormonal disorders [6,25,33,48-51]. Similarly, dyslipidemia was also observed in individuals affected with infection [52]. HCD rats supplemented with melatonin, CrPic, and combination of melatonin+CrPic showed restoration of normal histology of lobes, central vein and sinusoids with concomitant reduction in hyperglycemia and dyslipidemia [6]. Therefore, it is not hypothetical to confirm these data to dyslipidemia which en route for altered architecture. Earlier studies observed improvement in lipid profile parameters after supplementation of melatonin [53] and CrPic [24,25] in metabolic syndrome and T2DM individuals. Melatonin is known to dispose glucose from the blood in animal and human studies [54-56]. Absence of melatonin induces night time hepatic IR and increased gluconeogenesis [55,56]. Further, it has been shown that melatonin inhibits NF-kB and oxidative stress against thioacetamide induced liver damage in rats and experimental diabetic neuropathy [57,58]. Cr has also been shown to enhance glucose uptake by increasing GLUT-4 translocation and is useful in glucose disposal by enhancing phosphorylation of insulin receptor substrate-1 and protein kinase-B [25, 59-61].

NO is an intracellular second messenger that modulates vascular tone [62]. It is produced by constitutive and inducible NO synthase enzymes and expressed in many tissues including cardiac, neuronal, and endothelium [63,64]. The reports suggest that chronic hyperglycemia contributes to the regulation of NO synthase expression [64-68]. Studies demonstrated increased NO in fructose-fed rats [65], in human umbilical vein endothelial cells exposed to high concentrations of glucose [66]. On contrary, lower NO levels were observed in alloxan treated rats [50]. In the present study, in HCD-fed rats, we did not observe significant difference in serum NO levels compared with control rats. Our observation, however, differs from that of Consentino et al. [67] in as much as that they used fructose to induce diabetes in rats which were hyperinsulinemic, but we used HCD to induce diabetes and the rats were hypoinsulinemic. Further, in another report [25] on streptozotocin plus fat food induced T2DM model, the authors reported significant vacuolar degeneration of renal tubular cells with displaced pycnotic nuclei. This report also demonstrated tubulointerstitial nephropathy with significant mesangial proliferation in the glomeruli. However, the present differs in the type of T2DM induction process. In another study [6] on the similar present study T2DM model showed glomeruli degeneration with wider Bowman's spaces and diffuse vacuolation of the tissues. The present study finding differs with the study [6], nevertheless, in nitric oxide concentration in HCD rats. Nitric oxide is responsible for the dilatation of blood vessels. Histological examination of administration of melatonin or CrPic either in single or in combination to HCD rats after 8 weeks showed relatively normal Bowman's space and normal glomeruli with no visible lesion in these structures. This infers that melatonin and CrPic either in single or in combination are not toxic to kidney.

Several studies have shown that chromium is a potent hypoglycemic compound with anti-inflammatory activity apparently mediated by inhibition of NF-kB and GLUT-2 in the levels of T2DM rats [8,26,69,70]. It is also postulated that the earlier investigations that amelioration of IR by CrPic might directly or indirectly associated with modulation of NF-kB expression. Moreover, melatonin supplementation decreased the activity of NF-kB by virtue of increase in the expression of Akt, which is responsible for glucose transport into the cell [71-73]. Earlier the authors have discussed how ROS induce IR through NF-kB and TNF-α.

Conclusion

The present study clearly shows that HCD-fed male Wistar rats that are destined to attain IR and T2DM through diet can be prevented by giving melatonin and CrPic administration in alone or in combination.

Promoting this area of research through in-vitro and in-vivo studies will help us to understand melatonin+CrPic mechanistic insights that may ultimately assist clinicians in controlling hyperglycaemia by supplementing as an add-on therapy. Finally, a large multicentric study is required to validate the findings of the present study as melatonin and CrPic are inexpensive and efficacious to control the high costs associated with diabetes and its complications as an add-on therapy to the existing anti-diabetic drugs to achieve glycemic targets of HbA1c <7%.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Doddigarla Zephy: Acquisition of the data, data Analysis, data interpretation, drafting of the manuscript, critical revision, approval of the article.

Prof. Iqbal Parwez: Data Analysis, data interpretation, drafting of the manuscript, critical revision, approval of the article.

Dr. Subuhi Abidi: Data interpretation, critical revision, approval of the article.

Prof. Jamal Ahmad: Acquisition of the data, data Analysis, data interpretation, drafting of the manuscript, critical revision, approval of the article.

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