Original Article

EXAGGERATION OF TYPE 2 DIABETES DUE TO CAFFEINE-NICOTINE CO-ADMINISTRATION: A STUDY IN RATS

SUBHASH T. KUMBHAR[1], HEMANT D. UNE[2], ANAGHA M. JOSHI[3], PRALHAD B. WANGIKAR[4]

[1]CRD, PRIST University, Thanjavur, TN, India and Indira College of Pharmacy, Pune, MS, India, [2]YB Chavan College of Pharmacy, Aurangabad, MS, India, [3]Indira College of Pharmacy, Pune, MS, India, [4]Pharmaceuical Chemistry, PRADO Preclinical Pvt. Ltd, Ravet, Pune, MS, India
Email: profsdune@gmail.com

ABSTRACT

Objective: This study evaluated the toxic effect of simultaneously injected normal doses of caffeine and nicotine in diabetic lab animals.

Methods: A study was conducted for three weeks in seven rat groups (n=6); viz. first non-diabetic group treated with caffeine (20 mg/kg, ip) twice daily, second with nicotine (0.4 mg/kg, ip) twice daily and third with both treatments simultaneously; whereas other three groups treated in the same way but inducing diabetes; and employing the seventh group as diabetic control. Type 2 diabetes was induced by high fatty diet prior for two weeks and a single streptozotocin injection on 1th day of study in all diabetic groups. Blood and urine samples were collected weekly to estimate blood parameters. Animals were sacrificed, and organs were collected for histopathology analysis.

Results: Most blood parameters showed a rapid increase in diabetes in co-addiction group compared with their single addiction or non-addiction control groups. Caffeine-nicotine co-addiction group showed about 60-80 mg/dl (p<0.05) rise in serum glucose, 15-20 U/l in AST (p<0.01), 80-100 U/l in ALT (p<0.01), 20-30 mg/dl in Urea (p<0.01), 02 mg/dl in creatinine (p<0.05), 12-15 mg/dl (p<0.01) in LDL-C, 6-9 mg/dl in VLDL-C (p<0.01) and 60-90 mg/dl in TC levels (p<0.01) when compared with non-addicted diabetic control. There was a significant reduction in HDL-C (p<0.01) while the less significant rise in triglycerides in the case of co-addiction as compared to non-addiction diabetic control group. Histopathology results exhibited moderate to severe tissue damage in agreement with clinical biochemistry results.

Conclusion: Nicotine-caffeine co-addiction harms exceptionally more in type 2 diabetes greater than their single addiction or non-addiction.

Keywords: Streptozotocin, Caffeine, Nicotine, Type 2 diabetes, Insulin resistance

INTRODUCTION

The addition of either caffeine or nicotine is common in today's population of almost every country across the world. Both compounds belong to the plant origin and available in the crude form in developing countries. Illiterate citizens of developing country consider these compounds safe, because of the routine habituation. As these compounds are incompletely banned by the government of these nations, they are mostly available for addiction.

Diabetes is lifestyle disease, and it is observed in most countries of the world [1]. As it is a chronic and slowly growing condition, people ignore it unless they face any serious symptom. Once diagnosed; most sufferers become serious about it and start treatment depending on the type of diabetes. Before; during, and sometimes after the diagnosis of diabetes, patient keep practicing addiction of caffeine-nicotine products. Generally, patients are habituated to these substances for the very long duration of their life; therefore quitting them instantly on the diagnosis of diabetes is difficult for them.

There are few studies published up to now claiming that, 'taking green tea in diabetes may prove helpful in its treatment' in human beings [2]. Generally, these authors conclude with finding that polyphenolic compounds like flavonoids are responsible for these effects and not the caffeine [3]. Few authors tested good or bad clinical effects of caffeine products in diabetes and concluded that, either it increase hyperglycemia in few tissues and/or decrease it in other tissues [4]. They also investigated the mechanism behind this glucose related activity in various isolated tissues to put a concrete outcome; but unfortunately, there is a lack of such breakthrough unbiased finding which will be important for clinical decision. 'Hyperglycemia is the immediate effect of caffeine product consumption,' is supported by most authors; but the effect in chronic addiction is unresolved. Few authors reported that caffeine products reduce insulin sensitivity of the tissues [5, 6]. Interestingly,

an isolated caffeine is still not injected by these researchers in diabetic animals to confirm hyperglycemic effect proposed by them.

Nicotine is another substance known to produce strong addiction. There are several forms available for addiction; suitable for either smoking, transmucosal or parenteral use. Nicotine products had frequently been tested for their possible hyper or hypoglycemic effect in human subjects or animals. Most authors accept that it is hyperglycemic substance; as it reduces insulin sensitivity of tissues in normal human subjects and animals [7]. Net or direct effect on glucose distribution still remains less significant, and there may still be controversy to some extent. Doctors advise the diabetic patient to quit smoking; to minimize hazardous effects of nicotine and hypoxic damage due to smoke contents [7]. As nicotine creates damage to the liver, kidney, and cardiovascular system; it contributes diabetic complication as well [8]. There is still a lack of direct observation that, nicotine harm the pancreatic islets progressively to worsen diabetes. Most authors state that 'nicotine reduce insulin sensitivity' and produce hyperglycemia through a mechanism other than pancreatic islet damage. Here also an isolated nicotine is not tested in diabetic animals to observe the effect.

Though there is large population addicted to caffeine-nicotine products; surprisingly there is no study where both these isolated compounds tested simultaneously in chronic diabetic condition, and therefore we felt this aspect was novel. We were carrying intuition that, there could be additive or synergistic damage caused by these two compounds when they are co-addicted by diabetic patient [9]. We created co-addiction situation in type 2 diabetic animal by injecting caffeine-nicotine in normal doses twice daily. Results of addicted diabetic groups were compared with non-addicted diabetic groups and further tested to prove this hypothesis. Studies in this area are clinical observations on a diabetic patient, either with caffeine or nicotine in their crude form. This study was first protocol so far, where caffeine-nicotine simultaneous addiction was induced in diabetic animals to observe chronic effects.

MATERIALS AND METHODS

Chemicals/reagents/diagnostic kits

Streptozotocin (Sisco Research Lab, Mumbai, India) Caffeine and Nicotine (Tokyo Chemical Industry, Japan). Hematology reagent kit (Biolab diagnostics, Mumbai); All other chemicals of analytical grade were used.

Study approval

All the procedures used in the study were as per the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA) as published in The Gazette of India, December 15, 1998. Approval of the Institutional Animal Ethics Committee (IAEC) was obtained before initiation of the study (IAEC-15-001).

Animals

Sprague dawley (SD) rats were procured from CPCSEA approved supplier from Pune, India; and kept in quarantine for seven days to acclimatize with animal house facility. After acclimatization, animals were kept in 12 h auto controlled light-dark cycles in a separate room. Except during experiments, animals were kept on the standard diet (Nutrivet Life Sciences, India) and water *ad libitum*. Animals were taken randomly, and±20% weight variation from mean, was a selection criterion. Rats of both the gender having age 8-10 w, and body weight 150-175 gm were taken for the study.

Study protocol

Animals were divided into seven groups; containing six animals in three non-diabetic groups, while ten animals in four diabetic groups; such as D (diabetic control), C (caffeine alone), N (nicotine alone), CN (caffeine-nicotine both), DC (diabetic caffeine), DN (diabetic nicotine), and DCN (diabetic caffeine-nicotine) groups. All animals were kept on a normal diet before, during and after the experiment; except diabetic animals were fed with high fat diet (HFD) for two weeks prior STZ treatment. Six treatment groups were treated with caffeine (20 mg/kg, ip) and/or nicotine (0.4 mg/kg, ip), while D group was treated with equal volume of vehicle (Sodium chloride in distilled water); twice daily for three weeks. Group D, DC, DN and DCN were intravenously injected with a single dose (50 mg/kg) of freshly dissolved streptozotocin in citrate buffer on the first day of the study, while other groups were injected with citrate buffer vehicle. At the end of the first week, based on the serum glucose level, stable six animals were selected out of ten from each diabetic group and included in the study. Overnight fasted animals were subjected to the blood withdrawal and body weight measurement on 1, 7, 14 and 21st day of study. On the 21st day of study; animals were anesthetized and sacrificed for terminal blood and organ collection [10].

Haematology

Each animal was anesthetized to the appropriate level using diethyl ether and blood was withdrawn from orbital plexus by capillary method. Approximately, one ml of blood was collected in eppendorf tube containing K_2-EDTA anticoagulant. Blood parameters were immediately estimated using Sysmex XP-100 (Sysmex Corporation, UK) auto hematology analyzer. Erythrocyte count (RBC), total leukocyte count (WBC), hematocrit (HCT), mean corpuscular volume (MCV), hemoglobin concentration (HGB), mean hemoglobin concentration (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) were determined [11].

Clinical biochemistry

Blood was collected from each animal in a vial containing sodium heparin anticoagulant and centrifuged at 4000 rpm for 10 min using R-8C centrifuge (Remi, India). Plasma were separated and analyzed using EM Density180 Clinical Chemistry Analyzer (ERBA Diagnostics, Germany). Ready-to-use reagents were employed to estimate serum levels of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRE), urea (BU), triglycerides (TG), high density lipid cholesterol (HDL-C), low-density lipid cholesterol (LDL-C) and total cholesterol (TC) [11].

Necropsy; organ collection; histopathology

On 21st day of study, all animals were anesthetized and sacrificed by the cervical dislocation method. Animals were dissected to remove pancreas, liver, kidneys; and these organs were fixed in 10% buffered formalin for histopathological examination. After 48 h, organs were fixed in paraffin blocks and sectioned approximately 3-5 µ thick using microtome (Panchal Scientific Corporation, India). Selected sections were carefully taken on slides and stained with hematoxylin-eosin and observed under suitable magnification using a compound microscope (Labomed, US). Histopathology results were evaluated under microscopy by toxicology expert.

Statistical analysis

Each of the seven groups taken was containing six animals to generalize the outcome statistically. Results were presented as mean±SEM, and diabetic groups were analyzed against diabetic control (running parallel) using one-way ANOVA and Dunnett's t post-test. Significance was determined in terms of p-value; and values of p<0.05 were considered significant. Different levels of significance utilized in the study were *(p<0.05), **(p<0.01), ***(p<0.001) for test.

RESULTS

Hematology

Hematology analysis was conducted to track possible toxic effects of diabetes induction and caffeine-nicotine co-administration in rats. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were the two deviated parameters deserving expression here, while others were ignorable. It was observed that diabetes induction increases MCV and MCH in diabetic rats as compared to non-diabetic rats. Caffeine and nicotine did not elevate MCV and MCH in C, N, and CN groups; whereas they did elevate MCV and MCH significantly (p<0.05, P<0.01) in DN and DCN groups. MCH was showing little more difference and more significance as compared to MCV in a later week. This increase in the MCV and MCH may be due to dehydration and hypovolemia caused due to diabetes and simultaneous caffeine-nicotine administration. Co-addiction harms diabetic rats more than nicotine or caffeine single addiction during the latter two weeks of study (table 1).

Table 1: Hematology (n=6)

Mean corpuscular volume (fl)				Mean corpuscular haemoglobin (pg)			
Group	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 1**	**Day 7**	**Day 14**	**Day 21**
D	46±1.6	58±1.7	54±1.4	59±1.5	18.5±0.7	19.9±0.6	18.6±0.6	19.7±0.7
C	49±1.4	54±1.1	51±1.0	50±1.6	19.3±1.1	20.2±0.9	19.4±0.8	20.5±0.9
N	50±1.3	51±1.3	50±1.4	53±1.4	19.3±0.9	20.3±1.8	18.9±0.5	20.6±0.4
CN	53±1.5	49±1.9	53±1.7	54±1.2	20.1±0.7	19.8±0.9	22.1±0.8	21.4±0.7
DC	53±1.8	56±1.7	57±1.7	61±1.9	19.5±0.7	21.4±0.5	23.1±0.9	22.9±0.6
DN	49±1.2	50±1.5	56±1.4	59±1.6*	19.9±0.5	21.8±0.6	21.5±0.5*	21.6±0.8**
DCN	50±1.5	49±1.6	58±1.0*	62±1.8*	18.7±0.8	21.8±0.9	23.1±0.7*	24.3±0.6**

F1**:** D = Diabetic control group (STZ 50 mg/kg, ip.). C= Caffeine alone (20 mg/kg, ip.). N= Nicotine alone (0.4 mg/kg, ip.). CN=Nicotine-caffeine both simultaneously (20 mg/kg and 0.4 mg/kg, ip.). DC= Diabetic caffeine group. (STZ 50 mg/kg, and caffeine 20 mg/kg, ip.). DN=Diabetic nicotine group (STZ 50 mg/kg, and Nicotine 0.4 mg/kg, ip.). DCN= Nicotine-caffeine both simultaneously in diabetic rats (STZ 50 mg/kg, and Caffeine 20 mg/kg and Nicotine 0.4 mg/kg, ip.). Results expressed as mean±SEM. Difference analyzed amongst groups using one-way ANOVA and Dunnett's test as post-test. Day 7, 14, and 21, results taken simultaneously for ANOVA.* The difference in groups when compared with diabetic control significant at p<0.05. ** Difference in groups when compared with diabetic control significant at p<0.01.

Clinical biochemistry

Serum glucose levels

Serum glucose level was considered as the main direct determinant of type 2 diabetes. Fasting glucose level was estimated by early morning blood withdrawal from overnight fasted animals. Diabetic animals exhibited a distinguishable rise in mean serum glucose level presenting successful diabetes induction. There was an insignificant rise in mean serum glucose level in DC and DN groups compared to DCN group. DCN group showed significantly (p<0.05) rise in blood level during later two weeks of study as compared to D group. This difference was accelerating throughout the study, and it was as maximum as 60-80 mg/dl at the end of study (table 2).

Liver function test

Serum AST and ALT levels were estimated to evaluate the extent of liver damage. Diabetic animals showed a progressive increase in AST, ALT levels throughout the study; approximately 3 times greater than the normal. In diabetic animals, DC and DN groups showed negligible and insignificant acceleration in AST, ALT mean levels but DCN group exhibited as maximum as 15-20 and 80-100 (U/l) rise

(p<0.01, p<0.01) in these levels, respectively. There was no significant change in ALT/AST ratio throughout this study (table 2).

Kidney function test

Serum urea and creatinine levels were determined to observe the extent of kidney damage in diabetes. D group showed the clear progressive elevation of these parameters due to disease-induced kidney damage in later two weeks. DC and DN group presented a negligible rise in urea, and creatinine means levels; whereas DCN group created much intense rise about 20-30 and 0.2 mg/dL (p<0.01, p<0.05) in these parameters respectively when compared with D group (table 2).

Lipid profile

Diabetes being a metabolic disorder elevates triglycerides in the blood and alters the distribution of cholesterol in different types of lipid viz. HDL-C, LDL-C, VLDL-C, TC. There was a marked increase in blood triglycerides in diabetic animals while no change this level in non-diabetic animals. In DC, DN and DCN groups there were negligible and less significant differences in triglyceride levels compared with D group (table 2).

Table 2: Clinical biochemistry and body weight (n=6)

Gr/Day	Serum glucose level (mg/dl)				Serum AST levels (U/l)			
	Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
D	90±12	425±19	459±24	474±22	39±2.9	87±2.9	92±3.6	96±3.5
C	89±11	109±12	113±12	105±15	32±2.9	46±3.4	48±3.6	43±3.4
N	94±13	108±14	112±11	109±14	33±3.1	43±3.5	41±3.5	42±3.5
CN	92±15	111±16	115±15	123±13	37±3.5	39±3.4	43±3.3	45±3.6
DC	91±13	437±23	459±23	485±21	41±3.2	84±3.7	88±3.4	92±3.7
DN	96±10	449±25	467±19	470±26	40±3.3	81±2.9	87±3.1	89±3.8
DCN	97±14	490±21	543±20*	556±19*	39±3.0	102±3.2*	109±3.7**	114±3.8**
Gr/Day	Serum ALT levels (U/l)				Serum urea (mg/dl)			
	Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
D	118±9.4	308±16.4	324±17.4	333±19.4	16±3.4	62±4.4	67±4.7	70±4.1
C	125±6.7	126±15.3	122±8.7	128±9.7	18±3.6	23±3.2	21±3.5	24±4.1
N	106±8.7	118±14.6	126±9.8	127±7.4	17±3.3	19±3.6	24±3.1	27±3.2
CN	109±7.8	164±14.2	159±8.6	162±8.6	21±3.5	25±3.3	23±3.4	27±4.2
DC	117±9.4	269±13.8	359±15.4	364±16.4	19±4.6	67±4.8	76±4.9	79±4.7
DN	127±8.6	303±14.3	381±16.4	389±18.7	17±4.3	66±4.4	77±4.7	78±4.1
DCN	130±4.5	294±15.4	406±13.4**	438±17.9**	19±4.1	79±4.7*	88±4.1**	91±4.4**
Gr/Day	Serum creatinine (mg/dl)				Serum HDL cholesterol (mg/dl)			
	Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
D	0.36±0.04	0.67±0.05	0.73±0.05	0.71±0.05	58±3.6	48±4.1	49±4.2	47±4.7
C	0.35±0.04	0.36±0.04	0.39±0.04	0.41±0.04	61±3.6	63±3.1	56±3.6	54±3.4
N	0.32±0.04	0.38±0.04	0.39±0.04	0.38±0.04	53±3.7	49±3.5	48±3.6	56±3.1
CN	0.39±0.04	0.48±0.04	0.46±0.04	0.49±0.04	62±3.4	64±3.7	58±3.1	51±3.3
DC	0.36±0.04	0.73±0.05	0.78±0.05	0.81±0.05	48±3.1	50±4.1	46±3.9	42±3.9
DN	0.43±0.04	0.74±0.05	0.75±0.05	0.79±0.05	52±3.1	51±3.7	45±3.8	40±3.6*
DCN	0.31±0.04	0.81±0.05	0.89±0.05	0.91±0.05*	58±3.5	53±3.9	42±3.8*	37±3.8**
Gr/Day	Serum LDL cholesterol (mg/d)				Serum VLDL cholesterol (mg/d)			
	Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
D	66±6	125±10	135±10	133±9	19±1.3	28±1.4	31±1.1	31±1.5
C	55±6	77±7	78±6	69±7	20±1.6	23±1.2	21±1.3	20±1.3
N	61±6	69±8	74±8	73±7	18±1.2	26±1.1	28±1.4	29±1.4
CN	72±5	62±7	77±7	79±8	20±1.2	27±1.6	26±1.7	28±1.7
DC	58±9	118±10	129±8	141±9	20±1.4	29±1.6	31±1.7	34±1.9
DN	67±8	120±9	134±8*	139±10*	21±1.5	30±1.7	30±1.9	34±1.8
DCN	69±9	137±8*	147±10**	149±10**	21±1.4	34±1.8*	37±1.5*	40±1.7**
Gr/Day	Serum total cholesterol(mg/d)				Change in body weight (gm)			
	Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
D	61±6	153±6	159±7	163±6	-01±2.91	-34±2.32	-47±2.91	-61±3.31
C	68±8	74±9	77±6	73±7	-05±2.26	-13±2.36	-03±2.16	-08±2.07
N	67±8	73±7	65±8	70±9	+6±2.28	+09±2.84	+11±2.66	+03±2.29
CN	73±7	73±8	77±7	83±7	-03±2.33	-13±2.12	+02±2.39	-07±2.04
DC	66±9	143±9	151±9	157±10	-04±2.42	-16±2.16	-49±2.27	-83±2.32*
DN	69±8	177±9	176±11	179±9*	+1±2.22	-24±2.32	-59±2.19	-77±2.22
DCN	68±9	193±10*	190±9*	199±11**	+3±2.22	-25±2.42	-61±2.45	-89±2.29*

F2: D = Diabetic control group (STZ 50 mg/kg, ip.). C= Caffeine alone (20 mg/kg, ip.). N= Nicotine alone (0.4 mg/kg, ip.). CN=Nicotine-caffeine both simultaneously (20 mg/kg and 0.4 mg/kg, ip.). DC= Diabetic caffeine group. (STZ 50 mg/kg, and caffeine 20 mg/kg, ip.). DN=Diabetic nicotine group (STZ 50 mg/kg, and Nicotine 0.4 mg/kg, ip.). DCN= Nicotine-caffeine both simultaneously in diabetic rats (STZ 50 mg/kg, and Caffeine 20 mg/kg and Nicotine 0.4 mg/kg, ip.). Results expressed as mean±SEM. Difference analyzed amongst groups using one-way ANOVA and Dunnett's t-test as post-test. Day 07, 14, and 21, results taken simultaneously for ANOVA.*Difference in groups when compared with diabetic control significant at p<0.05. **Difference in groups when compared with diabetic control significant at p<0.01.

There was a progressive reduction in HDL-C in all diabetic groups as a part of metabolic complication, whereas the negligible change in HDL-C in all non-diabetic groups. In DCN group there was about 6-10 mg/dl mean reduction in HDL-C (p<0.01) than that of D group.

There was a marked increase in mean bad cholesterol, LDL-C in diabetic groups and almost no change in this type of cholesterol in non-diabetic groups. In DCN group, there was considerable (12-15 mg/dl) and significant (p<0.01) increase in mean LDL-C compared to D group. Co-addiction enhanced bad cholesterol more than that of any single addiction group (DC and DN).

There were similar results for LDL-C and VLDL-C. Diabetic groups showed a rise in VLDL-C; whereas non-diabetic groups didn't showed such rise. DCN group showed much clear and significant increase in mean VLDL-C (6-9 mg/dL) (p<0.01) compared to D and DC/DN group.

There was clear and much significant rise in total cholesterol in diabetic animals as compared to non-diabetic animals. There was greater (60-65 mg/dl) and much significant (p<0.01) rise in mean TC in DCN group as compared to DC/DN group and D group (table 2)

Histopathology analysis

Diabetic pancreas

Tail portions of the pancreas were dissected and observed under high power magnification using a compound microscope. Non-diabetic animals showed the normal histological architecture of pancreatic tissue, while diabetic groups exhibited oxidative damage due to STZ administration. In diabetic groups, D and DC/DN group showed limited damage; while DCN group animals showed more damage to endocrine pancreas at the end of the study. There were more frequent vacuolations and much focal degeneration of pancreatic tissue in diabetes due to co-addiction (fig. 1. A).

Diabetic kidneys

Kidney sections were taken in such a way that both cortex and medullary regions of it were covered. Kidneys of non-diabetic animals from C, N, and CN groups were normal and did not exhibit significant loss of the tissue architecture. Diabetic groups due to STZ injection showed a significant abnormality in tissue architecture of kidney. In diabetic groups, D and DC/DN groups showed a similar degree of damage, while DCN showed severe structural damage of tissue. In DCN group there was great focal degeneration with prominent vacuolation. There was much significant inflammatory cell infiltration in kidney tissue at the end of the study in DCN group (fig. 1. B).

A. Pancreas histopathology: (n=6)
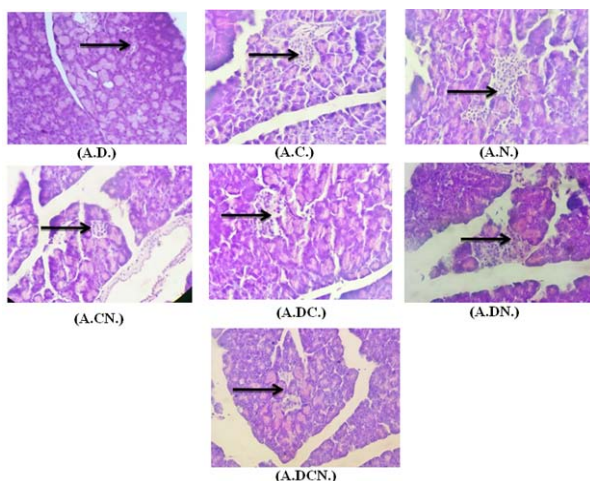
Fig. 1A: F3: A. D.: Moderately damaged pancreatic islets. A. C.: Normal pancreatic islets. A. N.: Normal pancreatic islets. A. CN.: Minimally affected pancreatic islets. A. DC.: Minimally affected pancreatic islets. A. DN: Moderately affected pancreatic islets. A. DCN.: Diabetes and addiction affected distorted pancreatic islets. (HandE 40X)

Diabetic liver

All non-diabetic groups showed eminent hepatocytes and normal central vein; whereas, diabetic groups showed different degrees of damage depending upon the nature of addiction. DC/DN group showed minimal or negligible damage while DCN group showed much intense damage of hepatocytes. Addiction created a different kind of damages; like a focal loss, intermittent vacuolation, and infiltration of inflammatory cells and leukocytes (fig. 1. C).

B. Kidney histopathology: (n=6)
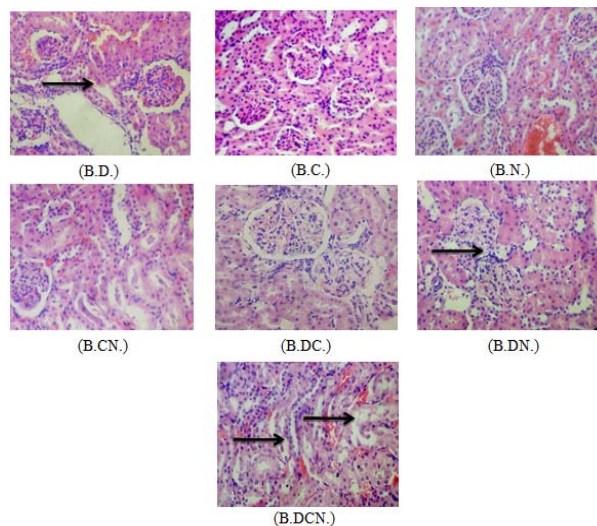
Fig. 1B: F4: B. D.: Partly damaged the kidney. B. C.: Normal Kidney. B. N.: Normal kidney. C. N.: Normal tissue with minimal vacuolation. B. DC.: Minimal damage to kidney tissue. B. DN.: Distorted kidney tissue and congested blood vessels. B. DCN.: Fully distorted kidney tissue. (HandE 40X.)

C. Liver histopathology: (n=6)
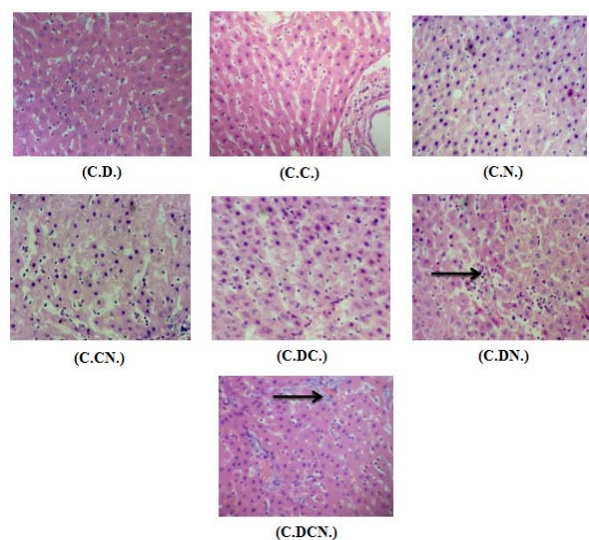
Fig. 1C: F5: C. D.: Moderate Focal degeneration and inflammatory cell infiltration; C. C.: Caffeine affected liver; C. N.: Nicotine affected liver; C. DN.: Moderate vacuolations and inflammatory cell infiltration; C. CN.: Minimal vacuolation and inflammatory cells infiltration; C. DC.: Caffeine affected diabetic liver; C. DN.: Moderate vacuolation and inflammatory cell infiltration; C. DCN.: Damaged diabetic liver with inflammatory cell infiltration. (HandE 40X)

DISCUSSION

This study is based on the assumption that, the HFD/STZ model induces type 2 diabetes in SD rats, and it alters the blood parameters in quite comparable and reproducible manner in these rats. This study also assumes that the caffeine and nicotine injected in animal induces an addiction, similar like an addiction in a human being. These two aspects may be considered as principal limitations of the study.

It is difficult fully to simulate the human conditions of type II diabetes in an animal by using chemical induction. However, there are few reliable models developed by the researchers where chemical induction creates a condition that very closely resemble the condition of type II diabetes. Assuming that these models really do their purpose, we selected a high-fat diet/streptozotocin (HFD/STZ) model to induce type II diabetes in SD rats [12]. For diabetic animals, standard diet was mixed with the vegetable source ghee in such a concentration that, approximately 40% kcals were contributed by the fatty content. STZ (assay>98%) was instantly diluted in cold citrate buffer and injected intraperitoneally in rats to induce type 2 diabetes. The induction of diabetes was confirmed by polyphagia and polyuria in the animals.

This study was structured in such a way that, it allowed us to compare parameters of non-diabetic groups with diabetic groups on one side; while non-addiction group, single addiction, and double addiction group with each other on another side. This design also allowed us to compare results of each time point with successive week time point, facilitating cross-over analysis. One way ANOVA was employed to compare the parameters of non-addiction, single addiction and double addiction groups within the diabetic block to prove proposed a hypothesis of exaggeration of diabetes.

Many authors investigated the effect of various components of coffee and tea in type 2 diabetes. Such studies had been conducted a few in rats, less in mouse and mostly in human beings. In most studies, it is observed that the components of tea or coffee (mostly polyphenolic), other than caffeine are beneficial in type 2 diabetes [13]. Sang-Hyun Ihm, et. al proposed that decaffeinated green tea extract is beneficial in hypertension and insulin resistance [14]. Caffeine consumption had been found to increase glucose and insulin resistance in type 2 diabetes and deteriorated overall situation [15]. This effect of caffeine in developing insulin resistant is said to be attributed to its antagonist action on A1 adenosine receptor and adrenergic effect induced by it [4, 15]. Other authors proposed that hyperglycemic effect of caffeine is due to stimulation of alpha-amylases [4]. Few authors explained that by different mechanisms, caffeine reduce insulin sensitivity in tissues [15, 16]. On these grounds, caffeine was selected for the study; though there are few studies opposing these claims for caffeine.

Nicotine is being known the substance of abuse and form strong addiction compared to that of caffeine. Nicotine is used by the addicts in different ways. In connection to diabetes, most authors observed nicotine-induced hyperglycemia and decrease in insulin sensitivity with an increase in nicotine addiction [17]. Nicotine induces insulin resistance in skeletal muscle by activating mammalian target of rapamycin [3]. Smoking enhances the risk of type 2 diabetes, and chances of cardiovascular disease and obesity [18]. Long-term use of nicotine gum is associated with hyperinsulinemia and insulin resistance [19] Wu Y, *et al.* (2012) proposed adenosine monophosphate kinase α2 as an essential mediator of nicotine-induced whole-body insulin resistance in spite of the reduction in adiposity [20]. Smoking cessation is associated with improvement in insulin resistance and nicotine is the main ingredient in cigarette smoke causing insulin resistance [21]. Insulin resistant rats undergo a unique neurobiological change that disrupts insulin signaling and promotes the rewarding effects of nicotine [22]. Positive dose-dependent associations and independent effects of postnatal exposure to nicotine suggest the involvement of environmental tobacco smoke in the risk for development of insulin resistance in children [23]. Smoking is one of the main risk factors for cardiovascular disease. The smokers exhibit different degrees of insulin resistance [24].

Caffeine (assay>98%) and nicotine (assay>95 %) were diluted in normal saline and administered intraperitoneally twice daily in their normal doses to create addiction simulation [25]. Oral route was considered inappropriate for addiction due to its limitations like variable absorption, prehepatic and hepatic metabolism, etc. When two drugs are injected simultaneously, they generally do not interfere the pharmacological effects of each other, unless they act on the same system or same molecular targets, excluding exceptions [9]. Nicotine and caffeine both are nervous system stimulant drugs and known for their addiction in a human being. There are a significant amount of people addicted to these to drugs knowingly or unknowingly. As per our observation, both these drugs together enhance insulin resistant exceptionally more than that of their individual addiction do. There is additive damage they produce when administered together in diabetic rats, through some unknown mechanism not yet explored by any research team. Bringing the awareness about this hidden harm caused by these abused compounds, was the principle target of this study; which we could, fortunately, able to demonstrate.

Biochemical changes substantially and clearly expressed the outcome of the study that, simultaneous administration of these compounds exaggerated type 2 diabetes in rats. Histopathology results exhibited damage to the liver, which was exceptionally more in the case where both the compounds were injected; suggesting that, liver inflammation possibly have some connectivity with rapid progression of type 2 diabetes. Kidneys showed relatively more damage in co-administration group compared to the individual administration group. There were significant inflammatory cell infiltration and focal loss in co-administration cases.

CONCLUSION

On the basis of results obtained; we suggest that diabetes patient should strictly avoid taking caffeine and nicotine products together, but taking them separately will be less harmful when he cannot avoid them. This will avoid co-occurrence of these compounds in the blood and enhancement of insulin resistance. But, permanently leaving the addition will definitely be good and may reduce sufferings of the patient in the later lifetime. Whereas, taking these two compound-containing products at different time points can possibly avoid much of the damage; as single addiction creates less damage. We further suggest that there is a need to conduct a clinical trial on these lines to confirm our finding in type 2 diabetic patients [26]. It may be too early and immature to conclude the fact on the model animal basis alone.

ACKNOWLEDGEMENT

ABBREVIATION

SD: Sprague Dawley; STZ: Streptozotocin; DB: Diabetic control group; C: Caffeine group; N: Nicotine group; D: Diabetic group; CN: Caffeine and Nicotine both simultaneously group; DC: Caffeine treated diabetic animal group; DN: Nicotine treated diabetic animal group; DCN: Caffeine and Nicotine both simultaneously treated diabetic animal group; IAEC: Institutional Animal Ethics Committee; CPCSEA: Committee for the purpose of control and supervision of experiments in animals; SEM: Standard Error of Mean; ANOVA: Analysis of Variance; ip: Intraperitoneal; po: per oral.

CONFLICT OF INTERESTS

Authors explicitly declare that there is no conflict of interest

REFERENCES

1. Dhaliwall C, Erinmacpherson, Richardson J. Effectiveness of telephone-delivered interventions for increasing physical activity levels in persons with type 2 diabetes or hypertension: a systematic review. J Crit Rev 2015;2:06-11.

2. Malini P, Kanchana G, Rajadurai M. Antidiabetic efficacy of ellagic acid in streptozotocin-induced diabetes mellitus in albino Wistar rats. Asian J Pharm Clin Res 2011;4:125-8.

3. Van Dijk AE, Olthof MR, Meeuse JC, Seebus E, Heine RJ, Van Dam RM. Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance. Diabetes Care 2009;32:1023–5.

4. Egawa T, Hamada T, Kameda N, Karaike K, Ma X, Masuda S, *et al.* Caffeine acutely activates 5'adenosine monophosphate-activated protein kinase and increases insulin-independent glucose transport in rat skeletal muscles. Metabolism 2009;58:1609–17.

5. Akiba T, Yaguchi K, Tsutsumi K, Nishioka T, Koyama I, Nomura M, *et al.* Inhibitory mechanism of caffeine on insulin-stimulated glucose uptake in adipose cells. Biochem Pharmacol 2004; 68:1929–37.

6. Kashani AE, Yaghmaei P, Larijani B, Ebrahim HA. Xanthine derivatives as activators of alpha-amylase: a hypothesis on a link with the hyperglycemia induced by caffeine. Obesity Res Clin Practice 2013;7:e487–93.

7. Bergman BC, Perreault L, Hunerdosse D, Kerege A, Playdon M, Samek AM, *et al.* Novel and reversible mechanisms of smoking-induced insulin resistance in humans. Diabetes 2012;61:3156–66.

8. Li W, Hui R. Cigarette smoking induces insulin resistance: partly via ASP–C5L2 pathway? Biosci Hypotheses 2009;2:267–9.

9. Rajan AK, Kousalya K, Senthil NB, Valentina P. An outlook on the mechanisms of drug interactions with other drugs, fruits, herbs and their preventive measures. Asian J Pharm Clin Res 2016;9:10-8.

10. Skovsø S. Modeling types 2 diabetes in rats using high-fat diet and streptozotocin. J Diabetes Invest 2014;5:349–58.

11. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: an overview. Indian J Med Res 2007;125:451–72.

12. Divi SM, Bellamkonda R, Dasireddy SK. Valuation of the antidiabetic and antihyperlipidemic potential of aqueous extract of *Moringa oleifera* in fructose-fed insulin resistant and STZ induced diabetic wistar rats: a comparative study. Asian J Pharm Clin Res 2012;5:67-72.

13. Kim Y, Keogh JB, Clifton PM. Polyphenols and glycemic control. Nutrients 2016;8:17.

14. Ihm SH, Jang SW, Kim OR, Chang K, Oak MH, Lee JO. Decaffeinated green tea extract improves hypertension and insulin resistance in a rat model of metabolic syndrome. Atherosclerosis 2012;224:377–83.

15. Lane JD, Barkauskas CE, Surwit RS, Feinglos MN. Caffeine impairs glucose metabolism in type 2 diabetes. Diabetes Care 2004;27:2047–8.

16. Beaudoin MS, Robinson LE, Graham TE. An oral lipid challenge and acute intake of caffeinated coffee additively decrease glucose tolerance in healthy men. J Nutr 2011;141:574–81.

17. Van Dam RM, Hu FB. Coffee consumption and risk of type 2 diabetes: a systematic review. JAMA 2005;294:97–104.

18. Morgan TM, Crawford L, Stoller A, Toth D, Yeo KTJ, Baron JA. Acute effects of nicotine on serum glucose-insulin growth hormone and cortisol in healthy smokers. Metabolism 2004;53:578–82.

19. Chiolero A, Faeh D, Paccaud F, Cornuz J. Consequences of smoking for body weight, body fat distribution, and insulin resistance. Am J Clin Nutr 2008;87:801–9.

20. Eliasson B, Taskinen MR, Smith U. Long-term use of nicotine gum is associated with hyperinsulinemia and insulin resistance. Circulation 1996;94:878–81.

21. Wu Y, Song P, Zhang W, Liu J, Dai X, Liu Z, *et al.* Activation of AMPK [alpha] 2 in adipocytes is essential for nicotine-induced insulin resistance *in vivo*. Nat Med 2015;21:373–82.

22. Assali AR, Beigel Y, Schreibman R, Shafer Z, Fainaru M. Weight gain and insulin resistance during nicotine replacement therapy. Clin Cardiol 1999;22:357–60.

23. Richardson JR, Pipkin JA, O'Dell LE, Nazarian A. Insulin resistant rats display enhanced rewarding effects of nicotine. Drug Alcohol Depend 2014;140:205–7.

24. Eliasson B1, Attvall S, Taskinen MR, Smith U. The insulin resistance syndrome in smokers is related to smoking habits. Arterioscler Thromb 1994;14:1946-50.

25. Matta S, Balfour D, Benowitz N, Boyd RT, Buccafusco J, Caggiula AR, *et al.* Guidelines on nicotine dose selection for *in vivo* research. Psychopharmacology (Berl) 2007;190:269–319.

26. Gupta M. Impact of meal replacement on the health status of type ii diabetes. Int J Curr Pharm Res 2014;6:79-82.

How to cite this article

- Subhash T Kumbhar, Hemant D Une, Anagha M Joshi, Pralhad B Wangika. Exaggeration of type 2 diabetes due to caffeine-nicotine co-administration: a study in rats. Int J Pharm Pharm Sci 2016;8(9):277-282.