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ANTIOBESITY, ANTIHYPERLIPIDEMIC AND ANTIDIABETIC AGENTS OF PROTO-CATECHUIC ACID IN HIGH FATTY DIET ALONG WITH ALLOXAN INDUCED DIABETES

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Keywords:

Obesity,
High fatty diet, Alloxan,
Protocatechuic acid, Diabetes

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ABSTRACT: Aim: The present investigation was carried out by evaluating the effect of protocatechuic acid (PCA) on a high-fat diet (HFD) along with a low dose of alloxan-induced hyperglycemia in male Wistar rats. **Methods:** In the present study, administration of high fatty diet (HFD) for 45 days along with low dose of alloxan (80 mg/kg) in rats on 30 days produced significant high fatty diet in the, body weight, low density lipoproteins (LDL), triglycerides (TG) and blood glucose levels while decreases the high density lipoprotein (HDL) and serum insulin level. Protocatechuic acid (PCA) was started to administered at a dose of 50, 100, 200 mg/kg (orally) on day 45 of HFD administration and continue further 45 days. Glibenclamide was used as reference standard. **Results:** Results of PCA shows a significant decreased in body weight, blood glucose level after 45 days of treatment in diabetic animals. Result also indicated to increase in serum insulin level. The result of lipid profile indicated to normalize after 45 days treatment with PCA in diabetic rats. All the results are compared with reference standard glibenclamide. **Conclusion:** From this result, it concludes that PCA decreases the body weight, blood glucose level dose-dependently indicated antidiabetic and antiobesity activity. Antidiabetic activity of PCA is associated with increased in the level of serum insulin.

INTRODUCTION: Obesity is a metabolic disease of pandemic proportions mainly arising from positive energy balance, a consequence of sedentary lifestyle, conditioned by environmental and genetic factors. Obesity is characterized by the accumulation of excess fat in adipose tissues and results in various life upcoming complications such as cardiovascular diseases, Type 2 diabetes, and cancer [1].

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The modern lifestyle of increased intake of high-calorie cafeteria fast food associated with decreased energy expenditure also contributes to the current rising prevalence of obesity and type 2 diabetes [1, 2].

Insulin resistance plays a primary role in the development of Type 2 diabetes and is a characteristic feature of other health disorders including obesity, dyslipidemia, hypertension, and cardiovascular disease [3]. It is widely known that an elevation in circulating free fatty acid (FFA) levels impairs insulin action and leads to insulin resistance in animals and human [4]. This may represent a physiologic mechanism of insulin resistance because elevated FFA levels are generally observed in most human insulin resistance states [5].

The HFD causes insulin resistance in rats. The feeding of HFD produced insulin resistance syndrome in rats. It was characterized by the increased body weight (obesity), mild hypertension, hypertriglyceridemia, hypercholesterolemia and compensatory hyperinsulinemia [3, 4]. It is therefore important to prevent or abate obesity. A growing number of enzymes involved in lipid metabolic pathways are being identified and characterized. They represent a rich pool of potential therapeutic targets for obesity. To reduce these serious complications and negative outcome of the metabolic syndrome, the control not only of blood glucose but also of lipids profile is necessary [5].

Aim and Objective: In the current research, the study was aimed to investigate antiobesity, anti-hyperlipidemic and antidiabetic effect of proto-catechuic acid against experimentally high fatty diet (HFD) along with a low dose of alloxan-induced in the diabetic rat.

MATERIALS AND METHODS:

Animals: 36 male Wistar rats weighing (180-220 g) were used for the study. The animals were kept in standard cages at room temperature with 12 h light/dark condition in the animal house of the Department of Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) (Reg. CPCSEA/IAEC/PC-08/01-2K17). The animals were fed on a standard diet and had water *ad libitum*.

Methods: Obesity and diabetes were induced by administration of high fatty diet (HFD) for 45 days along with a single low dose of freshly prepared in normal saline alloxan monohydrate 80 mg/kg by intraperitoneal injection(on 30 days) in Wistar rat.

HFD Composition: The composition of a high-fat diet was considered as reported in the literature with appropriate modification. (6) Containing lard oil (25%), fructose (40%), protein powder (45%), cholesterol (2%), vitamin and fiber contains feed.

Experimental Methodology: After 45 days of HFD administration along with alloxan (on 30 days), blood samples and body weight were taken. The blood glucose level greater than 200 g/dl were considered diabetic and selected for further configuration. Group I: Normal (saline only), Group II: Diabetic control (saline only), Group III: Diabetic + Glibenclamide (600 µg/kg b.wt/day in saline), Group IV: Diabetic + PCA acid (50 mg/kg b.wt/day in saline), Group V: Diabetic + PCA (100 mg/kg b.wt/day in saline), Group VI: Diabetic + PCA (200 mg/kg b.wt/day in saline)

Biochemical Estimations: After 45 days of with PCA treatment samples blood was withdrawn from the retro-orbital plexus and the serum was separated and used for biochemical estimations of blood serum glucose level, serum insulin level, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG)

RESULTS:

TABLE 1: THE EFFECT OF PROTOCATECHUIC ACID (PCA) ON BLOOD GLUCOSE LEVEL IN HIGH FATTY DIET WITH LOW DOSE OF ALLOXAN INDUCED DIABETES

Group	0 Day	30 Days	45 Days	90 Days
Control	92.485550 ± 1.469921	91.522160 ± 0.413249	83.526010 ± 0.844274	94.171490 ± 0.589561
Negative Control	91.377650 ± 0.839039	109.248600 ± 1.096744	301.107900 ± 5.136826###	332.466300 ± 0.644825
Glibenclamide	90.558770 ± 1.654679	111.079000 ± 1.744776	324.566500 ± 5.642093###	111.632900 ± 0.832442*
PCA (50 mg/kg)	92.485550 ± 0.654823	109.682100 ± 2.101098	321.475900 ± 0.498735###	141.281300 ± 0.665019*
PCA (100 mg/kg)	89.932560 ± 1.140103	112.186900 ± 2.257811	310.934500 ± 5.127604###	132.851600 ± 0.609302*
PCA (200 mg/kg)	88.921010 ± 2.079676	112.957600 ± 3.72927	314..499000 ± 0.67849###	122.543400 ± 0.810624 *

n=6, Values are mean ± S.E.M., ###P<0.0001, as compared with 0 days reading of respective group; *P<0.05 compared to 45 days reading of respective groups. Data analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's Multiple Comparison Test.

TABLE 2: THE EFFECT OF PROTOCATECHUIC ACID (PCA) ON BODY WEIGHT IN HIGH FATTY DIET WITH LOW DOSE OF ALLOXAN INDUCED DIABETES

Group	0 Day	40 Days	90 Days
Control	179.333300	179.833300	179.500000
Negative Control	180.000000	268.500000$^{###}$	264.122600
Glibenclamide	179.166700	265.166700$^{###}$	221.345000**
Losartan	181.833300	264.666700$^{###}$	220.314500**
PCA (50 mg/kg)	181.833300	266.333300$^{###}$	233.415500**
PCA (100 mg/kg)	181.000000	267.666700$^{###}$	228.146300**
PCA (200 mg/kg)	180.500000	268.833300$^{###}$	218.314300**

n=6, Values are mean ± S.E.M., ###P<0.0001, as compared with 0 days reading of respective group; **P<0.001 compared to 40 days reading of respective groups. Data analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's Multiple Comparison Test.

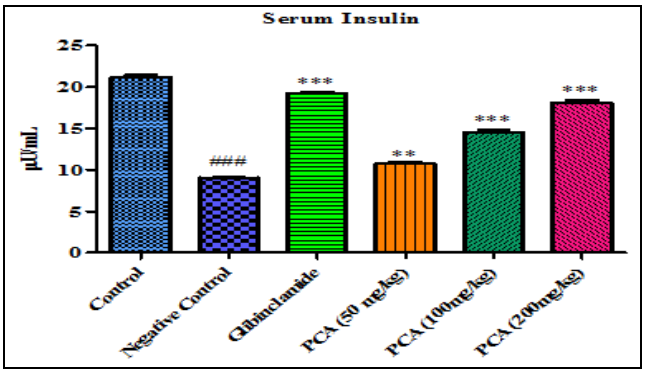

FIG. 1: EFFECT OF PCA ON SERUM INSULIN LEVEL IN HFD AND ALLOXAN INDUCED DIABETIC RATS

n=6, Values are mean ± S.E.M., ###P<0.0001, as compared to NC(Negative Control) to control group; ***P<0.0001, ***P<0.0001, ***P<0.0001, as compared to negative control, Data analyzed by one way Analysis of Variance (ANOVA) followed by Tukey's Multiple Comparison Test.

TABLE 3: THE EFFECT OF PROTOCATECHUIC ACID (PCA) ON LIPID PROFILE IN HIGH FATTY DIET WITH LOW DOSE OF ALLOXAN INDUCED DIABETES

Treatment Grp.	HDL level (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)
Control	27.22 ± 1.274	7.444 ± 0.2725	27.78 ± 0.3020
Negative Control	10.37 ± 0.5312 ###	16.72 ± 0.09239 ###	61.27 ± 0.5255 ###
Glibenclamide	18.59 ± 0.3150 ***	8.319 ± 0.05648 ***	31.41 ± 0.4163 ***
Losartan	19.43 ± 0.2846 ***	8.615 ± 0.07814 ***	30.30 ± 0.3959 ***
PCA (50 mg/kg)	13.23 ± 0.4577 *	14.37 ± 0.1218 ***	37.33 ± 0.4694 ***
PCA (100 mg/kg)	13.96 ± 0.1253 **	15.66 ± 0.1826 ***	35.67 ± 0.2532 ***
PCA (200 mg/kg)	14.24 ± 0.1083 **	12.77 ± 0.1022 ***	32.92 ± 0.4284 ***

n=6, Values are mean ± S.E.M., ###P<0.0001, as compared with Vehicle Control; ***P<0.0001, **p<0.001, *P<0.01 as compared to negative control, Data analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's Multiple Comparison Test

Histopathology Study: Pancreas:

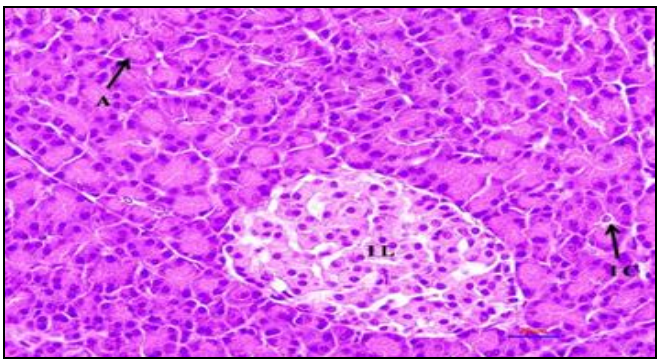

FIG. 2: CONTROL PANCREAS SHOWING NORMAL HISTOLOGY, ACINUS (A), INTERCALATED DUCT (IC), ISLET OF LANGERHANS (IL)

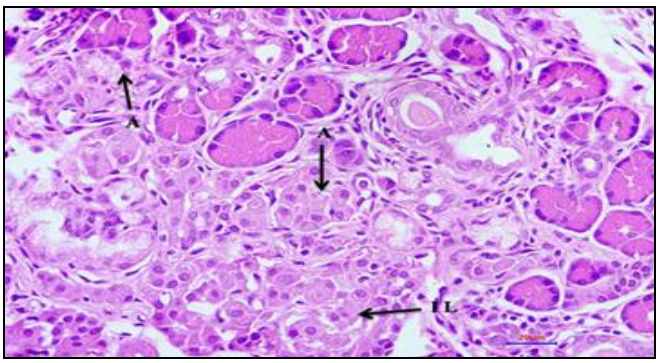

FIG. 3: CONTROL PANCREAS SHOWING DEGENERATION OF ACINUS (A) AND ISLET OF LANGERHANS (IL)

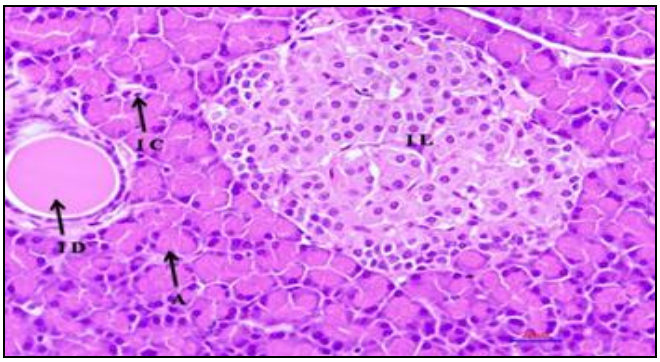

FIG 4: STD SHOWING NORMAL HISTOLOGY, ACINUS (A), INTERLOBULAR DUCT (ID), INTERCALATED DUCT (IC), ISLET OF LANGERHANS (IL)

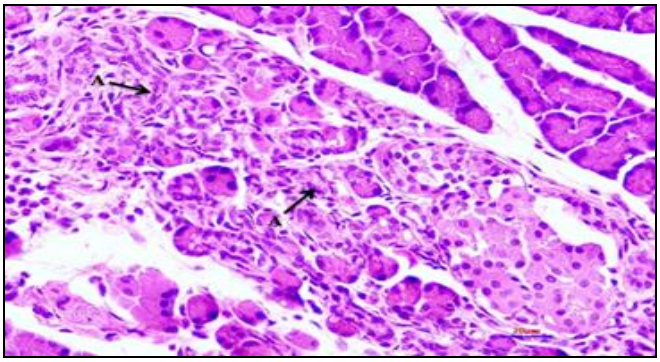

FIG. 5: PCA (50 mg/kg) SHOWING DEGENERATION OF ACINUS (A)

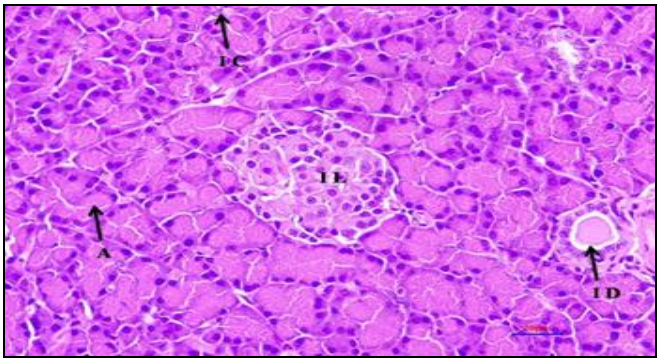

FIG. 6: PCA (100 mg/kg) SHOWING NORMAL HISTOLOGY, ACINUS (A), INTERLOBULAR DUCT (ID), INTERCALATED DUCT (IC), ISLET OF LANGERHANS (IL)

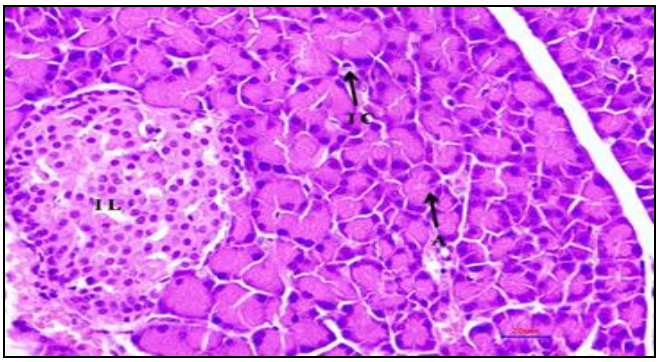

FIG. 7: PCA (200 mg/kg) SHOWING NORMAL HISTOLOGY, ACINUS (A), INTERCALATED DUCT (IC), ISLET OF LANGERHANS (IL)

Liver:

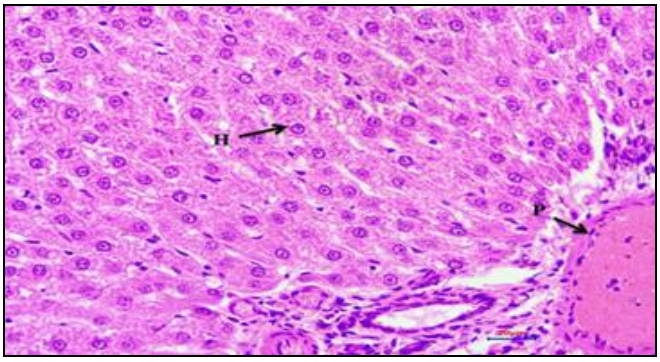

FIG. 8: CONTROL LIVER SHOWING NORMAL HISTOLOGY, PORTAL TRIAD (P) AND HEPATOCYTE (H)

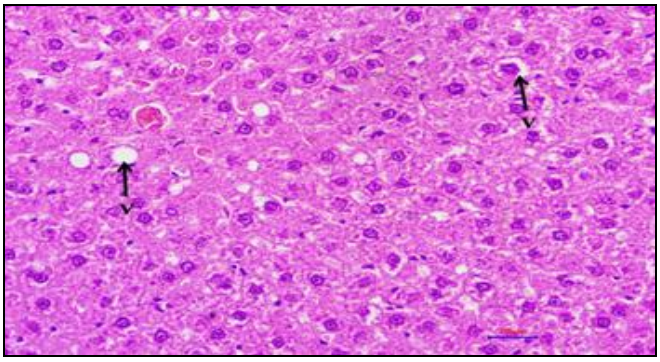

FIG. 9: NEGATIVE CONTROL SHOWING HEPATOCELLULAR VACUOLATION (V)

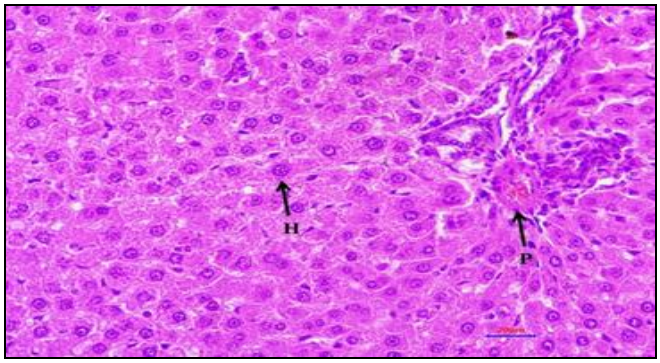

FIG. 10: STD SHOWING NORMAL HISTOLOGY, PORTAL TRIAD (P) AND HEPATOCYTE (H)

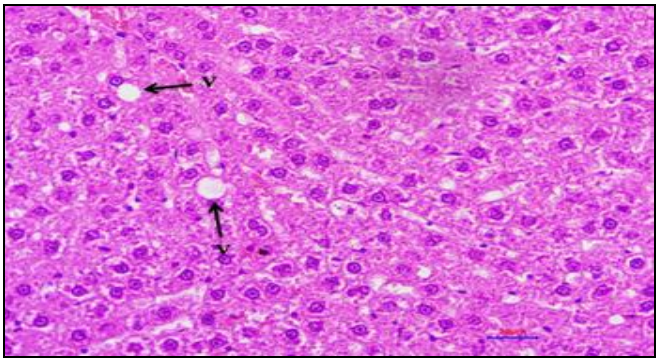

FIG. 11: PCA (50 mg/kg) SHOWING HEPATOCELLULAR VACUOLATION (V)

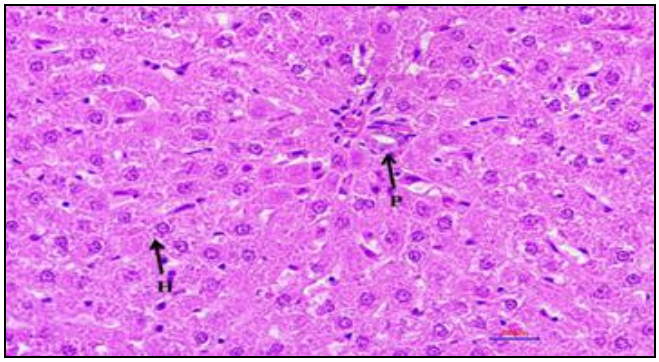

FIG. 12: PCA (100 mg/kg) SHOWING NORMAL HISTO-LOGY, PORTAL TRIAD (P) AND HEPATOCYTE (H)

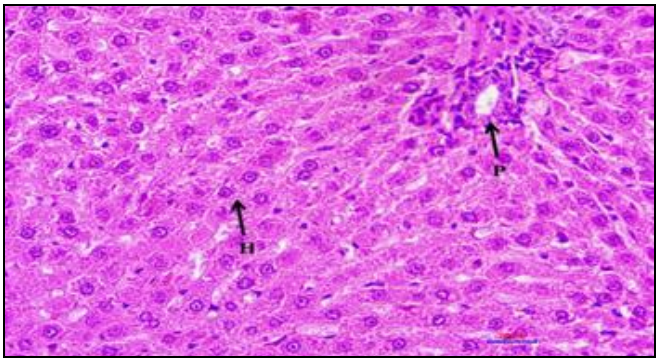

FIG. 13: PCA (200 MG/KG) SHOWING NORMAL HISTO-LOGY, PORTAL TRIAD (P) AND HEPATOCYTE (H)

DISCUSSION: Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency or deficiency of insulin secretion [7]. Obesity is more likely to increase the risk for hyperglycemia because of alteration in serum lipid profile like Serum Insulin level or blood glucose level [8]. Generally, a high-fat diet is one of the major factors causing obesity and that the long-term intake of high-fat diet evokes a significant increase in abdominal fat weight. Protocatechuic acid (PCA) is a phenolic compound, the main metabolite of anthocyanin, which has been reported to display various pharmacological properties [7]. In the present investigation, we proposed the hypothesis that PCA exerts antiobesity, antihyperlipidemic, antidiabetic effects in the high fatty diet with a low dose of alloxan-induced in diabetic rats [8, 9, 10, 11].

In light of this, the present study was undertaken to evaluate the effects of PCA on body weight, lipid profile and blood glucose level in the high fatty diet with a low dose of alloxan-induced body weight in diabetic rats [12, 13, 14, 15, 16]. Results of the present study indicated increased in body weight, and blood glucose level in the high fatty diet with a low dose of alloxan shows induction of obesity and diabetes on 45 days of study as compared to normal control animals [17]. Results of 45 days of oral administration of PCA indicates the decreased in body weight and normalization of blood glucose level demonstrates antiobesity and antidiabetic activity investigation [7]. The results suggest that insulin resistance and hyperinsulinemia are not induced in the excessive weight gain observed in their animal model. The decline in the level of insulin reflect difficult in the secretion of insulin because of degeneration of Langerhans cell indicates induction of type II model as indicated in the histopathology of pancreas [18, 19, 20, 21]. Other factors which may contribute to the increased in obesity and diabetes are altered of lipid profile. Considering the high lipid profile is risk factors in the development of obesity and complication in diabetes, the study further was undertaken to measure the level of LDL, HDL, triglycerides level [22]. The results of the study indicate to increase in the level of lipid profiles due to consumption of high fatty diet contributes in the development of obesity [23, 24]. While the 45 days of treatments with PCA indicates to significantly decreased in the LDL, triglycerides and elevates the level of HDL, these results reflects the preventing rate in development of cardiovascular complication as well as antiobesity in diabetic rats activity of protocatechuic acid in the high fatty diet with a low dose of alloxan-induced diabetic rats [25].

CONCLUSION: From this finding, it concludes that protocatechuic acid possesses antiobesity, anti-hyperlipidemic and anti-diabetic activity as reflected by a decline in body weight, blood glucose and normalization of lipid profile in HFD along with the single low dose of alloxan included diabetic rats. So, it would help treat obesity and its associated condition such as diabetes and its cardiovascular complication.

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CONFLICT OF INTEREST: The authors do not have any conflict of interest to declare.

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