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Hepatoprotective and hypolipidemic activities of *Caesalpinia bonduc* seed kernels and *Gymnema sylvestre* leaves extracts in alloxan-induced diabetic rats

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Abstract: The objective of the current research was to validate the hepatoprotective and anti-hyperlipidemic activities of *C. bonduc* seed kernels (CBSK) and *G. sylvestre* leaves (GSL) hydro-methanolic extracts, separately and in combination (CBSKE+GSLE) in alloxan-induced diabetic rat model for 28 days. Diabetes was induced by i.p. injection of alloxan monohydrate (140 mg/kg body weight) to albino Wistar rats. Six groups of rats (n=9) were used. Group 1 was the normal control; group 2 was diabetic control. After induction of diabetes metformin (150mg/kg), CBSKE (400mg/kg), GSLE (400 mg/kg) and CBSKE+GSLE (400mg/kg) were administered to diabetic rat groups 3, 4, 5 and 6 respectively for a period of 28 days. Diabetic rats exhibited an increase in serum blood glucose, liver function markers and lipid profile. Treatment of diabetic rats with metformin, CBSKE, GSLE and CBSKE+GSLE for 4 weeks significantly produced hepatoprotective and hypolipidemic effect via amelioration of raised serum glucose, liver profile, and lipid profile. The outcomes of this study suggest that *G. sylvestre* leaves and *C. bonduc* seed kernels have hepatoprotective and hypolipidemic potential which possibly help in managing diabetes-induced liver injury and hyperlipidemia.

Keywords: *Caesalpinia bonduc,* diabetes, *Gymnema sylvestre,* hepatotoxicity, hyperlipidemia.

INTRODUCTION

Diabetes is a metabolic disease described by prolonged hyperglycemia. In this disease, there is an alteration in lipid, protein, and carbohydrate metabolism. This metabolic disorder has become the leading cause of main complications such as oxidative stress, retinopathy, neuropathy, nephropathy, dyslipidemia and impaired liver functions. It is predicted that with the unexpected prevalence of diabetes type 2 worldwide, a noteworthy population could be suffering from liver impairment secondary to prolonged hyperglycemia (Ogurtsova *et al*., 2017).

Diabetes mellitus can be treated by diet, exercise and oral hypoglycemic agents and insulin. However, these drugs are costly and have side effects. Keeping this point in view, an appropriate pharmacological intervention is indispensable for the management of diabetes. World health organization director of traditional medicines reported that about 60% population of the world fulfills their primary health-associated requirements by using herbal drugs owing to their abundance, low cost, easy availability and safety (Nambirajan *et al*., 2018).

Caesalpinia bonduc (L.) Roxb. (Family: Caesalpiniaceae) is also known as "bonduc nut, fever nut and nicker nut". *C. bonduc* possesses broad therapeutic effects like antiviral, antipyretic, antibacterial and antioxidant. These effects are due to the presence of phytoconstituents such as polyphenols, saponins and flavonoids in various parts of *C. bonduc* such as seeds, leaves, roots and bark (Iftikhar *et al*., 2020).

Gymnema sylvestre (Family: Asclepiadaceae) also known as "Gudmar or Sugar destroyer" is a traditional medicinal plant that has been recognized with various pharmacological properties such as antioxidant, anti-diabetic, and anti-hyperlipidemic. *G. sylvestre* leaves have acidic glycosides, anthraquinone and their derivatives (Kim *et al.,* 2017). The current experiment was conducted to explore the effect of *C. bonduc* seed kernels extract (CBSKE) and *G. sylvestre* leaves extract (GSLE) alone and in combination on liver and lipid profiles of diabetic rats.

MATERIALS AND METHODS

Preparation of plants extracts

Caesalpinia bonduc seed kernels (CBSK) and *Gymnema sylvestre* leaves (GSL) were duly recognized and authenticated by a Taxonomist at the Department of Botany, University of Agriculture, Faisalabad. For future reference, specimen vouchers were assigned and preserved in herbarium vide No.248-1-2019 and 249-1-2019 for *C. bonduc* and *G. sylvestre* respectively. The extracts of both plants were prepared by macerating powdered CBSK and GSL each 1kg in methanol and water (7:3) for 7 days and were evaporated and concentrated by using a rotary evaporator.

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Experimental design and induction of diabetes

Male adult Wistar albino rats (n=54) weighing about 180-200 g were used. Rats were retained in an animal house at the Institute of Physiology and Pharmacology, University of Agriculture Faisalabad. The research was carried out by strictly following the guidelines for the use and care of animals, approved by the institutional bioethical committee after getting permission to vide letter number 1764. Animals were allocated into 6 groups (n=9). Group 1 was of normal control. Group 2 was of diabetic rats without treatment. Group 3, 4, 5 and 6 respectively were treated with metformin (150mg/kg), CBSKE, GSLE and CBSKE+GSLE (400 mg/kg each) on daily basis for 28 days. Rats were made diabetic by administering a single i.p. injection of alloxan monohydrate dissolved in normal saline at 140mg/kg b.w. The rats exhibiting fasting blood glucose levels of ≥ 250mg/dL were included in the research (Muzaffar *et al*., 2019).

Blood sampling

Rats were decapitated by cervical dislocation after completion of the experiment. Blood samples were collected and centrifuged at 4000 rpm for 10-15 minutes to separate serum samples.

Determination of biochemical parameters

A commercially available kit (SBio® Glucose Mono reagent diagnostic kit) was used for the measurement of serum glucose levels. Serum ALT, AST, ALP, albumin, total protein, total cholesterol, triglyceride and HDL-cholesterol levels were measured using commercially available kits (Q.C.A., Spain). While serum LDL-cholesterol was measured by using the following formula: LDL- cholesterol (mg/dL) = Total cholesterol-[Triglycerides/5 + HDL- Cholesterol] (Murad *et al*., 2020).

Histopathological examination

To preserve the tissue samples of the liver, a neutral buffered 10% formalin solution was used. Fixed liver tissue samples were dehydrated and fixed in paraffin. Staining was completed by using eosin and hematoxylin stains. All sections were observed under a light microscope by following previously established protocols (Naseer *et al*., 2019).

STATISTICAL ANALYSIS

One way ANOVA was used for statistical analysis of data by using Graph Pad Prism 6.01 software at a 5% level of significance. The significant variations among means were assessed by Tukey's multiple comparison test. Results were expressed as mean ± SD.

RESULTS

Effect of CBSKE, GSLE separately and in combination on serum glucose

Administration of alloxan monohydrate leads to an elevation of serum blood glucose levels that were maintained in the diabetic control group throughout the experimental period. Daily treatment with metformin, CBSKE, GSLE and CBSKE+GSLE for 28 days significantly reduced (P≤0.05) serum blood glucose levels (fig. 1).

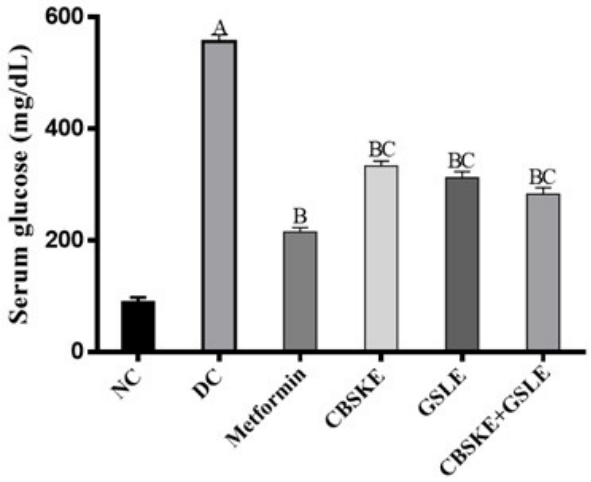

Fig. 1: Effect of metformin, CBSKE, GSLE, and CBSKE+GSLE on serum glucose levels (mg/dL) in alloxan-induced diabetic rats.

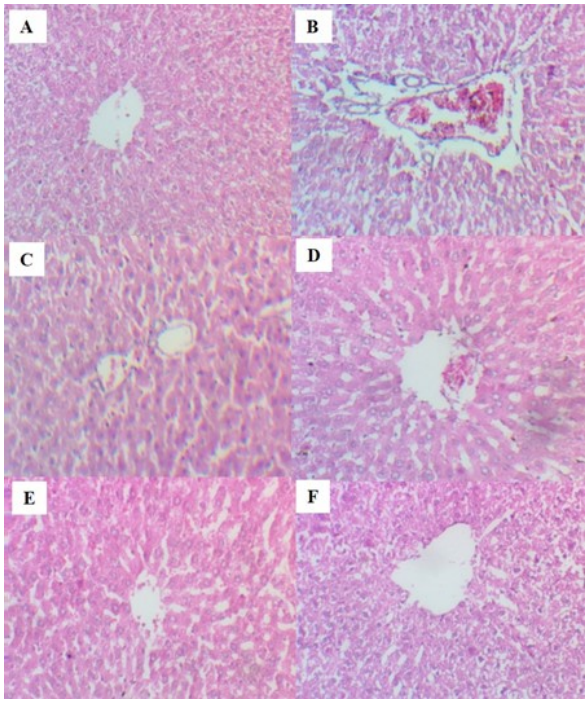

Fig. 2: Photomicrographs of liver rat tissue sections of (A) normal control(B) diabetic control(C) metformin(D) CBSKE(E) GSLE and (F) CBSKE+GSLE treated groups (H&E staining; 100X).

Effect of CBSKE, GSLE separately and in combination on serum liver function markers of diabetic rats

The diabetic control group has significantly (P≤0.05) increased the levels of ALT, AST, and ALP as compared to the normal control group. Rats treated with metformin,

Table 1: Effect of metformin, CBSKE, GSLE and CBSKE+GSLE on serum liver function markers ALT, AST, ALP, total protein and albumin levels in alloxan-induced diabetic rats.

Parameters					
Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total protein (g/dL)	Albumin (g/dL)
Normal control	33.57±4.44	44.38±6.6	116.67±17.8	7.62± 0.6	4.98±0.5
Diabetic control	67.56±6.7^{A}	121.44±13.9^{A}	188.40±20.0^{A}	4.40±0.7^{A}	2.73±0.7^{A}
Metformin	36.16±4.7^{B}	62.66± 7.8^{B}	132.22±14.7^{B}	7.07±0.4^{B}	4.63±0.5^{B}
CBSKE	47.84±6.7^{BC}	83.56±11.9^{BC}	155.56±18.7^{B}	6.57±0.5^{B}	4.43±0.6^{B}
GSLE	40.48± 3.6^{B}	76.31±14.4^{B}	143.89±23.3^{B}	6.92±0.4^{B}	4.52±0.4^{B}
CBSKE+GSLE	38.33± 6.9^{B}	72.22±10.8^{B}	137.78±18.2^{B}	7.17±0.6^{B}	4.60±0.8^{B}

Table 2: Effect of metformin, CBSKE, GSLE and CBSKE+GSLE on serum lipid profile TC, TG, HDL-cholesterol, and LDL-cholesterol levels in alloxan-induced diabetic rats.

Parameters				
Groups	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Normal control	75.89±17.5	56.89 ± 13.7	36.33±8.6	31.94±7.9
Diabetic control	127.44±23.6^{A}	110.44±11.6^{A}	22.56±5.4	77.92±13.9^{A}
Metformin	71.56±14.4^{B}	67±10.8^{B}	45.78±8.6^{B}	13.67±4.4^{B}
CBSKE	93.67±18.8^{B}	88.33±19.0^{B}	39.56±11.6^{B}	41.85±9.8^{BC}
GSLE	84.66 ±12.7^{B}	80.86±16.5^{B}	44.22±7.9^{B}	31.51±8.7^{BC}
CBSKE+GSLE	77.78±9.7^{B}	77.05±13.8^{B}	48.67±9.7^{B}	22.43±5.8^{B}

Results are compared with normal control, diabetic control, and standard drug. $^{A-C}$Mean values within a column, having a different superscript differed significantly from each other at P≤0.05. Values are the mean ±S.D.

CBSKE, GSLE and CBSKE+GSLE significantly (P≤0.05) restored the normal liver enzyme levels. While all treatment groups exhibited significant (P≤0.05) revert to normal serum ALT, AST and ALP concentrations but groups treated with GSLE and CBSKE+GSLE showed better results, which were comparable to (standard) metformin. The albumin and total protein levels were significantly (P≤0.05) declined in the diabetic control group in contrast to the normal control group, while CBSKE, GSLE and CBSKE+GSLE treatment considerably (P≤0.05) restored the albumin and total protein levels. However, no significant change was detected among all groups of extract-treated and when compared with metformin (table 1).

Effect of CBSKE, GSLE separately and in combination on lipid profile of diabetic rats

A noteworthy rise (P≤0.05) in serum total cholesterol, triglyceride and LDL-cholesterol levels and a noteworthy (P≤0.05) decrease in HDL-cholesterol was detected in the diabetic control group in comparison to the normal control group, while treatment with metformin, CBSKE, GSLE, and CBSKE+GSLE showed a significant improvement in lipid profile (table 2).

Effect of CBSKE, GSLE separately and in combination on histology of liver

(A) Photomicrograph of the liver of normal control group rat revealed normal hepatocytes with a well-preserved nucleus, cytoplasm, and central vein. (B) Diabetic control group rat's liver revealed hepatocytes inflammation, accumulation of lipid, the central vein was prominent and congested. Pycnotic nucleus and hemorrhage were also seen. (C) Liver tissue of the metformin-treated group showed reverse degeneration of hepatocytes and central vein. (D) CBSKE treated group depicted significant improvement and restoration of the histological structure of the liver. (E) Liver tissue of GSLE treated group displayed improvement in degenerative effect in comparison to the diabetic control group (F) Liver tissue of CBSKE+GSLE treated group showed restoration of the normal architecture of liver hepatocytes and central vein (fig. 2A-F).

DISCUSSION

Diabetes is linked with hepatic complications such as acute liver disease, raised liver enzyme levels and nonalcoholic fatty liver (Khan *et al*., 2019). The current study has explored the effect of CBSKE and GSLE separately and in combination on hepatic and lipid profiles in alloxanized diabetic rats.

A significant (P≤0.05) decline towards normal serum glucose levels was detected in CBSKE, GSLE, CBSKE+GSLE, and metformin treatments as compared to the diabetic control group (Akhtar *et al*., 2019). Metabolic disorders mainly diabetes mellitus can cause harm or injury to hepatocytes resulting in the release of liver enzymes into the circulation has been reported earlier (EL-Kassaby *et al*., 2019). In our research, liver cell degeneration with the fragmented nucleus, necrotic hepatocytes and congested central vein were evident in diabetic control rats similar to earlier research (Omodanisi

et al., 2017). However, treatment with metformin, CBSKE, GSLE and CBSKE+GSLE restored some of these histopathological alterations, indicating a defensive role of CBSKE and GSLE in hyperglycemia linked to liver injury. In the existent study, the concentration of liver enzymes presented a significant (P≤0.05) increase in the levels of serum ALT, AST and ALP in diabetic control rats in contrast to the normal control group. It might be possible due to the leakage of enzymes from the tissues into circulation. However, treatment with CBSKE, GSLE, and CBSKE+GSLE significantly reduced serum levels of these enzymes suggesting the hepato-protective activity of both herbs. Our results further showed that diabetic control rats had a significant (P≤0.05) drop in serum total protein and albumin levels in comparison to normal control rats suggesting hyperglycemia-induced hepatic injury. Treatment with metformin, CBSKE, GSLE, and CBSKE+GSLE significantly restored serum levels of total protein and albumin. For hepatocytes inflammation and damage, hypoalbuminemia is a strong clinical biomarker. These findings were followed preceding studies (Osigwe *et al*., 2017; Almalki *et al*., 2019).

Diabetes is linked with a wide variation in serum lipid profiles. In the present study, a significant (P≤0.05) rise in levels of mean serum LDL cholesterol, total cholesterol, triglyceride, and a significant decrease (P≤0.05) in HDL-cholesterol levels was seen in diabetic rats in contrast to normal control. While a significant (P≤0.05) improvement in the levels of lipid profile was observed in metformin, CBSKE, GSLE and CBSKE+GSLE treated diabetic rats. It was noticed that capability of CBSKE, GSLE and CBSKE+GSLE treatment to lower lipid concentration in serum is linked with their potential to recover hyperglycemia and antioxidant activities. Our study results were consistent with the former study (Mansi *et al*., 2019).

Although our research has not depicted clearly the mechanism by which CBSKE and GSLE could be exerting their hepatoprotective and hypolipidemic effects in alloxanized diabetic rats. It has earlier been suggested that both plants are rich in phenolics and flavonoids, which could act as a free radical scavenger that ameliorates inflammation and oxidative stress. However, which phytochemicals could be responsible for these effects need to be recognized and more studies are suggested.

CONCLUSIONS

Treatment of alloxan-induced diabetic rats with CBSKE, GSLE either alone or in combination showed a noteworthy reduction in serum glucose and improvement in the hepatic function markers and lipid profile. However, CBSKE and GSLE combination treatment showed better but not statistically significant results than separate treatment. Hence, this study results suggested that *C. bonduc* seed kernels and *G. Sylvestre* leaves extracts alone and in combination could be used as hepatoprotective and hypolipidemic in hyperglycemia-induced liver injury.

REFERENCES

Akhtar P, Khan H, Ambreen, Viqar S, Ara J and Ehteshamul-Haque S (2019). Hypoglycemic potential of some seaweeds from Karachi coast of Pakistan. *Pak. J. Pharm. Sci.,* **32**(4): 1599-1605.

Almalki DA, Alghamdi SA and Al-Attar AM (2019). Comparative study on the influence of some medicinal plants on diabetes induced by streptozotocin in male rats. *Biomed. Res. Int.*, p.11.

EL-Kassabya MI, Salama AAA, Mourada HH and Abdul-Wahab KG (2019). Effect of lemon balm (*Melissa officinalis*) aqueous extract on streptozotocin-induced diabetic rats. *Egypt. Pharm. J*., **18**(4): 296-303.

Iftikhar A, Aslam B, Iftikhar M, Majeed W, Batool M, Zahoor B, Amna N, Gohar H and Latif I (2020). Effect of *Caesalpinia bonduc* polyphenol extract on alloxan-induced diabetic rats in attenuating hyperglycemia by upregulating insulin secretion and inhibiting jnk signaling pathway. *Oxid. Med. Cell. Longev.,* p.14.

Khan F, Sarker MMR, Ming LC, Mohamad IN, Zhao C, Sheikh BY, Tsong HF and Rashid MA (2019). Comprehensive review on phytochemicals, pharmacological, and clinical potential of *Gymnema sylvestre*. *Front. Pharmacol*., **10**: p1223.

Kim HJ, Kim K, Lee AY,Jang Y, Davaadamdin O, Hong SH, Kim JS and Cho MH (2017). The effects of *Gymnema sylvestre* in high-fat diet-induced metabolic disorders. *Am. J. Chin. Med*., **45**(4): 1-20.

Mansi K, Abu-al-basal M and Aburjai T (2019). The hypoglycaemic and hypolipidemic effects of aqueous extract of *Alkannastrigosa* in alloxan induced diabetic rats. *J. Dis. Med. Plants*., 5:60-68.

Murad S, Khan M, Fatima F and Murad SS (2020). Kalonji, fenofibrates and triglycerides. *Res. Rev*. **1**(1): 27-30.

Muzaffar H, Faisal MN, Khan JA and Aslam B (2019). High protein diet improves biochemical and metabolic hormonal profile in alloxan-induced diabetic rats. *Pak. Vet. J.*, **39**(2): 231-23.

Nambirajan G, Karunanidhi K, Ganesan A, Rajendran R, Kandasamy R, Elangovan A and Thilagar S (2018). Evaluation of the antidiabetic activity of bud and flower of Avaram Senna (*Cassia auriculata* L.) in high-fat diet and streptozotocin-induced diabetic rats. *Biomed. Pharmacother*., **108**: 1495-1506.

Naseer A, Hussain A, Aslam B, Muhammad F, Mohsin M, Bari M, Sultana A and Masood A (2020). Vitamin E and selenium attenuate hepatotoxicity, nephrotoxicity and oxidative stress induced by rifampicin in rabbits. *Pak. Vet. J*., **40**(3): 277-282.

Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE and Makaroff LE (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes. Res. Clin. Pract*., **128**: 40-50.

Omodanisi El, Aboua, YG, Chegou NN and Oguntibeju OO (2017). Hepatoprotective, antihyperlipidemic and anti-inflammatory activity of *Moringa oleifera* in diabetic-induced damage in male Wistar rats. *Pharmacogn. Res*., **9**(2): 182-187.

Osigwe CC, Akah PA and Nworu CS (2017). Biochemical and hematological effects of the leaf extract of New*bouldia laevis* in alloxan-induced diabetic rats. *J. Biosci. Med*., **5**(6): 18-36.