EP-11

Evaluation of antidiabetic activity of novel triterpenoids isolated from ethyl acetate extract of *Cassia fistula* stem bark through *in vivo* and *in silico* analysis

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BACKGROUND

In recent years, plant-based drugs are very effective in the treatment of diabetes mellitus due to low cost and negligible side effects. Here, we identified the mechanisms of action of antidiabetic activity of novel compounds isolated from *Cassia fistula* (*C. fistula*) stem bark in STZ-diabetic animals.

HYPOTHESIS

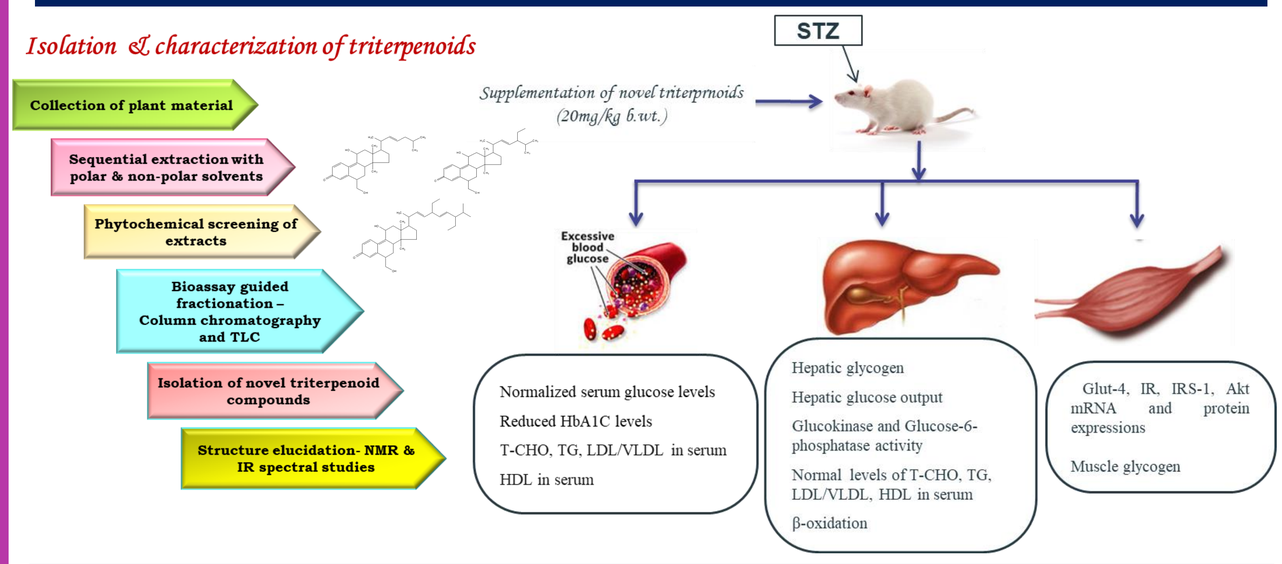
Novel triterpenoids isolated from ethyl acetate extract of *C. fistula* stem bark may have potential to ameliorate diabetic conditions in STZ-induced diabetic albino male Wistar rats through insulin signaling pathway.

OBJECTIVES

Novel triterpenoid compounds (C1, C2 and C3) were treated to STZ-administered diabetic animals at a concentration of 20mg/kg body weight orally for 60 days to assess their effects on plasma glucose, plasma insulin/C-peptide, serum lipid markers and the enzymes of carbohydrate metabolism, glucose oxidation, HbA1c glycemic indicator, and insulin signaling molecules.

MATERIALS & METHODS

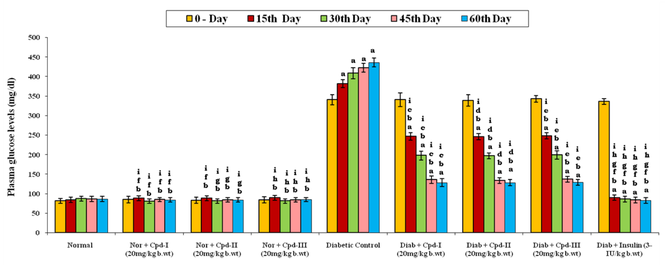
Isolation & characterization of triterpenoids



RESULTS & DISCUSSION

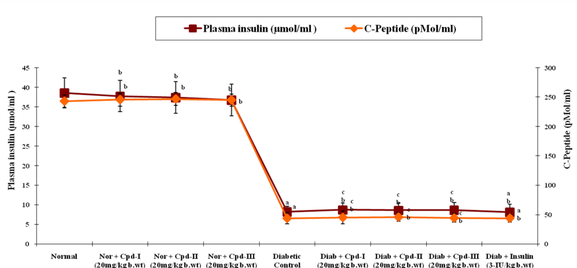
Oral administration of novel triterpenoid compounds to STZ-diabetic animals significantly decreased ($p<0.05$) the plasma glucose concentration on the 7th, 15th, 30th, 45th and 60th days in a duration-dependent manner ($p<0.05$). Plasma insulin ($p<0.0001$)/C-peptide ($p<0.0006$), tissue glycogen ($p<0.0034$), glycogen phosphorylase($p<0.005$), glucose 6-phosphatase ($p<0.0001$) and lipid markers were significantly increased ($p<0.0001$) in diabetic rats, whereas glucokinase ($p<0.0047$), glycogen synthase ($p<0.003$), glucose oxidation ($p<0.001$), GLUT4 mRNA ($p<0.0463$), GLUT4 protein ($p<0.0475$) and the insulin-signaling molecules IR mRNA ($p<0.0195$), IR protein ($p<0.0001$), IRS-1 mRNA ($p<0.0478$), p-IRS-1Tyr612 ($p<0.0185$), Akt mRNA ($p<0.0394$), p–AktSer473($p<0.0162$), GLUT4 mRNA ($p<0.0463$) and GLUT4($p<0.0475$) were decreased in the gastrocnemius muscle. Oral administration of novel triterpenoids has also restored the HbA1c levels, altered levels of enzymes involved in liver function to near normal. The antidiabetic targets in the insulin signaling pathway were docked with triterpenoids using SeeSAR software. C1–C3 possessed promising antidiabetic activity by regulating insulin signaling mechanisms and carbohydrate metabolic enzymes. *In silico* analysis of C1–C3 with IRK protein coincided with *in vivo* findings.

Fig 1. Effect of triterpenoids on plasma glucose levels in STZ-induced diabetic male albino Wistar rats



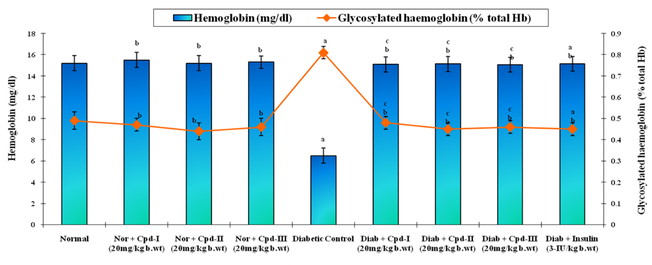
Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 2. Effect of triterpenoids on plasma insulin & C-peptide levels in STZ-induced diabetic male albino Wistar rats



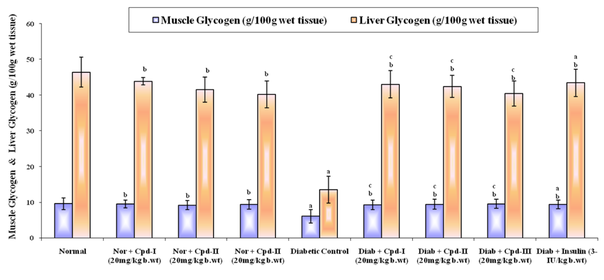
Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 3. Effect of triterpenoids on hemoglobin & glycosylated hemoglobin levels in STZ-induced diabetic male albino Wistar rats



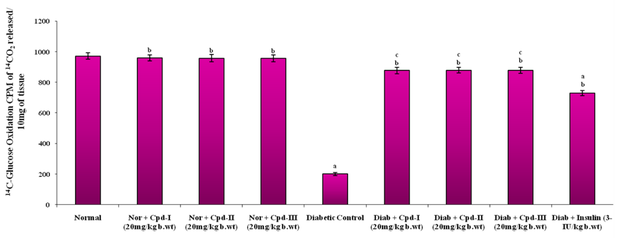
Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 4. Effect of triterpenoids on liver and muscle glycogen content in STZ-induced diabetic male albino Wistar rats



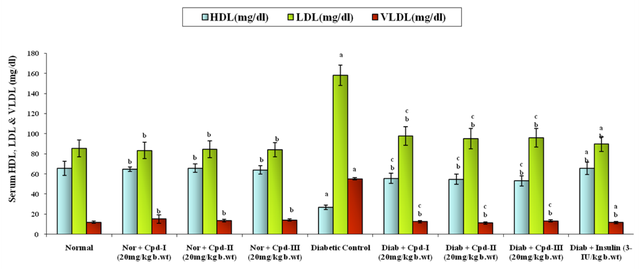
Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 5. Effect of triterpenoids on ^{14}C-glucose oxidation in STZ-induced diabetic male albino Wistar rats



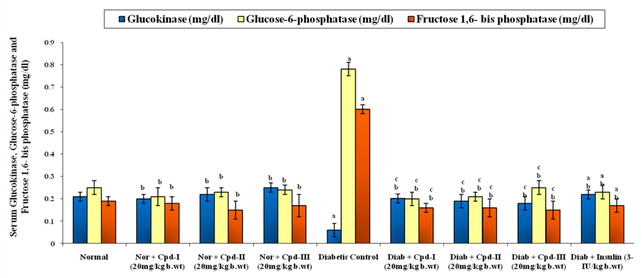
Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 6. Effect of triterpenoids on HDL, LDL & VLDL levels in STZ-induced diabetic male albino Wistar rats



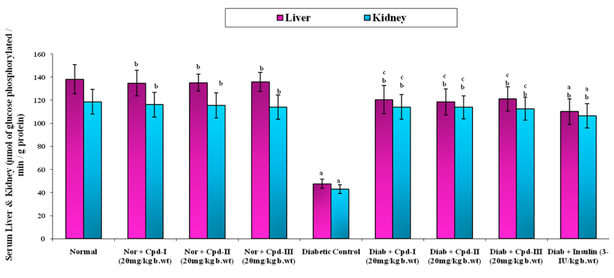
Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 7. Effect of triterpenoids on carbohydrate metabolizing enzyme levels in serum of STZ-induced diabetic male albino Wistar rats



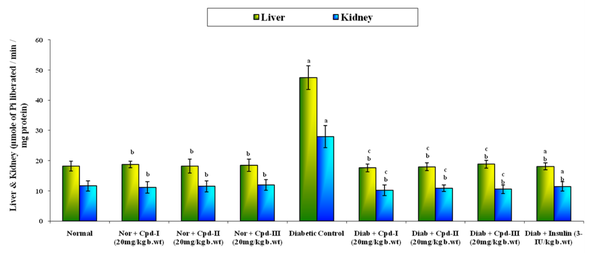
Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 8. Effect of triterpenoids on tissue Glucokinase levels in STZ-induced diabetic male albino Wistar rats



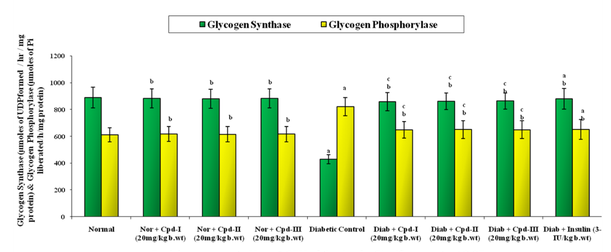
Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 9. Effect of triterpenoids on tissue Glucose-6-phophatase levels in STZ-induced diabetic male albino Wistar rats



Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 10. Effect of triterpenoids on tissue glycogen synthase and glycogen phosphorylase levels in STZ-induced diabetic male albino Wistar rats



Each bar represents Mean ± SEM of 6 animals. Significance at $p< 0.05$;
a- compared with normal control; b- compared with diabetic control; c- compared with diabetic+ insulin treated rats

Fig 11. Effect of triterpenoids on IR mRNA & protein expression in STZ-induced diabetic male albino Wistar rats

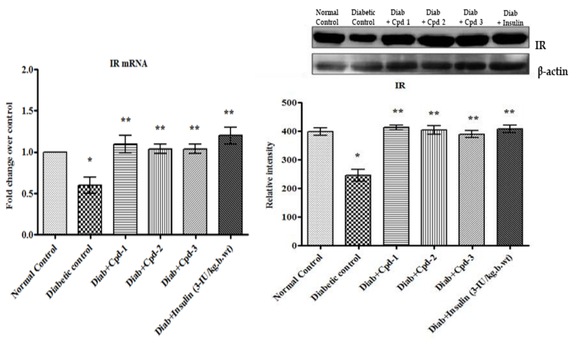


Fig 12. Effect of triterpenoids on IRS-1 mRNA & protein expression in STZ-induced diabetic male albino Wistar rats

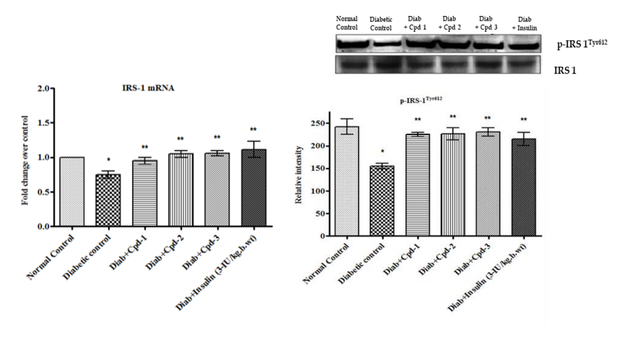


Fig 13. Effect of triterpenoids on AKT mRNA & protein expression in STZ-induced diabetic male albino Wistar rats

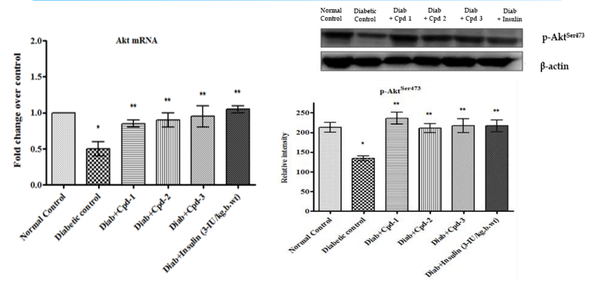
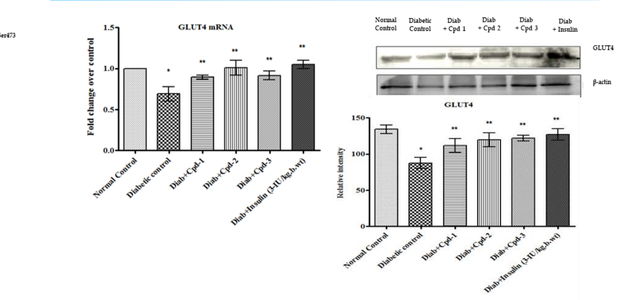


Fig 14. Effect of triterpenoids on GLUT 4 mRNA & protein expression in STZ-induced diabetic male albino Wistar rats



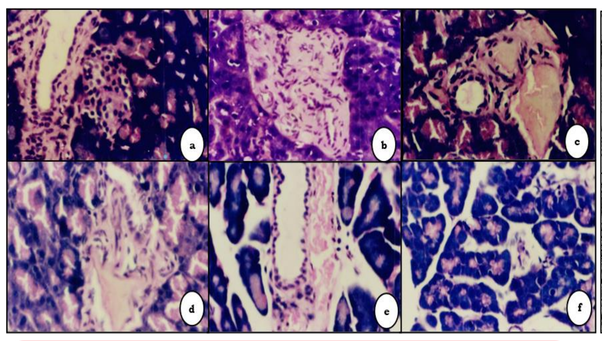


Fig.15 Hematoxylin and eosin sections (x 400) of an islet of Langerhans
(a) normal control rat (nuclei of the islet cells are round or ovoid),
(b) diabetic control rat (showing pyknotic nuclei and cellular boundary has been disrupted) in the islet cells, (c) insulin-treated diabetic rat, (d) Cpd-1 treated diabetic rat,
(e) Cpd-2 treated diabetic rat, (f) Cpd-3 treated diabetic rat,
Is- islet of Langerhans; Ac- acinar tissue, C- capillary.

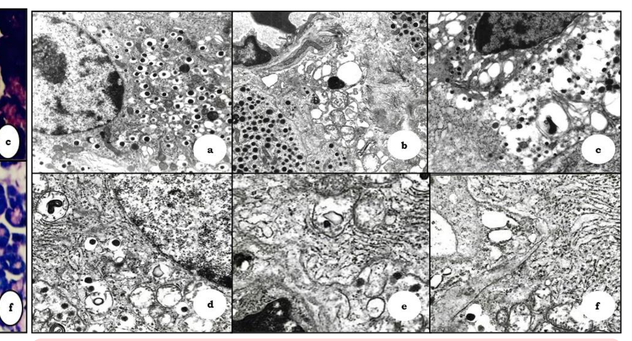
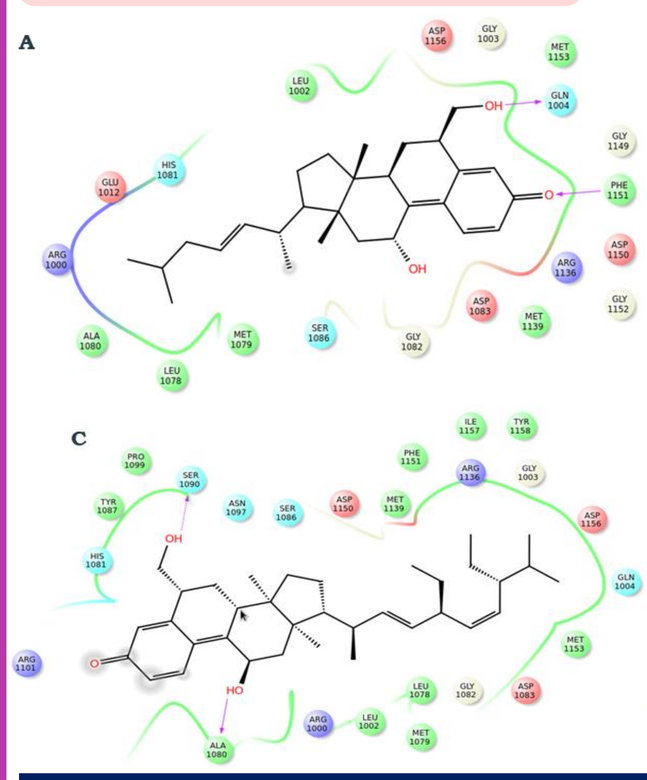


Fig.16 Transmission Electron Microscopy of β-cell (x 15,000) (a) normal control rat,
(b) diabetic control rat, (c) insulin-treated diabetic rat,
(d) Cpd-1 treated diabetic rat, (e) Cpd-2 treated diabetic rat, (f) Cpd-3 treated diabetic rat.



Compound	Glide gscore (kcal/mol)	Glide energy (kcal/mol)	Interacting amino acids
Cpd 1	-10.36	-98.84	GLN 1004 PHE 1151
Cpd 2	-10.21	-98.01	ALA 1080 PHE 1151 ASP 1083
Cpd 3	-10.57	-106.28	SER 1090 ALA 1080

Fig. 17. Two-dimensional view of ligand-receptor molecular interactions between triterpenoids compounds and IRK

CONCLUSION

Thus, these investigations indicated therapeutic efficacies of novel triterpenoids against diabetes. Novel triterpenoids isolated from ethyl acetate extract of *C. fistula* stem bark enhanced insulin-stimulated phosphorylation of IR tyrosine and/or Akt in the skeletal muscle showing insulin mimetic action, thus these compounds could be developed as hypoglycemic drug for diabetes mellitus.

ACKNOWLEDGEMENT

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✓**Indu S,** Ireen C, Vijayalakshmi P, Rajalakshmi M. **Molecular docking analysis of tetracyclic triterpenoids from *Cassis fistula L.* with targets for diabetes mellitus**. *Bioinformation*. 2022; 18(3):200-205. https://doi.org/10.6026/97320630018200 . JCI: 0.31 *(G-II WoS indexed in UGC-CARE journal list)*