P-0666 Thymol protects diabetic kidney by attenuating hyperglycemia induced oxidative stress

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Background

- Chronic hyperglycemic milieu of diabetes mellitus produces oxidative stress-a key factor in the development of diabetic vascular complications such as diabetic nephropathy.
- In addition to the conventional treatments for intensive blood glucose control, it is essential to decrease oxidative stress to prevent the development of diabetic complications.
- NADPH oxidase has been identified as a major source of intrarenal oxidative stress in the diabetic kidney

Rationale for drug selection

Aqueous extract of *Tracyspermum ammi*
Rat aldose reductase inhibition
(IC₅₀=0.65 mg/ml)

Antioxidant, anti-inflammatory,
Anti-lipid peroxidative, hepatoprotective

Renoprotective effect of Thymol in STZ induced diabetes

Male wistar rats weighing 200-250 g, Protocol No: ICT/IAEC/2013/P12

Fasted for 6 hrs
↓
Streptozotocin (45 mg/kg) in cold citrate buffer *i.p.*

- Blood glucose determination after 72 hrs
- Rats with blood glucose level >250 mg/dL considered diabetic

- Diabetic rats were treated with Thymol at graded doses (1mg/kg, 5mg/kg and 10mg/kg *i.p.*) for 28 days.
- Metformin (500mg/kg *p.o.*) was used as standard control. Normal and diabetic control groups were also maintained

Statistical analysis: One way ANOVA post Dunnett's test. ###p<0.001 when compared with normal control, ***p<0.001, **p<0.01, *p<0.05 when compared with diabetic control.

Effect on biomarkers of renal injury

Effect on renal oxidative stress

Serum AGE

Kidney Hypertrophy Index

Hematoxylin and Eosin Staining

(A) Control, (B) Diabetic control, (C) Thymol 1mg/kg, (D) Thymol 5mg/kg, (E) Thymol 10mg/kg, (F) Metformin groups. D: Degeneration of PCT; VD: vacuolar degeneration; SC: Scarring of tubule; G: Glomeruli; H: Hylanisation of vessels; TC: Tubular Collapse

Periodic acid-Schiff Base Staining

PP: PAS positivity; BM: basement membrane thickness; +: mild change, ++: moderate change & 0: no change

In vitro NADPH oxidase inhibitory activity

- Resident macrophages obtained from peritoneal cavity of rats were grown in 96-well plates (1hr, 37°C, 5%CO₂).
- Rotenone (10 nM) was added as activator of NADPH oxidase in presence or absence of thymol. Apocynin was standard control.
- Activation of NADPH oxidase was measured in terms of release of superoxide anions by reduction of nitro blue tetrazolium to insoluble blue formazan.

Thymol showed marked NADPH oxidase inhibition of 72.5 % at a concentration of 1.5 μg/ml

Conclusion

- Thymol restored renal functions which were altered in the diabetic state demonstrating the protective effect on diabetic kidney by decreasing hyperglycemia induced oxidative stress.
- *In vitro* study demonstrated NADPH oxidase inhibition potential of Thymol which was comparable with the standard inhibitor Apocynin.
- Thus the renoprotective effect of Thymol may be attributed to inhibition of renal NADPH oxidase.
- Further *in vivo* studies are warranted to elaborate the effect of Thymol on renal NADPH oxidase.

References: Divya K et al. *Pharmacological reports*. 2016; 68 (2):370-377.
Chem W et al. *Oxidative medicine and cellular longevity*. 2016; 2016:7813072.