RESULTS

Administration of insulin (0.6 IU/kg/day.,s.c), YS 49 (1,2,3,4-Tetrahydro-1-(1-naphthalenylmethyl)-6,7-Isoquinolinediol hydrobromide monohydrate) (1.6 mg/kg/day.,i.p), DAQ B1(Demethylasteraquinone B1) (5mg/kg/day.,i.p) and atorvastatin in normal rats did not produce any marked effect on various parameters assessed in present study. Wortmannin, [inhibitor of phosphoinositidyl-3-kinase (PI3K)], UCN-01(Phosphoinositide dependent kinase inhibitor), API-2 (AKT inhibitor), also did not produce any significant effect on various parameters in normal rats. DMSO (1%; 0.5 ml DMSO) used as vehicle to prepare solution of DAQ B1, API-2, L-NAME, UCN-01 and wortmannin did not produce any marked effect on various parameters.

5.1. Effect of pharmacological interventions on serum glucose level in diabetic rats.

Serum concentration of glucose was significantly increased in diabetic rats administered streptozotocin, in comparison to normal control. Administration of insulin (0.6 IU/kg/day.,s.c), YS 49 (1,2,3,4-Tetrahydro-1-(1-naphthalenylmethyl)-6,7-Isoquinolinediol hydrobromide monohydrate) (1.6 mg/kg/day.,i.p), DAQ B1(Demethylasteraquinone B1) (5mg/kg/day.,i.p), significantly decreased serum concentration of glucose in diabetic rats. This ameliorative effect of insulin was significantly blocked by Wortmannin, [inhibitor of phosphoinositidyl-3-kinase (PI3K)], UCN-01(Phosphoinositide dependent kinase inhibitor), API-2 (AKT inhibitor) and L-NAME [endothelial nitric oxide synthase inhibitor] (Fig 1).

Sodium orthovanadate (24 mg/kg p.o.) and atorvastatin(30 mg/kg p.o.) treatments, also significantly reduced serum glucose concentration in diabetic rats. However effect of Insulin was significantly more in comparison to
atorvastatin (Fig1). Both L-NAME and UCN-01 (0.35 mg/kg, p.o.) significantly blocked the ameliorative effect of SOV (24 mg/kg, p.o.) in diabetic rats (Fig 2).

5.2. Effect of pharmacological interventions on serum lipid profile in hypercholesterolemic rat: Administration of cholesterol rich diet (4 weeks),
significantly increased serum concentration of cholesterol, TG-c (triglyceride) and decreased HDL-c (high density lipoproteins). Administration of insulin (0.6 IU/kg/day., s.c) significantly reduced serum concentration of cholesterol and increased serum HDL-c concentration in hypercholesterolemia rats. However, there was no significant decrease in serum TG-c levels by insulin treatment. Atorvastatin (30 mg/kg/day., p.o) significantly reduced serum concentration of cholesterol (Fig 3), TG-c (Fig 5) and increased the HDL-c (Fig 4) in hypercholesterolemia rats.

The administration of sodium orthovanadate (24 mg/kg p.o) significantly reduced serum concentration of cholesterol and increased serum HDL-c concentration in hypercholesterolemia rats. Also, there was a significant decrease in serum TG-c levels by sodium orthovanadate treatment. UCN01 significantly attenuated the cholesterol lowering effect of SOV (24 mg/kg., p.o) (Fig 6, 7, 8). Moreover L-NAME (25 mg/kg, p.o) treatment significantly attenuated the effect of sodium orthovanadate (24 mg/kg p.o) on serum concentration of cholesterol, TG-c and HDL-c in hypercholesterolemic rats.

![Fig 3: Effect of pharmacological interventions on serum cholesterol level in hypercholesterolemic (Hch) rats (a) P<0.05 vs control; (b) P<0.05 vs Hch. All values represent mean ± SD (n=6).](image)
Fig 4: Effect of pharmacological interventions on serum HDL level in hypercholesterolemic (Hch) rats (a) P<0.05 vs control; (b) P<0.05 vs Hch. All values represent mean ± SD(n=6).

Fig 5: Effect of pharmacological interventions on serum TG level in hypercholesterolemic (Hch) rats (a) P<0.05 vs control; (b) P<0.05 vs Hch; (c) P<0.05 vs Hch. All values represent mean ± SD(n=6).
Fig 6: Effect of Pharmacological Interventions in Hch rats on serum cholesterol level (a) P<0.001 vs control (b) P<0.001 vs Hch (c) P<0.05 vs Hch (d) P<0.001 vs Hch+SOV (e) P<0.001 vs Hch+SOV. All values represent mean ± SD(n=6).

Fig 7: Serum HDL level in hypercholesterolemic rats (a) P<0.05 vs control; (b) P<0.001 vs Hch; (c) P<0.001 vs Hch; (d) P<0.001 vs Hch+SOV; (e) P<0.001 vs Hch+SOV. All values represent mean ± SD(n=6).
5.3. Effect of Pharmacological interventions on serum homocysteine level in hyperhomocysteinemia.

L-Methionine administration in rats, for 4 weeks, significantly increased serum homocysteine concentration in comparison to normal control. Administration of insulin (0.6 IU/kg/day, s.c) and atorvastatin significantly reduced serum concentration of homocysteine in hyperhomocysteinemic rats (Fig 9).

Administration of sodium orthovanadate (24 mg/kg p.o.) and atorvastatin (30 mg/kg p.o.) also significantly reduced serum concentration of homocysteine in hyperhomocysteinemic rats (Fig 10).
5.4. Effect of Pharmacological interventions on mean arterial blood pressure in hypertensive rats. Administration of insulin (0.6 IU/kg/day.,s.c), YS 49 (1,2,3,4-
Tetrahydro-1-(1-naphthalenylmethyl)-6,7-Isoquinolinediol hydrobromide monohydrate (1.6 mg/kg/day, i.p) ,DAQ B1 (Demethylasteraquinone B1) (5 mg/kg/day, i.p) and atorvastatin significantly reduced MABP in hypertensive rats (Fig 11). The ameliorative effect of insulin was blocked by wortmannin, [inhibitor of phosphoinositidyl-3-kinase (PI3K)], UCN-01 (Phosphoinositide dependent kinase inhibitor), API-2 (AKT inhibitor) and L-NAME (eNOS inhibitor).

Atorvastatin (30 mg/kg p.o.) also significantly reduced MABP in hypertensive rats (Fig 12).
Effect of Pharmacological interventions on serum Nitrite/Nitrate Concentration

Diabetes mellitus, hypercholesterolemia, hyperhomocystenemia and hypertension significantly reduced serum nitrite/nitrate concentration. Administration of insulin (0.6 IU/kg/day, s.c), YS 49 (1,2,3,4-Tetrahydro-1-(1-naphthalenylmethyl)-6,7-Isoquinolinediol hydrobromide monohydrate) (1.6 mg/kg/day, i.p), DAQ B1 (Deoxyethylasteraquinone B1) (5mg/kg/day, i.p) significantly improved serum nitrate/nitrite level, This ameliorative effect of insulin was blocked by Wortmannin, [inhibitor of phosphoinositidyl-3-kinase (PI3K)], UCN-01 (Phosphoinositide dependent kinase inhibitor), API-2 (AKT inhibitor) and L-NAME (eNOS inhibitor) (Fig 13,14,15,16).

Treatment with SOV (24 mg/kg p.o) or atorvastatin (30 mg/kg, p.o) also significantly attenuated diabetes mellitus (Fig 17), hypercholesterolemia (Fig 18), and hypertension (Fig 20)-induced decrease in serum nitrite/nitrate concentrations. Both UCN-01 and L-NAME administration significantly attenuated the SOV-induced
increase in serum nitrate/nitrite concentration. However in hyperhomocystenemic (Fig 19) rats effect of insulin is significantly reduced by L-NAME but not with UCN-01.

Fig 13: Effect of pharmacological interventions on serum nitrate/nitrite conc. in Diabetic (Db) rats (a) P<0.05 vs control; (b) P<0.05 vs Db; (c) P<0.05 vs Db +Insulin; (e) P<0.05 vs Db +Insulin. All values represent mean ± SD(n=6)

Fig 14: Effect of pharmacological interventions on serum nitrate/nitrite conc. in hypercholesterolemic (Hch) rats (a) P<0.05 vs control; (b) P<0.05 vs Hch; (c) P<0.05 vs Hch +Insulin. All values represent mean ± SD(n=6).
Fig 15: Effect of pharmacological interventions on serum nitrate/nitrite conc. in Hyperhomocysteinemic (Hhcy) rats: (a) P<0.05 vs control; (b) P<0.05 vs Hhcy; (c) P<0.05 vs Hhcy+Insulin. All values represent mean±SD (n=6).

Fig 16: Effect of pharmacological interventions on serum nitrate/nitrite conc in hypertensive (HT) rats: (a) P<0.05 vs control; (b) P<0.05 vs HT; (c) P<0.05 vs HT; (d) P<0.05 vs HT+Insulin. All values represent mean±SD (n=6).
Fig 17: Effect of pharmacological interventions on Serum Nitrate/Nitrite conc. in Diabetic rats (a) P<0.001 vs control; (b) P<0.001 vs Db; (c) P<0.001 vs Db+SOV; (d) P<0.001 vs Db+SOV All values represent±SD(n=6).

Fig 18: Effect of pharmacological interventions on Serum Nitrate/Nitrite conc. in Hypercholesterolemic rats (a) P<0.001 vs control ;(b) P<0.001 vs Hch; (c) P<0.001 vs Hch; (d) P<0.001 vs Hch+SOV All values represent mean ± SD(n=6).
5.6. Effect of Pharmacological interventions on acetylcholine-induced endothelium dependent and sodium nitroprusside-induced endothelium independent relaxation.

Acetylcholine and sodium nitroprusside produced endothelium-dependent and independent relaxation respectively, in a dose-dependent manner, in phenylephrine
(3 X10^6) precontracted isolated rat aortic ring preparation of normal rats. Diabetes mellitus, hypercholesterolemia, hyperhomocysteinemia and hypertension significantly attenuated acetylcholine-induced endothelium-dependent relaxation. Administration of insulin (0.6 IU/kg/day.,s.c), YS 49 (1,2,3,4-Tetrahydro-1-(1-naphthalenylmethyl)-6,7-Isoquinolinediol hydrobromide monohydrate) (1.6 mg/kg/day.,i.p) ,DAQ B1(Demethylasteraquinone B1) (5mg/kg/day.,i.p) significantly improved acetylcholine-induced endothelium-dependent relaxation in diabetes, hypercholesterolemic, hyperhomocysteinemic and hypertensive rats. This ameliorative effect of insulin was significantly blocked by Wortmannin, [inhibitor of phosphoinositidy1-3-kinase (PI3K)], UCN-01(Phosphoinositide dependent kinase inhibitor), API-2 (AKT inhibitor) and L-NAME (eNOS inhibitor) (Fig 21,22,23,24).Administration of sodium orthovanadate(24 mg/kg ,p.o.) or atorvastatin (30 mg/kg.,p.o.) also significantly attenuated diabetes, hypercholesterolemia, hyperhomocysteinemia and hypertension-induced decrease in acetylcholine induced endothelium dependent relaxation. The effect of SOV was significantly blocked by both UCN-01(0.35 mg/kg p.o.) and L-NAME (25mg/kg, p.o.) (Fig 25,26,27,28). Diabetes(Fig29,33),hypercholesterolemia(Fig30,34),hyperhomocysteinemic(Fig31,35 ) and hypercholesterolemic (Fig32,36) did not effect sodium nitroprusside-evoked endothelium-independent relaxation, in comparison to normal control .
Fig 21: Effect of pharmacological interventions in Ach induced EDR in PE. Precontracted aorta. Diabetic (Db) rats (a) P < 0.05 vs control; (b) P < 0.05 vs Db; (c) P < 0.05 vs Db; (d) P < 0.05 vs Db + Insulin; (e) P < 0.05 vs Db + Insulin. All values represent mean ± SD (n = 6).

Fig 22: Effect of pharmacological interventions in Ach induced EDR in PE precontracted IA. Hypercholesterolemic (Hch) rats (a) P < 0.05 vs control; (b) P < 0.05 vs Hch; (c) P < 0.05 vs Hch; (d) P < 0.05 vs Hch + Insulin; (e) P < 0.05 vs Hch + Insulin. Values represent mean ± SD (n = 6).
Fig 23: Effect of pharmacological interventions in Ach induced EDR in PE precontracted IARP Hcy rats. (a) P<0.05 vs control (b) P<0.05 vs Hcy (c) P<0.05 vs Hcy + Insulin. All values represents mean±SD (n=6).

Fig 24: Effect of pharmacological interventions in Ach induced EDR in PE precontracted IARP HT rats. (a) P<0.05 vs control; (b) P<0.05 vs HT; (c) P<0.05 vs HT; (d) P<0.05 vs HT + Insulin; (e) P<0.05 vs HT + Insulin. All values represents mean±SD (n=6).
Fig 25: Effect of pharmacological interventions in Ach Induced EDR in PE precontracted IARP) Db rats. (a) P<0.05 vs control; (b) P<0.05 vs Db; (c) P<0.05 vs Db; (d) P<0.05 vs Db + SOV; (e) P<0.05 vs Db + SOV. All values represent ± SE (n=6).

Fig 26: Effect of pharmacological interventions in Ach Induced EDR in PE precontracted IARP) Hch rats. (a) P<0.05 vs control; (b) P<0.05 vs Hch; (c) P<0.05 vs Hch; (d) P<0.05 vs Hch + SOV; (e) P<0.05 vs Hch + SOV. All values represent ± SD (n=6).
Fig 27: Effect of pharmacological interventions in (Ach induced EDR in PE precontracted IARP) hyperhomocysteinemic (Hcy) rats (a) \( P < 0.05 \) vs control; (b) \( P < 0.05 \) vs Hcy; (c) \( P < 0.05 \) vs Hcy; (d) \( P < 0.05 \) vs Hcy+SOV. All values represent ± SD (n=6).

Fig 28: Effect of Pharmacological interventions in (Ach induced EDR in PE precontracted IARP) Hypertension (HT) rats (a) \( P < 0.05 \) vs control; (b) \( P < 0.05 \) vs HT; (c) \( P < 0.05 \) vs HT; (d) \( P < 0.05 \) vs HT + SOV; (e) \( P < 0.05 \) vs HT + SOV. All values represent ± SD (n=6).
Fig 29: Effect of pharmacological interventions in (SNP induced EDR in PE precontracted IARP) Diabetic (Db) rats. All values represent ± SD (n=6).

Fig 30: Effect of Pharmacological interventions in (SNP induced EDR in PE Precontracted IARP) Hypercholesterolemic (Hch) rats. All values represent mean ± SD (n=6).
Fig 31: Effect of pharmacological interventions in (SNP induced EDR in PE precontracted IARP) hyperhomocysteinemic (Hhcy) rats. All values represent mean ± SD(n=6).

Fig 32: Effect of pharmacological interventions in (SNP induced EDR in PE precontracted IARP) hypertensive (HT) rats. All values represent mean ± SD(n=6).
Fig 33: Effect of pharmacological interventions in SNP induced EDR in PE precontracted IARP)Diabetic rats. All values represent mean ± SD (n=6).

Fig 34: Effect of pharmacological interventions (SNP induced EDR in PE precontracted IARP) in Hypercholesterolemic rats. All values represent mean ± SD (n=6).
Fig 35: Effect of pharmacological interventions in (SNP induced EDR in PE precontracted IARP) hyperhomocysteinemic (Hhcy) rats. All values represent ± SD (n=6).

Fig 36: Effect of pharmacological interventions in (SNP induced EDR in PE precontracted IARP) hypertension (HT) rats. All values represent ± SD (n=6).
5.7. Effect of Pharmacological Intervention on mRNA expression of eNOS

Diabetes mellitus, hypercholesterolemia, hyperhomocystenemia and hypertension significantly decreased the expression ratio of eNOS/GAPDH. The administration of insulin (0.6 IU/kg/day.,s.c), YS 49 (1,2,3,4-Tetrahydro-1-(1-naphthalenylmethyl)-6,7-Isoquinolinediol hydrobromide monohydrate) (1.6 mg/kg/day.,i.p) ,DAQ B1(De(methyl)asteraquinone B1) (5mg/kg/day.,i.p) significantly improved mRNA expression of eNOS. Similar results were observed following the administration of atorvastatin (30mg/kg, p.o.).The ameliorative effect of insulin was blocked by Wortmannin, [inhibitor of phosphoinositidyl-3-kinase (PI3K)], UCN-01(Phosphoinositide dependent kinase inhibitor), API-2 (AKT inhibitor) and L-NAME (eNOS inhibitor) (Fig 37,38,39,40).

The administration of SOV (24 mg/kg, p.o.) also significantly attenuated the diabetes mellitus, hypercholesterolemia, hyperhomocysteinemlia and hypertension induced decrease in expression ratio of eNOS/GAPDH. Administration of L-NAME (25mg/kg., p.o.), significantly attenuated the SOV- induced increase in expression ratio of eNOS/GADPH (Fig 41,42,43,44).
Fig 37: Effect of pharmacological interventions on eNOS/Gadph ratio in Diabetic (Db) rats (a) P<0.05 vs control; (b) P<0.05 vs Db; (c) P<0.05 vs Db +Insulin. All values represent mean ± SD(n=6).

Fig 38: Effect of pharmacological interventions on eNOS/Gadph ratio in hypercholesterolemic (Hch) rats (a) P<0.05 vs control; (b) P<0.05 vs Hch; (c) P<0.05 vs Hch +Insulin. All values represent mean ± SD(n=6).
**Fig 39:** Effect of Pharmacological interventions on eNOS/Gadph ratio in Hyperhomocysteinemic (HhcY) rats. (a) P<0.05 vs control; (b) P<0.05 vs HhcY; (c) P<0.05 vs HhcY+Insulin. All values represent mean ± SD (n=6).  

**Fig 40:** Effect of Pharmacological interventions on eNOS/Gadph ratio in Hypertensive (HT) rats. (a) P<0.05 vs control; (b1,b) P<0.05 vs HT; (c) P<0.05 vs HT+Insulin. All values represent mean ± SD.
**Fig 41:** Effect of pharmacological interventions on eNOS/GADPH ratio in Diabetic rats. (a) P<0.05 vs control; (b) P<0.05 vs Db; (c) P<0.05 vs Db+SOV. All values represent mean ±SD (n=6).

**Fig 42:** Effect of Pharmacological interventions on eNOS/Gadph ratio in Hypercholesterolemic rats. (a) P<0.05 vs control; (b) P<0.05 vs Hch; (c) P<0.05 vs Hch+SOV. All values represent mean ±SD (n=6).
Fig 43: Effect of pharmacological interventions on eNOS/Gadph ratio in hyperhomocysteinemic (Hhcy) rats (a) P<0.05 vs control; (b) P<0.05 vs Hhcy; (c) P<0.05 vs Hhcy + SOV. All values represent ± SD (n=6).

Fig 44: Effect of pharmacological interventions on eNOS/Gadph ratio in hypertension (HT) rats (a) P<0.05 vs control; (b) P<0.05 vs HT; (c) P<0.05 vs HT + SOV. All values represent mean ± SD (n=6).
5.8. Effect of Pharmacological interventions on integrity of vascular endothelium

Diabetes mellitus (Fig 45B I), hypercholesterolemia (Fig 45C I), hyperhomocystenemia (Fig 45D I), and hypertension (Fig 45E I), impaired the integrity of vascular endothelial lining of thoracic aorta, as compared to normal control (Fig 45 A). Administration of insulin (0.6 IU/kg/day, s.c) significantly improved impairment of integrity of vascular endothelial lining in diabetic (Fig 45 B II), hypercholesterolemia (Fig 45C II), hyperhomocystenemia (Fig 45D II), and hypertension (Fig 45E II). This ameliorative effect of insulin was blocked by wortmannin in diabetes mellitus (Fig 45B V), hypercholesterolemia (Fig 45C V), hyperhomocystenemia (Fig 45D V), and hypertension (Fig 45E V). UCN-01 also prevented the ameliorative effect of insulin in diabetes mellitus (Fig 45B VI), hypercholesterolemia (Fig 45C VI), hyperhomocystenemia (Fig 45D VI), and hypertension (Fig 45E VI). Improvement of endothelial lining by insulin was prevented by API-2 in diabetes mellitus (Fig 45B VII), hypercholesterolemia (Fig 45C VII), hyperhomocystenemia (Fig 45D VII), and hypertension (Fig 45E VII). L-NAME also prevented the effect of insulin in diabetes mellitus (Fig 45B VIII), hypercholesterolemia (Fig 45C VIII), hyperhomocystenemia (Fig 45D VIII), and hypertension (Fig 45E VIII).

YS 49 (1.6 mg/kg/day, i.p) significantly improved impairment of integrity of vascular endothelial lining in diabetic (Fig 45B III), hypercholesterolemia (Fig 45C III), hyperhomocystenemic (Fig 45D III), and hypertension (Fig 45 E III). DAQ B1 (Demethylasteraquinone B1) (5mg/kg/day, i.p), significantly improved impairment of integrity of vascular endothelial lining in diabetes mellitus (Fig 45B IV), hypercholesterolemia (Fig 45C IV), hyperhomocystenemia (Fig 45D IV), and hypertension (Fig 45E IV). Electron
micrographs of endothelial lining of aorta isolated from diabetes mellitus (Fig 45B IX), hypercholesterolemia (Fig 45C IX), hyperhomocystenemia (Fig 45D IX), and hypertension (Fig 45E IX) rats treated with atorvastatin showed significant improvement and reendothelization.

SOV (24 mg/kg p.o.) markedly prevented diabetes (Fig 46Ia), hypercholesterolemia (Fig 46IIa), hyperhomocystenemia(Fig 46IIIa) and hypertension(Fig 46 IV a) induced impairment of the integrity of vascular endothelial lining of thoracic aorta, as compared to normal control. UCN-01 prevented the amiolartative effect of SOV in Diabetes (Fig 46I b), hypercholesterolemia (Fig 46IIb), hyperhomocystenemia(Fig 46IIIb) and hypertension(Fig 46 IVb). Administration of L-NAME significantly blocked the effect of SOV in respective groups (Fig Ic,IIc,IIIc and IVc).