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EFFECT OF *SOLANUM TORVUM* ON THE CONTRACTILE RESPONSE OF ISOLATED TISSUES PREPARATION IN FRUCTOSE FED RAT

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ABSTRACT

The present study was aimed to determine the effect of ethanolic extract of *Solanum torvum* (100 and 300 mg/kg; p.o. for 6 weeks) on isolated strip of ascending colon, Fundus and Vasdeferens in fructose (10%) fed rat. Chronic administration of *S. torvum* extract (100, 300 mg/kg/day, p.o.) in fructose fed rats significantly ($p<0.05$) shifted the CCRC of Angiotensin (Ang-II, 10 ng/ml) and 5-Hydroxytryptamine (5-HT, 10 µg/ml) to the right with suppression of maxima as compared to CCRC of fructose fed rats on isolated ascending colon and fundus strip respectively. CCRC of Phenylephrine (PE, 10 µg/ml) using isolated Vas deferens was significantly ($p<0.05$) rise in rats cotreated with *S. torvum* (100, 300 mg/kg/day, p.o.). The results clearly suggest that *S. torvum* could block the Ang-II and 5-HT receptor and it also activate α_1-adrenergic pathway in this *in-vitro* model study.

KEYWORDS ;Angiotensin II, 5-Hydroxytryptamine, *Solanum torvum,* Fructose, CCRC

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INTRODUCTION

Solanum torvum Swartz (Solanaceae) is a small tree of about 4 m tall, evergreen and widely branched. It has white flowers with yellow stamens. The fruit is round berry with numerous seeds. It is native and found cultivated in Africa and West Indies [1]. The fruits and leaves are widely used in Camerooninan folk medicine. The plant is cultivated in the tropics for its sharp tasting immature fruits. It is used in the treatment of stomach pain and skin infections [2]. Phytochemical investigations have shown that the plant is rich in triacontane derivatives [3], chlorogenone and neochlorogenone [4], isoflavonoid sulfate and steroidal glycosides [5, 6], 22-β-O-spirostanol oligoglycosides [7], 26-O-β-glucosidase [8]. Pharmacological studies revealed that Solanum torvum possesses Antimicrobial [9, 10], Antiviral [11], Immuno-secretory [12], Antioxidant [13], Analgesic and Anti-inflammatory [14] and Anti-ulcerogenic activities [15]. It also possesses cardiovascular [16], Nephroprotective [17] and Antidiabetic activities [18].

Metabolic syndrome is a pathophysiological entity characterized by insulin resistance, hyperinsulinemia, dyslipidemia, hypertension and obesity [19]. The macronutrient content of the diet has been linked to the metabolic syndrome. Recently, consumption of dietary fructose has been suggested to be one of the environmental factors contributing to the development of obesity and the accompanying abnormalities of the metabolic syndrome [20]. In fact a well-known experimental model of metabolic syndrome is induced by high consumption of fructose; this model induces hypertension, hypertriglyceridemia, hyperinsulinemia, and insulin resistance in rats [21]. Fructose feeding also activates the renin-angiotensin system (RAS), sympathetic nervous system and it can be affect the serotonergic system [22].

The present study was undertaken to determine the effect of ethanolic extract of Solanum torvum (S. torvum) on isolated strip of ascending colon, Fundus and Vasdeferens in fructose (10%) fed rat.

MATERIALS AND METHODS

(i) Extract preparation :

Dried fruits of Solanum torvum Sw. (Solanaceae) were purchased locally and authenticated by Dr. S.C. Pal, NDMVP Samaj's College of Pharmacy, Nashik, India. The voucher specimen (1731) has been deposited at Agharkar Research Institute, Pune, India. Mature fruits were dried in shade, and grounded. The powder obtained (1 kg) was defatted using pet ether (60–80^0 C). The marc was macerated in ethanol for 3-4 days at room temperature. The filtrate was concentrated under reduced pressure to obtain 120 g of extract (12.0%, w/w). The total flavonoids content of ethanolic extract of Solanum torvum was found to be 85.26±0.02 µg rutin equiv. /mg of extract [23]. The total phenolic content of ethanolic extract of Solanum torvum was found to be 99.52±0.42 µg gallic acid equiv. /mg of extract [24]. Appropriate concentrations of the extracts were made in distilled water. The phytoconstituents present in the crude extract were flavonoids, alkaloids, tannins and saponins [25].

(ii) Animals :

Laboratory breed Wistar albino rats of either sex weighing between 150 and 200 g, maintained under standard laboratory conditions of 25 ± 1 ^{0}C and photo period (12 h dark/12 h light) were used for the experiment. Commercial pellet diet (Amrut laboratory rat and mice feed, Sangli, India) and water were provided ad libitum. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee.

(iii) Chemicals :
Angiotensins (Ang II), Serotonin (5-HT), Phenylepherine (PE) were purchased from Sigma, Mumbai. Fructose was obtained from Merck (India). Fructose (10%) was freshly prepared in distilled water.

(iv) Experimental Protocol :
The adult male rats were divided in to 7 groups of 5 rats each.
Group I: vehicle treated (Control) group.
Group II: *S. torvum* (100 mg/kg, p.o.) daily for 6 weeks.
Group III: *S. torvum* (300 mg/kg, p.o.) daily for 6 weeks.
Group IV: 10% fructose solution instead of drinking water ad libitum for 6 weeks.
Group V: 10% fructose solution instead of drinking water, ad libitum + *S. torvum* (100 mg/kg/day, p.o.) for 6 weeks.
Group VI: 10% fructose solution instead of drinking water, ad libitum + *S. torvum* (300 mg/kg/day, p.o.) for 6 weeks.
Group VII: 10% fructose solution instead of drinking water, ad libitum + Nifedipine (10 mg/kg/day, p.o.) for 6 weeks.

(v) Effects of S. torvum on contractile response of treated animals :
After completion of the treatment schedule, rats from individual groups were sacrificed. Cumulative concentration response curve (CCRC) of Ang II (10 ng/ml), 5-HT (10 µg/ml) and PE (10 µg/ml) were performed using ascending colon, Fundus and Vas deferens respectively with the help of thermo-regulated organ bath, containing 50 ml Krebs's solution. The physiological salt solution had the following composition (mM) NaCl (118); KCl (4.7); CaCl2 (2.5); MgSO4 (1.2); NaHCO3 (25); KH2PO4 (1.2) and glucose (11). The physiological salt solution had a pH of 7.4. It was warmed to 37^0 C and aerated by using carbogen (95% oxygen and 5% carbon dioxide). One end was tied to an aerator tube and other end to the lever. Each strip was placed under optimum resting tension (1g) and allowed to equilibrate for 30 min. Contractile response to each dose of Ang II, 5-HT and PE was recorded for 60 sec, 90 sec and 60 sec respectively [26, 27].

(vi) Statistical analysis :
The mean ± SEM values were calculated for each group. One-Way ANOVA followed by Dunnett's multiple comparison tests were used for statistical analysis. Values of $p < 0.05$ were considered statistically significant.

RESULTS

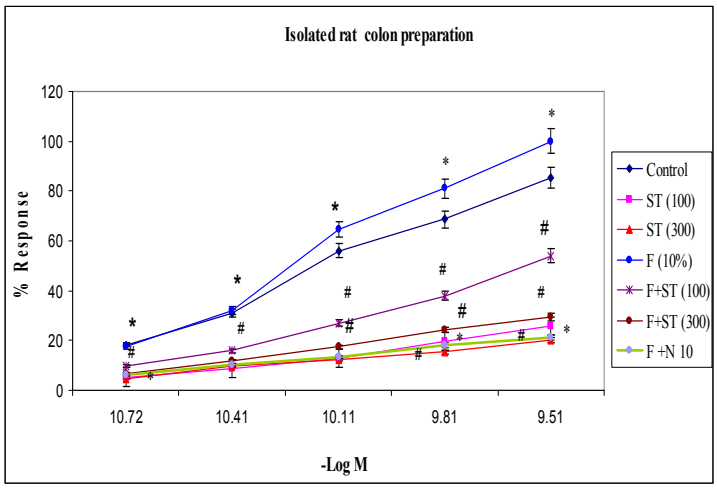
1. Effect of S. torvum on isolated ascending Colon :
Isolated ascending colon showed notable Ang-II contraction in molar concentrations 1-16 ng/ml. For Ang-II induced contraction response in Fructose fed rat at 16 ng/ml, average height 5.8 cm (n=5) was taken as 100%. Chronic administration of *S. torvum* (100, 300 mg/kg/day, p.o.) antagonized Fructose induced contraction responses significantly ($P<0.05$). Significant relaxation observed in only *S. torvum* (300 mg/kg/day, p.o.) treated animals as shown in Graph 1.

Table 1

Effect of S. torvum extract on height of Response in isolated ascending colon preparation

Sr. No.	Con. Of Ang-II (ng/ml)	Height of Response (cm) and Treatment groups (mg/kg)						
		Control	ST(100)	ST(300)	F(10)	F+ST(100)	F+ST(300)	F+N(10)
1.	1 ng	0.67 ±0.023	0.56 ±0.021	0.20 ±0.019*	1.20 ±0.045*	0.98 ±0.036#	0.69 ±0.025#	0.64 ±0.028#
2.	2 ng	1.32 ±0.021	1.11 ±0.024	0.72 ±0.022*	2.15 ±0.017*	1.12 ±0.016#	0.82 ±0.018#	0.75 ±0.016#
3.	4 ng	2.52 ±0.022	1.21 ±0.043*	1.13 ±0.023*	3.38 ±0.032*	1.76 ±0.022#	1.52 ±0.012#	1.40 ±0.021#
4.	8 ng	3.35 ±0.036	2.15 ±0.012*	1.70 ±0.023*	4.78 ±0.014*	2.21 ±0.017#	1.67 ±0.011#	1.50 ±0.023#
5.	16 ng	4.72 ±0.021	2.21 ±0.012*	1.98 ±0.022*	5.8 ±0.034*	2.31 ±0.016#	1.76 ±0.031#	1.41 ±0.051#

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to fructose fed group. F- Fructose, ST- S. torvum extract



Graph 1

Effect of S. torvum extract (100, 300 mg/kg, p.o., for 6 weeks) on CCRC of Ang-II on isolated rat ascending colon in Fructose (10%) fed rats

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to fructose fed group. Vertical lines represent SEM. F- Fructose, ST- S. torvum extract

2. Effect of S. torvum on isolated Fundus strip :

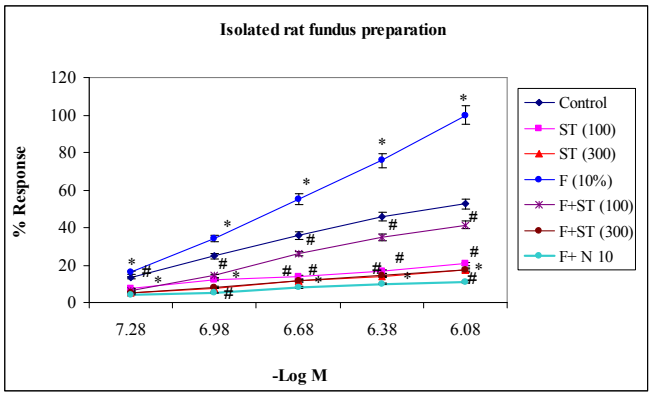
Fructose fed rats showed significant rise in 5-HT (10 µg/ml) induced CCRC height as compared to control group. Fundus strip from rats cotreated with *S. torvum* (100, 300 mg/kg/day, p.o.) showed a significant relaxation as shown in Graph 2.

Table 2

Effect of S. torvum extract on height of Response in isolated fundus strip preparation

Sr. No.	Con. Of 5-HT (µg/ml)	Height of Response (cm) and Treatment groups (mg/kg)						
		Control	ST(100)	ST(300)	F(10)	F+ST(100)	F+ST(300)	F+N(10)
1.	1 µg	0.77 ±0.021	0.66 ±0.031	0.22 ±0.016*	1.52 ±0.035*	0.58 ±0.032#	0.39 ±0.025#	0.30 ±0.026#
2.	2 µg	1.62 ±0.023	0.71 ±0.026	0.42 ±0.012*	2.95 ±0.016*	1.17 ±0.016#	0.71 ±0.016#	0.65 ±0.011#
3.	4 µg	2.52 ±0.022	1.21 ±0.043*	1.13 ±0.023*	3.38 ±0.032*	1.76 ±0.022#	1.52 ±0.012#	1.40 ±0.021#
4.	8 µg	4.10 ±0.032	2.25 ±0.014*	1.75 ±0.043*	5.11 ±0.024*	2.31 ±0.027#	1.97 ±0.031#	1.30 ±0.023#
5.	16 µg	4.8 ±0.021	2.24 ±0.012*	2.1 ±0.022*	5.6 ±0.034*	2.33 ±0.016#	1.86 ±0.031#	1.71 ±0.051#

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to fructose fed group. F- Fructose, ST- S. torvum extract



Graph 2

Effect of S. torvum extract (100, 300 mg/kg, p.o., for 6 weeks) on CCRC of 5-HT on isolated stomoch fundus strip in Fructose (10%) fed rats

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to fructose fed group. Vertical lines represent SEM. F- Fructose, ST- S. torvum extract

3. Effect of S. torvum on isolated Vas deferens :

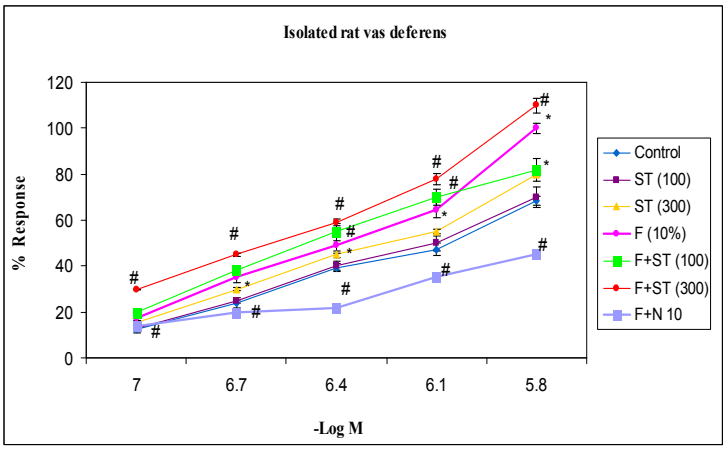
In Vas deferens chronic treatment of *S. torvum* (100, 300 mg/kg/day, p.o.) showed significant rise in CCRC in Fructose fed rat as shown in Graph 3. Animals cotreated with *S. torvum* (300 mg/kg/day, p.o.) showed a high reactivity as compared to Fructose fed rat.

Table 3

Effect of S. torvum extract on height of Response in isolated Vas deferens preparation

Sr. No.	Con. Of PE (µg/ml)	Height of Response (cm) and Treatment groups (mg/kg)						
		Control	ST(100)	ST(300)	F(10)	F+ST(100)	F+ST(300)	F+N(10)
1.	1 µg	0.62 ±0.015	0.76 ±0.021	1.20 ±0.026*	1.82 ±0.025*	2.48 ±0.033#	2.92 ±0.035#	0.80 ±0.016#
2.	2 µg	1.20 ±0.013	1.71 ±0.026*	2.10 ±0.022*	2.81 ±0.016*	3.21 ±0.026#	3.96 ±0.056#	1.2 ±0.011#
3.	4 µg	2.21 ±0.024	2.71 ±0.023*	3.43 ±0.043*	3.58 ±0.012*	4.16 ±0.032#	4.52 ±0.022#	1.70 ±0.032#
4.	8 µg	3.20 ±0.012	3.45 ±0.014*	3.74 ±0.043*	4.51 ±0.014*	4.91 ±0.022#	5.47 ±0.031#	1.80 ±0.022#
5.	16 µg	4.10 ±0.022	4.39 ±0.012*	4.80 ±0.022*	5.10 ±0.034*	5.43 ±0.012#	5.76 ±0.032#	2.21 ±0.052#

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to fructose fed group. F- Fructose, ST- S. torvum extract



Graph 3

Effect of S. torvum extract (100, 300 mg/kg, p.o., for 6 weeks) on CCRC of PE on isolated Vas deferens in Fructose (10%) fed rats

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to fructose fed group. Vertical lines represent SEM. F- Fructose, ST- S. torvum extract

DISCUSSION

It has been shown that sucrose feeding increases nor-epinephrine excretion and enhances sympathetic nerve responses in rats. This may cause blood pressure elevation and impairment of blood flow to skeletal muscle, which would favor the development of insulin resistance. However, hyperinsulinemia may also be associated with a general impairment of agonist-mediated relaxation mechanisms, shifting the balance of insulin's vascular actions toward vasoconstriction [28].

Fructoses fed rats are characterized by elevated total mesenteric vascular endothelin-1 [29] (ET-1) content and an altered arterial reactivity to ET-1. Thus sympathetic hyperactivity can lead to an increase in blood vessel ET-1 levels. Several lines of evidence support the hypothesis that Ang-II stimulates the production and release of ET [30] also ET-1 through activation of ET_A receptors can stimulate NADPH oxidase dependent superoxide production [31]. It is proved by previous study that *S. torvum* is rich in flavonoid content [32]. Flavonoids have the ability to inhibit the synthesis of ET-1. It is quite possible that *S. torvum* prevents fructose induced hypertension by its Antioxidant activity to suppress the production of superoxide anion which annihilates NO function and its ability to strongly inhibit ET-1 receptor [33].

It is well known that the magnitude of vascular responses to Ang-II and 5-HT was significantly ($p<0.05$) enhanced in high fructose fed rats. Increased Ang-II vascular responses in high fructose fed rats may be due to upregulation of Ang-II receptors as observed in hyperinsulinemia. Contractions to 5-HT can also be related to $5\text{-}HT_{2A}$ upregulation as observed in spontaneously hypertensive rats or due to serotonin acting through alpha adrenoreceptors [34]. Chronic administration of *S. torvum* (100, 300 mg/kg/day, p.o.) for 6 weeks in fructose rats significantly ($p<0.05$) shifted the CCRC of Ang-II and 5-HT to the right with suppression of maxima as compared to CCRC of fructose fed rats on isolated ascending colon and fundus strip respectively. Contractile response of PE significantly increase in fructose fed rats. The important part of the contraction appears to be a direct musculotropic activity. A part from contractile effects of agents released by endothelium and their agonists, the direct smooth muscle contraction can be induced by alpha-1 adrenergic agonists, purinergic (P2X) receptor agonists, intracellular contractile machinery activators or calcium influx/calcium release activators. The contractile effect of *S. torvum* was inhibited in the presence of prazosin (an antagonist of alpha-1 receptors) or verapamil (an inhibitor of calcium influx) [35].

CONCLUSION

The present study provides the first experimental evidence for *S. torvum* relaxant activity on the Ascending Colon and Stomach smooth muscle. *S. torvum* possess potent *in vitro* vasocontractile activity on Vas deferens. These results suggest that S. torvum may be acting both through alpha-1 adrenergic pathway and calcium influx activation. In conclusion, oral chronic administration of *S. torvum* induced potentiation of α_1-adrenergic pathway and it could also block the Ang-II and 5-HT receptor.

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