Effect of Aqueous Extract of Defatted Flaxseeds (Linum Usitatissimum Linn) on Fructose-Induced Hypertension in Rats by Inhibiting Angiotensin-Converting Enzyme (ACE)

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Abstract: Linum usitatissimum Linn. is a widely acclaimed medicinal plant used in every household. The seeds of L. usitatissimum have both medicinal and nutraceutical properties. The study was conducted to evaluate the anti-hypertensive effect of aqueous extract with Secoisolariciresinol Diglucoside (SDG) enriched fraction (SEF) of defatted flaxseeds on fructose-induced hypertension in rats. Hypertension was induced by administration of 10% v/v solution of fructose orally in drinking water. Hypertension developed was confirmed by Systolic blood pressure (SBP) measured after 2 weeks of administration. SEF was given orally once daily at the doses of 50, 100 and 200 mg/kg. Captopril was given as standard at the dose regimen of 0.05 mg/kg once daily for consecutive two weeks. SBP and diastolic blood pressure were measured weekly along with plasma level of glucose, cholesterol, and triglyceride after dissection. Fructose feeding for 4 weeks induced hypertension, increased triglyceride level and increased plasma glucose levels in Male Wistar rats. The SEF experienced a significant reverse in hypertension by restoring SBP and also significant reduction on high plasma glucose, high cholesterol and triglyceride level and were observed in dose dependent manner and not observed in captopril group. In in vitro study SEF significantly inhibited Angiotensin-converting enzyme (ACE) when compared to standard. These results suggested that the extract of defatted flaxseed possess hypotensive effects. The anti-hypertensive activity of SEF is probably mediated through ACE inhibition, interaction with the renin-angiotensin system (RAS).

Keywords: Angiotensin converting enzyme, Flaxseed, Hypertension, Lignans, Secoisolariciresinol Diglucoside.

Abbreviations: Angiotensin-converting enzyme (ACE), Carboxy methyl cellulose (CMC), Institute of Medical Sciences, Banaras Hindu University (IMS-BHU), Hydrochloric Acid (HCl), Renin-angiotensin system (RAS), SDG enriched fraction (SEF), Secoisolariciresinol Diglucoside (SDG), Systolic blood pressure (SBP).

I. Introduction

Linum usitatissimum Linn. is a widely acclaimed medicinal plant used in every household. The seeds of L. usitatissimum have both medicinal and nutraceutical properties. L. usitatissimum Linn., versatile and blue flowering rabi crop belonging to Linaceae family. The plant is commonly known as Flaxseed or Linseed in English and Alsi, Tisi, Javas, Aksebjia in Indian languages. Flaxseed is a rich source of essential fatty acids. These essential oils are the key components that have healing properties (Kaithwas et al., 2013). Flaxseed also contains 28% dietary fiber by weight, a third of which is soluble fiber. Flaxseed is the richest known source of dietary lignans, an emerging class of phytoestrogens believed to have lipid-lowering and antioxidant properties (Bloedon et al., 2008). Phenolics are phytoconstituents having different functions, adding color to the flowers and attracting insects (Amarovicz et al., 1997). They possess anticancerous and antioxidant property (Murphy et al., 2002; Simmons et al., 2011 and Dashwood, 2007). It has anti-inflammatory (Kaithwas et al., 2013), anti-cancerous (Kitts et al., 1999), anti-diabetic, cardio-protective (Tuteja et al., 2014), estrogenic (Sacco et al., 2014; Richter et al., 2010) and hepatoprotective activity (Shakir et al., 2007). Different types of the bioactive compound have been isolated from this medicinal plant possesses immense value in medicine.

Flaxseed regulates cholesterol by decreasing LDL levels in the liver (Khanchandani, 2015). It is advocated by Ayurveda as a liver tonic. The extracts of Flaxseeds are known to help in strengthening the heart muscles, relieving stress, and hypertension (Tuteja et al., 2014). Flaxseed is also thought to fortify the central nervous system and to quicken individual reflexes (Boyles et al., 2012). The seeds of Flaxseed have been used in indigenous system of medicine for different ailments. The seeds are said to be hot, pungent, heavy, purgative and strengthening. Its use has been advocated in cancer, arthritis, wounds, abscesses, diabetes, digestive disorders, bronchitis, fever, cough, inflamed lymph nodes etc. as mentioned in Ayurveda procedures (Goyal et al., 2014).

Benefits of flaxseed is that it can be Ushna or Agneya (hot), Drukghni or Achakshushya (not good for eyes), Shukraghini (decreases sperm/semen), Vataghni (useful in treatment of Vata Dosha imbalances such as neuralgia, paralysis, constipation, bloating etc.), Kaphapitta prakopini (increases Kapha and Pitta Dosha),
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Raktapitta prakopana (not ideal in bleeding disorders such as nasal bleeding, heavy periods etc.). Along with these flaxseed oil is Balya (improves strength and immunity), Malakrut (increases bulk of faeces), Grahi (absorbent, useful in diarrhoea, IBS etc.), Twakkoshahara (detoxifies skin on external application) and also can be useful in Basti chikitsa (enema), Paana (oral consumption), Abhyanga (massage) and Karnapoorana (ear drops) (Goyal et al., 2014).

Flaxseed is recommended as an important cardiotonic which is known to promote healthy functionality of heart and regulate blood pressure (Tuteja et al.,2014). High fructose and high sucrose diet increase blood pressure in experimental rats (Bunnag et al., 1997; Cosenzi et al., 1999). Hypertension develops in Wistar rats fed with high fructose diet after 2 weeks of initiation (Vasdev et al., 1994). Hypertension is associated with metabolic abnormalities like hyperinsulinemia, uric acid pathway failure, hypertriglyceridemia and insulin resistance (Lee et al., 1995; Iyer and Katovich, 1996 and Navarro-Cid et al., 1996). The aim of present study is to evaluate the effect of aqueous extract with Secoisolariciresinol Diglucoside (SDG) enriched fraction (SEF) of defatted flaxseeds on fructose-induced hypertension in rats, resolved through ACE inhibition, interaction with the renin-angiotensin system (RAS).

II. Material and Methods

2.1 Animals

The experimental animal protocol was prepared as per CPCSEA guidelines, and it was approved by Institutional Animal Ethics Committee, Institute of Medical Sciences, Banaras Hindu University (IMS-BHU), Varanasi, India. Adult Wister rats’ male were obtained from the Animal House, IMS-BHU, weighing between 250±25 g. They were kept in the departmental animal house at 26±5°C and relative humidity 44-56%, light and dark cycles of 10 and 14 hours respectively, for a week before and during the experiments. Animals were provided with standard rodent pellet diet (Hind Liver), and the food was withdrawn 18-24 hours before the experiment and water were allowed ad libitum (Dahayanake et al., 2001).

2.2 Preparation of extract

The seeds of Linum usitatissimum Linn. was bought from Akhand Aushadhi Bandar, Indore in the month of August 2015. The identification and authentication of seeds were done by Prof. N.K Mishra and Mr. Abhishek, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi.

250 gm of dried flaxseed was collected and mechanically breakdown. The defatting of the seeds was done by soxhleting it with 250 ml of n-hexane for 7 hours. The defatted meal was then extracted with a mixture of ethanol: dioxane (1:1) in ratio of 1:8 for 24 hours. After that, the filtrate was collected and concentrated on rota evaporator at 40°C to get crude lignan extract. The lignan was alkaline hydrolyzed to pH 8.5 by using 0.01N sodium hydroxide and kept for 6 hours. The suspension was acidified to pH 3.0 using concentrated hydrochloric acid (2M) and concentrated on rota evaporator at 45°C. The concentrated crude extract was then liquid partitioned with a mixture of water: ethyl acetate (8:1). The aqueous part was collected, concentrated and stored at 4°C.

The formulation was prepared by accurately weighing the required quantity of SEF, sorbitol, sucrose and transferred it into a clean mortar. To this Tween 80 and small amount of CMC were added and mixed thoroughly to form a smooth paste. The remaining portion of CMC was then added to form a slurry and transferred into a clean volumetric flask(100ml). The mortar was rinsed with water and suspension brought to volume (100ml) with thererequisite amount of water. (Table 1)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Function</th>
<th>SEF I</th>
<th>SEF II</th>
<th>SEF III</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEF</td>
<td>Active ingredients</td>
<td>1.00 g</td>
<td>2.00 g</td>
<td>4.00 g</td>
</tr>
<tr>
<td>CMC</td>
<td>Thickener and stabilizer</td>
<td>2.00 g</td>
<td>2.00 g</td>
<td>2.00 g</td>
</tr>
<tr>
<td>Tween 80</td>
<td>Suspending agent</td>
<td>0.1 g</td>
<td>0.1 g</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Sweetening agent</td>
<td>10 g</td>
<td>10 g</td>
<td>10 g</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>Sweetening agent</td>
<td>5.00 g</td>
<td>5.00 g</td>
<td>5.00 g</td>
</tr>
<tr>
<td>Purified water (q.s)</td>
<td>Vehicle</td>
<td>100 ml</td>
<td>100 ml</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Each ml of formulation contain 10mg, 20mg and 40mg active ingredient per ml.

2.3 Pharmacological analysis

2.3.1 Fructose-induced hypertension and effect of flaxseed

The animals were weighed, and blood pressure was measured using Tail cuff instrument before administration of fructose to induce hypertension. 10% w/v solution of fructose was given in drinking water to all the treatment group for 14 days. The blood pressure was again measured and then animals were administered orally with carboxymethylcellulose (CMC) 2% w/v suspension of SEF for 14 days daily at the doses of 50 (SEF I),100 (SEF II), 200 (SEF III) mg/kg/day along with 10% w/v solution of fructose. Blood pressure readings were
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takentwice a week continued until the end of the tentative schedule (Dahayanake et al., 2001). Control rats received the ordinary drinking water along with the vehicle (CMC 2% w/v Suspension).

2.3.2 Blood pressure measurement procedures
Tail cuff method is a common and convenient way to measure systolic pressure in rats. The tail cuff is inflated and then deflated. Pulsations disappear when the cuff is inflated. When the cuff is deflated pulsations start appearing, when the pressure in the cuff equals systolic pressure. The cuff is attached to a tail cuff sphygmomanometer or more commonly to pressure transducer and BP is recorded on a chart using BIOPAC systems Inc. NIB200A, Small Animal Tail Non-invasive Blood Pressure System. Training the animal and warming the tail are required for this method. Computerized tail cuff instrument can also be used. (Vogel, 2002 and Susalit et al., 2011).

2.3.3 Biochemical measurements
All animals will be sacrificed by decapitation, and blood will be collected in EDTA coated tubes kept on ice and centrifuged at 1000×g for 20 min at 4°C to separate plasma (Compufuge CPR-30 Plus, with Rotor No. 8; REMI, India). Plasma will be separated, and aliquots will be stored at -70°C for biochemical estimations. Blood glucose level, Triglyceride level were estimated in Parul Pathology Clinic, BHU Naria Road, Lanka, Varanasi (U.P) with self-supervision. Immediately after blood collections, kidney and heart of the animals will be removed and weighed (Kapur et al., 2010).

2.4 Angiotensin Converting Enzyme Assay (In-vitro)
The rats were sacrificed under anaesthesia, and lungs were collected after dissection. The organs were cleaned of fatty tissues, rinsed with normal saline (pH 9) and homogenized in cold trizma- HCl buffer (pH 7.4). The homogenate was centrifuged at 1000 rpm at 4°C for 15 minutes, until pelleting stopped. Supernatants were stored at -40°C, until used.
Partial purification of ACE was done using ammonium sulphate solution. The supernatant was transferred to a cold beaker kept in an ice bath, and precipitation was done by adding ammonium sulphate solution at increasing concentration from 30-80%. The mixture was then centrifuged at 1000rpm at 4°C for 15 minutes, and the supernatant was discarded. The pellet was reconstituted in 7 ml buffer (pH 7.8) and ACE activity was determined.

ACE inhibitor activity was determined with 20μL of the sample solution added in 50μL of 8mM HHL as substrate and 10μL of ACE solution (0.25 U/mL). The mixture was mixed well and incubated for 1 hour at 37°C. The reaction was stopped by adding 62.5 μLHCl 1M. The hippuric acid formed was extracted with 375 μL of ethyl acetate. Finally, ethyl acetate layer was dried in a vacuum oven and 4mL of water was added. The absorbance of hippuric acid was measured by using UV-Visible spectrophotometer at 228 nm. Blanks were measured by replacing ACE with water, while 100% activity value was determined by replacing sample with 20 μL of water. Captopril was used as control in concentration 25µg/ml.

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\text{Percentage inhibition of ACE activity} = \frac{100 \times (A - B) - (C - D)}{(A - B)}
\]
Where A is absorbance of control, B is absorbance of control blank, C is absorbance of the sample, and D is absorbance of the control sample.

2.5 Statistical analysis
The data was statistically analyzed by one-way ANOVA, followed by Bartlett’s test. Values in tables are expressed as mean ±SEM. Values are significantly different from the control group at P< 0.05. Values with P<0.05 was acknowledged as statistically significant.

III. Results

3.1 Systolic blood pressure
The effect of fructose and SEF of flaxseed on SBP is shown in Figure 1. Fructose treatment was related to the significant increase (P<0.05) in SBP. Prolonged treatment with the SEF of flaxseed decreases the blood pressure in fructose-fed rats. SBP was significantly lower in groups treated with doses 50,100,200 mg/kg/day compared with thefructose-fed group. The hyperactivity of rats was also found to be decreased. Body weight increased due to fructose feeding. No significant difference was observed when compared with control group. (Table 2)
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Figure 1: Systolic Blood Pressure in mmHg in six groups of rats *

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
<th>Group F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>198 ± 3.65</td>
<td>197.16 ± 5.92</td>
<td>208.83 ± 2.47</td>
<td>202 ± 2.14</td>
<td>186.66 ± 2.18</td>
<td>191.16 ± 2.55</td>
</tr>
<tr>
<td>Heart rate (b/min)</td>
<td>335 ± 8</td>
<td>334 ± 9.7</td>
<td>347 ± 1.12</td>
<td>349 ± 1.2</td>
<td>378 ± 8.75</td>
<td>334 ± 2.3</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>189.5 ± 1.23</td>
<td>245.5 ± 3.45</td>
<td>258.5 ± 2.45</td>
<td>258 ± 1.78 ^*</td>
<td>163.5 ± 1.23 ^*</td>
<td>295 ± 3.21 ^*</td>
</tr>
<tr>
<td>Plasma triglyceride (mg/dl)</td>
<td>184.5 ± 2.12</td>
<td>194 ± 1.41 ^*</td>
<td>232.6 ± 3.88 ^b</td>
<td>180.5 ± 2.82</td>
<td>166 ± 1.02 ^c</td>
<td>239 ± 2.85</td>
</tr>
<tr>
<td>Kidney weight (mg)</td>
<td>605 ± 1.56</td>
<td>557 ± 1.09</td>
<td>505 ± 2.75</td>
<td>575 ± 2.34</td>
<td>590 ± 0.78</td>
<td>500 ± 2.46</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>300 ± 2.82</td>
<td>285.2 ± 1.97</td>
<td>267.5 ± 3.28</td>
<td>245 ± 3.63</td>
<td>230 ± 1.41</td>
<td>200 ± 2.28</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SEM for six animals per group. Group A- Control; Group B- Fructose plus Captopril (0.05mg/kg); Group C- Fructose plus SEF I; Group D- Fructose plus SEF II; Group E- Fructose plus SEF III; Group F- Fructose.

3.2 Biochemical parameters

The plasma glucose level and triglyceride levels were estimated and it was found to be that effect of SEF was dose dependent, optimum activity was observed at 200mg/kg, and it behaves like the animals present in the control group. The organs were separated after dissection of rats and weighed, the kidney was affected much because of the fructose treatment and the higher concentration of SEF is more effective in maintaining the anatomy of the kidney. Heart weight was also affected by fructose but the extract did not have much effect on its anatomy.

3.3 Angiotensin Converting Enzyme Assay (In-vitro)

ACE converts HHL into hippuric acid and histidil-leusin. The activity on ACE inhibition was evaluated based on the level of hippuric acid by measuring its absorbance using a spectrophotometer. The activity was calculated quantitatively in the presence or absence of the extract. Captopril was used as the positive control. Captopril showed high percentage inhibition with a value of 74.5% at 25 μg/ml concentration. Captopril is the potent ACE inhibitor for regulating blood pressure. ACE is related to endothelial dysfunction in hypertension and the development of atherosclerosis. Obstruction to ACE activity can prevent the progression of atherosclerosis and reduce the cardiovascular event. SEF showed significant inhibition activity at a concentration in a dose-dependent manner. The highest inhibition percentage showed at final concentration 100 μg/ml, the IC_{50} value of SEF was found to be 28.7 μg/ml. (Figure 2)
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Figure 2: Angiotensin Converting Enzyme Assay

IV. Discussion

Several anti-hypertensive drugs significantly decrease the increased blood pressure induced by fructose diet (Bhanot et al., 1994; Iimura et al., 1995 and Rosen et al., 1997). The results of this study demonstrate that 2 weeks high fructose diet resulted in hypertension in rats that was treated with the aqueous extract of flaxseed with Secoisolariciresinol Diglucoside (SDG) enriched fraction (SEF). Extract decreases the increased SBP in a dose-dependent manner.

The results of the study indicated that fructose feeding can induce hypertension in rats. Plasma glucose and triglyceride levels were higher in these rats. Hypertriglyceridemia, high glucose or both, responsible for the development of hypertension in rats (Erlich and Rosenthal, 1996; Bunnag et al., 1997 and Fang and Huang, 1998). The increased mobility and high blood pressure indicated the failure of Renin Angiotensin System and SEF act as an inhibitor of ACE responsible for the conversion of AT₁ to AT₂ because by the administration of a high amount of fructose the salt balance get disturbed inside the body which results in the excess secretion of uric acid. Excess of uric acid activates the juxtaglomerular cells of the kidney which secretes renin. Renin activates the liver cell to secrete high amount of AT 1 in the body. Hypertension is directly linked to the RAS involving AT₁, AT₂ and ACE. SEF inhibit the action of ACE, responsible for the conversion to AT 2 which further decrease the developed hypertension. Other mechanism includes sodium-water retention, sympathetic nerve stimulation, changes in transmembrane ion traffic and direct stimulation of smooth muscle cell growth (Ferrannini and Natali, 1991; Suzuki et al., 1997).

A significant reverse the hypertension was observed in SEF in dose dependent manner induced due to fructose feeding and not in captopril group. It was observed that SEF significantly protects the organ vitality and prevent it from damage occurs due to fructose feeding but this such effect was not observed with captopril. No withdrawal symptoms and side effects were observed with SEF group. Thus, treatment with SEF was considered superior to captopril for the maintenance of hypertension induced by fructose.

V. Conclusion

The study indicates that after taking a high fructose diet for 6 weeks significantly develops hypertension in normal rats with an increase in plasma glucose and triglyceride levels. Treatment with SEF of flaxseed decrease the elevation of blood pressure and also affects the plasma glucose and triglyceride level in a dose-dependent manner.

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Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Reference

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