ANTI-HYPERLIPIDEMIC ACTIVITY OF AQUEOUS EXTRACT OF SEEDS OF VIGNA RADIATA

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ABSTRACT

The present investigation is proposed to study the effect of various doses of seed extract of Vigna radiata in Triton-X-100 induced hyperlipidemia using normal healthy wistar rats as experimental model. To test the drug as a therapeutic agent, a major breakthrough in the management of hyperlipidemia induced by Triton-X-100 may be envisaged.

On the basis of my literature survey, no attempt has been made to investigate the anti-hyperlipidemia activity of aerial parts of Vigna radiata till date. So present work is an attempt to study seeds extracts of Vigna radiata to determine its anti-hyperlipidemia activity.

The study is planned with the following objectives:

i. To induce the hyperlipidemia in male wistar Rats by Triton-X-100.
ii. To investigate anti-hyperlipidemia effect of Vigna radiata.
iii. To compare the anti-hyperlipidemia effect of Vigna radiata to standard antihyperlipidemic drug Atorvastatin.

Keyword: Hyperlipidemia, Vigna Radiata, Extract, Triton-X-100, Toxicity, Wistar rats

1. INTRODUCTION

Hyperlipidemia, hyperlipoproteinemia or hyperlipidaemia involves abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It is the most common form of dyslipidemia (which includes any abnormal lipid levels).

Lipids (fat-soluble molecules) are transported in a protein capsule. The size of that capsule, or lipoprotein, determines its density. The lipoprotein density and type of apolipoproteins it contains determines the fate of the particle and its influence on metabolism.

Hyperlipidemias are divided into primary and secondary subtypes. Primary hyperlipidemia is usually due to genetic causes (such as a mutation in a receptor protein), while secondary hyperlipidemia arises due to other underlying causes such as diabetes. Lipid and lipoprotein abnormalities are common in the general population, and are regarded as a modifiable risk factor for cardiovascular disease due to their influence on atherosclerosis. In addition, some forms may predispose to acute pancreatitis.

2. EXPERIMENTAL WORK

2.1. Methodology:

1. Collection of plant material
2. Extraction
3. Phytochemical screening
4. Oral acute toxicity studies
5. Induction of hyperlipidemia
6. Evaluation of anti-hyperlipidemia activity of Vigna radiata

2.1.1. Collection of plant material

A specimen was deposited in the institutional herbarium of Rajasthan university campus, Jaipur, India. The collected plant material was made thoroughly free from any foreign organic matter and a part of the material was dried under shade. The taxonomic features of herbarium have Been Authenticated from “Botany Department of Rajasthan University” Jaipur. (Authentication number: RUBL211503). The seeds were powdered and were extracted with water by hot percolation.
2.2.2. Extraction

2.2.2.1. Direct aqueous extraction

It was done by the Soxhlet Apparatus. Water is used as a solvent.

Plant material was collected in bulk, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The plant material pulverized in a hand mill followed by sieving (sieve no. 40) to obtain coarse powder. The powdered seeds were extracted with water for 48 h in soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.

The marc obtained after petroleum ether was subjected to water in Soxhlet apparatus. The extraction was continued for the period of 48 hours. The extract was then concentrated and finally dried to a constant weight.

2.3. Phytochemical screening

200gm drug was taken and finely powdered. This powder was used further for the phytochemical screening.

2.3.1. Test for alkaloids:-

a) Mayer’s test:

To a few ml of filtrate, a few drops of Mayer’s reagent was added by the side of the tube. A creamy white precipitate indicates the presence of alkaloids.

2.3.2. Test for flavonoids:

a) Shinoda’s test:

To 5ml of the extract, 5-10 drops of dilute HCl and small piece of magnesium chloride was added and the solution was boiled for a few minutes. Appearance of reddish pink color or dirty brown color indicates the presence of flavonoids.

2.3.3. Test for carbohydrates:

a) Benedict’s Test:

To 0.5ml of the filtrate, 0.5 ml of Benedict’s reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic red colour precipitate indicates the presence of sugar.

2.3.4. Test for saponins:

a) Froath test:

To 0.0 5ml of filtrate, added 5ml of distilled water and shaken vigorously for a stable persistence froth. Frothing which persisted on warming indicates the presence of saponins.

2.3.5. Test for tannins:-

a) Ferric Chloride test:

To 2 ml of extract, few drops of 5% ferric chloride solution was added. The appearance of violet color indicates the presence of tannins.

2.3.6. Test for phytosterols:

a) Libermann-Burchard’s test:

To 2 ml of the filtrate, 2 ml of acetic anhydride was added. Concentrated sulphuric acid was added along the sides of the test tube. A colour change from violet to blue indicates the presence of phytosterols.

2.3.7. Test for Terpenoids:

a) Salkowski Test:-

5 ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid was added to form a layer. A reddish brown colour indicates the presence of terpenoids.

2.3.8. Test for proteins:

a) Ninhydrin Test:

To 1 ml of extract few drops of Ninhydrin reagent was added and heated in a boiling water bath. A purple blue colour indicates the presence of proteins.

b) Biuret Test:

To 1ml of extract, equal volume of 5% NaOH solution and copper sulphate solution added. A blue colour indicates the presence of proteins.

2.3.9. Test for Anthraquinone:

To 5 ml of extract, added dilute Sulphuric Acid and 1 ml of diluted ammonia. Appearance of pink colour indicates presence of anthraquinone.

2.3.10. Test for polyphenols:
10 ml of plant extract was heated for 30 minutes in a water bath. 1 ml of 1% Ferric Chloride was added to the mixture followed by the addition of 1% potassium ferric cyanide. The mixture was filtered and formation of green-blue color indicates the presence of polyphenols.

**2.3.11. Test for glycosides:**

*a) Borntrarger’s test:*

To 0.5 ml of extract, few drops of Hcl was added and heated in a boiling water bath for few minutes and treated with chloroform. The chloroform layer was separated and added Equal volume of diluted ammonia. Appearance of pink color indicates the presence of glycosides.

**Phytochemical screening of chemicals in methanol**

<table>
<thead>
<tr>
<th>S. No</th>
<th>PHYTOCHEMICALS</th>
<th>Aqueous EXTRACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavanoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Terpenoids</td>
<td>+</td>
</tr>
</tbody>
</table>

(-) Indicates Absence  
(+) Indicates Presence  

**2.4. Acute oral toxicity**

The acute toxicity study for aqueous extract of Vigna radiate Linn. seed was performed using wistar rats, the extract at different doses (5-2000 mg/kg body weight) was administered orally to normal wistar rats for acute toxicity studies, and behavior changes, 1 hour post dosing and at least once daily for 14 day, the parameter such as hyper activity, grooming convulsion, sedation hypothermia and mortality was observed.

No mortality with oral administration of Vigna radiate Linn. Even at the highest dose (2000 mg/kg orally) both the doses of Vigna radiate Linn. Had no toxicity effect on the normal behavior of rats.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose(mg/kg)</th>
<th>Clinical observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5 mg</td>
<td>No treatment related toxicity sign &amp; symptoms /mortality was noticed.</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>No treatment related toxicity sign &amp; symptoms /mortality was noticed.</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>50mg</td>
<td>No treatment related toxicity sign &amp; symptoms /mortality was noticed.</td>
<td></td>
</tr>
<tr>
<td>100mg</td>
<td>No treatment related toxicity sign &amp; symptoms /mortality was noticed.</td>
<td></td>
</tr>
<tr>
<td>300mg</td>
<td>No treatment related toxicity sign &amp; symptoms /mortality was noticed.</td>
<td></td>
</tr>
<tr>
<td>500mg</td>
<td>No treatment related toxicity sign &amp; symptoms /mortality was noticed.</td>
<td></td>
</tr>
<tr>
<td>2000 mg</td>
<td>No treatment related toxicity sign &amp; symptoms /mortality was noticed.</td>
<td></td>
</tr>
</tbody>
</table>

Vigna radiate Linn. Seeds have not shown toxicity at any doses.

### 2.5. Induction of hyperlipidemia

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h.

### 2.6. Experimental Design

The animals were divided into five groups of six rats each. The first group was given standard pellet diet, water and orally administered with 10% dimethyl sulfoxide (DMSO). The second group was given a single dose of triton administered at a dose of 100 mg/kg, i.p. After 72 h of triton injection, this group received a daily dose of 10% DMSO (i.p) for 7 days. The third group was administered a daily dose of Vigna radiate aqueous extract (VRAE) 200 mg/kg dissolved in 10% DMSO, p.o., for 7 days, after inducing hyperlipidemia. The fourth group was administered a daily dose of VRAE 400 mg/kg dissolved in 10% DMSO, p.o., for 7 days, after inducing hyperlipidemia. The fifth group was administered with the standard atorvastatin 10 mg/kg, p.o. for 7 days.

### 3. GROUPING

**Group I:** Normal (10% DMSO)

**Group II:** Hyperlipidemic (Triton X-100) + (10% DMSO)

**Group III:** Hyperlipidemic (Triton X-100) + (VARE 200mg/kg)

**Group VI:** Hyperlipidemic (Triton X-100) + (VARE 400mg/kg)

**Group V:** Hyperlipidemic (Triton X-100) + Atorvastatin (10mg/kg)

### 4. COLLECTION OF BLOOD

On the 8th day, blood was collected by retro orbital sinus puncture, under mild ether anesthesia. The collected samples were centrifuged for 10 min, and then serum samples were collected and used for various biochemical experiments.

After completion of 30 days experiment period, the overnight fasted animals were sacrificed under protocol conditions. Blood was collected directly from cardiac puncture; serum was separated and stored at -20°C for serum biochemistry. Aorta was removed, cleaned and weighed. The removed aortas were fixed in 10% formalin solution for histopathological observations.

### 5. BIOCHEMICAL ANALYSIS

The serum extract was assayed for TG, TC, HDL, LDL, VLDL by using standard kits.

The fraction of LDL-C in the serum was calculated by using Friedewald’s equation as follows:

\[
LDL-C = \text{Total cholesterol} - (\text{HDL-C} + \text{VLDL-C})
\]
VLDL-C = Triglyceride/5

Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) activities were also measured.

### 6. GROUPING PARAMETERS

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>TG</th>
<th>TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>50.83 ± 1.16</td>
<td>26.5 ± 0.42</td>
<td>16.16 ± 0.60</td>
<td>74.83 ± 0.94</td>
<td>83.33 ± 0.95</td>
</tr>
<tr>
<td>Hyperlipidemic Control</td>
<td>25.33 ±0.88</td>
<td>96.16 ± 1.11</td>
<td>33.33 ± 0.61</td>
<td>161 ± 1.24</td>
<td>165.16 ± 2.46</td>
</tr>
<tr>
<td>VRAE(200mg/kg)</td>
<td>42.16 ± 1.07</td>
<td>60.33 ± 0.49</td>
<td>24.33 ± 0.92</td>
<td>127.5 ± 1.73</td>
<td>140.33 ± 1.02</td>
</tr>
<tr>
<td>VRAE(400mg/kg)</td>
<td>47.16 ±0.47</td>
<td>47.5 ± 0.76</td>
<td>21.16 ± 0.40</td>
<td>110.5 ± 1.11</td>
<td>106 ± 0.57</td>
</tr>
<tr>
<td>Atorvastatin (10mg/kg)</td>
<td>49.33 ± 0.76</td>
<td>40.33 ± 0.66</td>
<td>20.33 ± 0.66</td>
<td>93.16 ± 2.09</td>
<td>100.83 ± 0.47</td>
</tr>
</tbody>
</table>

**SERUM LIPID PROFILE**

- Normal
- Hyperlipidemic Control
- VRAE(200mg/kg)
- VRAE(400mg/kg)
- Atorvastatin (10mg/kg)
GROUPS | PARAMETERS (mg/dl) |  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG</td>
<td>HDL</td>
</tr>
<tr>
<td>Normal control</td>
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</tbody>
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7. RESULT AND CONCLUSION

The histological investigations of aorta of cholesterol fed rabbits showed a well-developed sized atherosclerotic plaque (containing foam cells and extra lipid content) and reduction was observed through Vigna radiate linn. seed extract treatment, as micro photographic figures.

Landmarks clinical studies have been demonstrated that lowering elevated total cholesterol and low-density lipoprotein cholesterol (LDL) significantly reduces the risk of coronary events, stroke and death in both primary and secondary coronary prevention in patients. Data from epidemiological studies support that potential of dietary antioxidants and flavonoids that are presents in several herbs to improve cardiovascular health. Ancient literature has been also reported that the use of Vigna radiate Linn. as folk medicine for various ailments.

Triton X-100 increases lipid profile. A triglyceride based index (AI) can significantly add value when assessing the cardiovascular risk.

REFERENCES

[1] Dorland’s Medical Dictionary for Health Consumers; 2007; Sanders; Elsevier; 765