ANTI-HYPERLIPIDEMIC ACTIVITY OF CUCUMIS MELO FRUIT PEEL DIFFERENT EXTRACT IN TRITON X-100 INDUCED HYPERLIPIDEMIA IN RATS

Dhanaji D Ghanwat*, Jayant S. Bidkar, Madhuri D. Bhujbal, Ganesh Y. Dama

Department of Pharmacognosy, Sharadchandra Pawar College of Pharmacy, Dumberwadi (Otur), Tal.Junner, Dist.Pune, 410504, Maharashtra, India.

KEYWORDS:
Antihyperlipidemic activity, Triton induced hyperlipidemia, Cucumis melo fruits peel.

For Correspondence:
Mr. Dhanaji D. Ghanwat*, Department of Pharmacognosy, Sharadchandra Pawar College of Pharmacy, Dumberwadi (Otur), Tal.Junner, Dist.Pune, 410504.

E-mail: dhanaji.ghanwat@rediffmail.com

ABSTRACT

Cucumis melo Linn. (Cucurbitaceae) fruits have been used, traditionally, for the treatment of various disorders of heart as Cardioprotective and antiobesity. The aim of the present study was to investigate the possible anti-hyperlipidemic activity of Cucumis melo fruit peel extract in triton induced hyperlipidemia in rats. Chloroform, Methanolic and aqueous extracts of were administered to the triton induced hyperlipidemic rats for 7 days to study antihyperlipidemic acivity. The acute toxicity value of chloroform, methanol and aqueous extract after oral administration in mice were found to be 5000 mg/kg. The results concluded that CMFP methanolic extract (500 mg/kg) have definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemia model and which is equipotent activity when compared with Atorvastatin treated group.
1. INTRODUCTION
Current predictions estimate that by the year 2020 cardiovascular diseases, notably atherosclerosis, will become the leading global cause of total disease burden, defined as the years subtracted from healthy life by disability or premature death an important factor in causing atherosclerosis is hyperlipidemia [1]. Hyperlipidemia is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood [2]. It is also called hyperlipoproteinemia because these fatty substances travel in the blood, attached to proteins and this is the only way that these fatty substances can remain dissolved while in circulation. It is also synonymously known as dyslipidemia [3]. The randomly collected data clearly demonstrated that some forms of dyslipidemias were very common in adults all over the world. During the past decade, a vast amount of evidence has confirmed the critical role played by the dyslipidemias in the pathogenesis of atherosclerosis, coronary artery disease etc [4]. The advantages of lowering lipid levels to satisfactory levels have been confirmed by several experimental and interventional studies indicating lower morbidity and mortality in coronary heart disease which commensurate with reduction of serum cholesterol [5].

*Cucumis melo* Linn (Syn. *Cucumis callosus* (Rottl.) Cogn. *Cucumis trigonus* Roxb. Family *Cucurbitaceae*) popularly known as Muskmelon [6]. *Cucumis melo* Linn (CM) is a pubescent or trailing herb with edible, polymorphous fruits. It is used for various ailments in Indian Traditional System of Medicine. Fruit and roots have medicinal value. The fruits are used in flatulence, leprosy, fever, jaundice, diabetes, antiobesity, cough, bronchitis, ascites, anaemia, constipation, other abdominal disorders and amentia [6, 7, 8]. In addition, fruit pulp is bitter, acrid, thermogenic, anthelmintic, liver tonic, cardio tonic, appetizer, expectorant and intellect promoting. Roots are used as emetic and purgative [7].

Earlier works on CM fruits showed effectiveness in preventing chemically induced hypothyroidism in rats [9]. Some other research studies are available on its urease inhibitory, antioxidant, anti-inflammatory, antiulcer and diuretic [10, 11, 12]. Phytochemical investigations revealed the presence of phenolic glycoside(E)-4-hydroxycinnmyl alcohol 4-O-(2'-O-β-D-apiofuranosyl)(1"→2")-β-D-glycopyranoside, benzyl O-β-Dglucopyranoside, 3,29-O dibenzoylemultiflor-8-en-3α,7β,29-triol and 3-O-p-amino-benzoyl-29-O benzoylmultiflor-8-en-3 α, 7β,29-triol was isolated and identified from *Cucumis melo* seeds [13].
CM fruits are used traditionally in Indian traditional system of medicine, and also various uses have been described including for obesity [8] but there is no much scientific study reported about antihyperlipidemic activity. Therefore present study was undertaken to establish the acute toxicity study and scientifically evaluate the antihyperlipidemic activity of the Chloroform, methanol and aqueous extracts of CM fruits peel (CMFP) in Triton X-100 induced hyperlipidemia model in Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant material:
The fresh trailing herb with fruits of *C.melo* Linn was collected from Pune district (M.S.) in the months of July-August. The trailing herb was authenticated by Mr. P. G. Diwakar, Deputy Director, Botanical Survey of India, Pune through comparing morphological features. The herbarium of the plant specimen was deposited at Botanical Survey of India. Voucher specimen number DDG-4 (Ref. No.BSI/WRC/Tech/2010/428 dated 09.09.2010). They were peeled off mechanically and good quality peels were air dried under shadow and then grounded into coarsely powdered in grinder and powder material was passed through 120 mesh to remove fine powders and coarse powder was used for extraction.

2.2 Protocol for Successive Extraction
The coarse powder of CMFP (100 gm) was extracted by using successive soxhlet extraction using solvents of varying polarity such as petroleum ether (60-80ºc), chloroform, methanol, distilled water (8.2, 10.1, 4.2, 8.5g respectively) for 72 hrs. After completion of extraction, solvent was distilled off and concentrated extract was air-dried [14]. Petroleum ether extraction was used defatting. Chloroform, Methanol, Aqueous extract was mixed with 5% CMC and which was used for the antihyperlipidemic activity.

2.3 Phytochemical screening:
The crude extract obtained by using various solvents were analyzed for alkaloids, tannins, saponins, steroids, flavonoids, and phenolic compound using standard procedure of analysis [15].
2.4 Chemicals

Triton was purchased from Merck Limited, Mumbai, India. Cholesterol kit (Enzymatic Method), HDL-C kit, Triglycerides were procured from Beacon Diagnostics PVT.LTD, Navasari, India. All solvents used for extraction procured from Merck Limited, Mumbai, India.

2.5 Animal

Wistar albino male rats weighing 200-220g and albino mice (Either sex) weighing 20-25g were selected and housed in polypropylene cages in a room where the congenial temperature was 27°C ±1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet (VRK nutritional solution, Pune) and water *ad libitum*.

2.6 Acute toxicity study

Albino mice (Either sex) of 10 animals per group and weighing 20-25 g were administered graded dose (100-5000 mg/kg body weight, p.o.) of the chloroform, methanolic and aqueous extracts of CMFP. After administration of the extract the mice were observed for toxic effects for 48 hr. The toxicological effects were observed in terms of mortality expressed as LD50. The number of animals dying during a period was noted. The LD50 of the extract was determined by Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949) method [16].

2.7 Anti-hyperlipidemic activity

Hyperlipidemia was induced in Wistar albino male rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline after overnight fasting for 18 hours [17, 18]. The rats were divided into nine groups of six rats in each group and were treated with single dose/day (*p.o.*) of standard drug or extracts of CMFP. The first group was given Standard pellet diet, 5% CMC, and water (Served as normal control). The second group was given a single dose of triton at a dose 100 mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 5 % CMC (*p.o.*), for 7 days (Served as Triton control). The third to eight group was administered a daily dose of CMFP Chloroform, Methanol and aqueous extract at a dose 250 mg/kg and 500 mg/kg suspended in 5% CMC, *p.o.* (served as treatment groups) for seven days, after inducing hyperlipidemia. Ninth group was administered with
Standard Atorvastatin 10 mg/kg p.o. for 7 days (served as standard).

2.8 Collection of blood samples
On 8\textsuperscript{th} day of treatment, the blood was collected by retro orbital sinus puncture, under mild ether anesthesia in plane tubes. Serum obtained by immediate centrifugation of blood samples using remi ultra cooling centrifuge at 3000 rpm for 5 minutes at room temperature and was directly used for estimating serum lipid profiles (serum TC, TG, LDL-C and HDL-C). All samples were stored at 4°C until analysis.

2.9 Biochemical analysis
Serum lipid levels include TC, TG and HDL-C, were carried out using respective diagnostic commercial kits from Qualigens diagnostics, Mumbai, India and LDL-C in plasma was calculated as per friedewald estimation [19], LDL-C= (TC-(TG/5+HDL)-C) mg/dl, VLDL=(TG/5), Atherogenic Index=TC/HDL-C.

2.10 Statistical analysis
The results were expressed as mean ± SD. The Triton control was compared with normal and the experimental results were compared with Triton control. Statistical analysis was carried out using paired t-test and one-way ANOVA followed by Dunnett test. Differences below P<0.05 implied statistically significance.

3. RESULTS
3.1 Phytochemical screening
The results of phytochemical screening in table 1. indicated the presence of maximum amount of steroids and Steroidal glycosides, in Chloroform extract., Carbohydrate, Flavonoids, Tannins, Saponins, steroids are present in Methanolic extract and Aqueous extract contains Carbohydrate, Flavonoids, Saponin and Tannins.

3.2 Acute toxicity study
The acute toxicity studies of chloroform, methanolic and aqueous extract of the CMFP were found to be non-toxic up to the dose 5000 mg/kg and did not show any mortality.
3.3 Anti-hyperlipidemic activity

The systemic administration of the surfactant Triton to fasted or non-fasted rats results in the elevation of plasma cholesterol and triglyceride levels. The dyslipidemia of the metabolic syndrome consists of hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol, and a higher proportion of small, dense low-density lipoprotein (LDL) particles. An increased risk of coronary heart disease is associated with a high serum concentration of total cholesterol, LDL and triglyceride [20].

The results of present study are given in Table 2-3. The rats treated with triton showed significant increase in serum cholesterol level from 54.08 mg/dl in normal rats to 137.53 mg/dl, triglyceride level from 59.53 mg/dl in normal rats to 111.51 mg/dl and LDL-C from 20.24 mg/dl in normal rats to 86.02 mg/dl. Treatment with CMFP different extract at the different doses of 250 mg/kg, and 500 mg/kg reduced the serum TC, TG & LDL-C levels & increased the serum HDL-C levels when compared to the hyperlipidemic control group. It is widely accepted that reduction in plasma HDL-C level is a risk factor for developing atherosclerosis [21]. The change in lipid levels in group number III-VIII, were comparable with group of Atorvastatin treated rats. Methanolic and aqueous extract showed a dose dependant decrease in the levels of cholesterol, Triglyceride, and LDL-C level. Among five groups (i.e. group number III-VIII), group number VI reduced the elevated lipid levels more significantly than the others while CMFP chloroform extract having very low hypolipidemic activity. Administration of methanolic extract of CMFP at a dose of 500 mg/kg and standard drug 10 mg/kg the cholesterol level is 60.13 mg/dl triglyceride level is 68.63 mg/dl and HDL-C level is 25.57 mg/dl as compare to standard drug Atorvastatin where decrease of cholesterol level is by 58.17 mg/dl triglyceride level is by 61.92 mg/dl and HDL-C level is 26.70 in triton induced hyperlipidemic rats. A significant increase in HDL-C which was related to a significant reduction of atherogenic index was observed (8.01 to 2.17).

4. DISCUSSION

Hyperlipidemia is widely known to be the major risk factor for the development of cardiovascular diseases. Coronary heart diseases, stroke, atherosclerosis and hyperlipidemia are the primary cause of death [22-23]. Therefore, prime consideration in the therapy for hyperlipidemia and arteriosclerosis is to attenuate the elevated blood serum/plasma levels of lipids. The currently availa-
-ble antihyperlipidemic therapy includes mainly HMG-CoA reductase inhibitors (Statins), Bile acid sequesterants (Resins), Activate lipoprotein lipase (Fibric acid derivatives), Inhibit triglyceride synthesis (Nicotinic acid) and others (Gugulipid, Ezetimibe, Policosanol) [26]. Though there are a large number of antihyperlipidemic agents used in the treatment, none of the existing one available worldwide are fully effective, absolutely safe and free from side effects [25]. So efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. Many herbs and plant products have been shown to have antihyperglycaemic and antihyperlipidemic properties [26].

*Cucumis melo* is a drug-plant used traditionally for the treatment of heart disorders as Cardioprotective. The purpose of this work was to evaluate the traditional claims of *Cucumis melo* on scientific basis in experimentally induced hyperlipidemia. Single administration of CMFP different extract in various doses (100-5000 mg/kg) did not show any mortality, this indicate the extracts safety and absence of toxicity in the doses studied. The results of our present study clearly indicate that CMFP methanolic and aqueous extract at a dose of 250 and 500 mg/kg significantly lowered serum cholesterol and triglyceride levels i.e. antihyperlipidemic activity which was found to be more effective in higher dose as compared to lower dose when administered orally in triton induced hyperlipidemic models. The serum cholesterol lowering effect of CMFP methanolic and aqueous extract may be due to the inhibition of cholesterol biosynthesis.

The methanolic and aqueous extract of CMFP decreased the serum triglyceride level may be due to increased catabolism of triglyceride and an inhibition of fatty acetyl-CoA activity and glycerophosphate acetyl transferase.

The HDL-C level is inversely related to total body cholesterol and reduction of plasma HDL concentration may accelerate the development of atherosclerosis, leading to ischaemic heart disease, by impairing the clearance of cholesterol from the arterial wall [27]. (References, Miller and Miller) In the CMFP methanolic and aqueous extract treated groups of animals showed slightly increase the serum HDL-C levels in triton induced hyperlipidemic model. The increase in HDL-C level may be due to the activity of LCAT and inhibition of the action of hepatic TG-lipase on HDL, which may contribute for rapid catabolism of blood lipids through extra hepatic tissues.
Total cholesterol / HDL-C ratio of > 4.5 is associated with increased coronary heart disease risk and the ideal ratio is ≤ 3.5 [28]. A significant decrease in the atherogenic index on CMFP methanolic and aqueous extract treatment shows that the protective efficacy of the extract against atherogenesis.

Preliminary phytochemical screening revealed the presence of flavonoids, tannins, steroids and steroidal glycosides in CMFP methanolic and aqueous extract. It is found that some saponins increase the permeability of mucosal cells in vitro, inhibit active mucosal transport and facilitate uptake of substances that are normally not absorbed [29]. Phytosterols are reported to displace intestinal cholesterol and reduce cholesterol absorption from intestine. Flavonoids have exhibited a variety of pharmacological activities, including the anti-atherogenesis and antioxidant effect [30]. Thus the present result strongly suggests that the hypolipidemic activity of this medicinal plant could be attributed to the presence of saponins, steroids and flavonoids in the extract.

CONCLUSION:

The results concluded that CMFP methanolic extract (500 mg/kg) have definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemia model and which is equipotent activity when compared with Atorvastatin treated groups. Further studies on this extract may lead to identify the possible mechanism of action and isolation of active principle from the same.

REFERENCES:


**Table 1: Phytochemical screening of CMFP different extracts**

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Chloroform extract</th>
<th>Methanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+: Presence of Chemical constituents, -: Absence of Chemical constituents, ++: Maximum presence of Chemical constituents.
Table 2. Effect of CMFP various extract on TC, TG and VLDL-C level in Serum of control and experimental Rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Total Cholesterol</th>
<th>Triglyceride</th>
<th>VLDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I Normal control</td>
<td></td>
<td>54.08±2.67</td>
<td>59.53±2.65</td>
<td>12.00±0.54</td>
</tr>
<tr>
<td>Group-II Triton control</td>
<td></td>
<td>137.53±2.77##</td>
<td>111.51±3.09##</td>
<td>22.30±0.62##</td>
</tr>
<tr>
<td>Group-III Triton + CMFP Chloroform extract 250 mg/kg</td>
<td></td>
<td>112.90±2.869**</td>
<td>92.48±2.02**</td>
<td>18.50±0.41**</td>
</tr>
<tr>
<td>Group-IV Triton + CMFP Chloroform extract 500 mg/kg</td>
<td></td>
<td>95.98±8.80**</td>
<td>81.85±3.88**</td>
<td>16.37±0.78**</td>
</tr>
<tr>
<td>Group-V Triton + CMFP Methanolic extract 250 mg/kg</td>
<td></td>
<td>75.38±3.57**</td>
<td>76.60±2.28**</td>
<td>15.32±0.46**</td>
</tr>
<tr>
<td>Group-VI Triton + CMFP Methanolic extract 500 mg/kg</td>
<td></td>
<td>60.13±2.84**</td>
<td>68.63±2.83**</td>
<td>13.73±0.57**</td>
</tr>
<tr>
<td>Group-VII Triton + CMFP Aqueous extract 250 mg/kg</td>
<td></td>
<td>81.25±2.59**</td>
<td>86.68±2.66**</td>
<td>17.33±0.53**</td>
</tr>
<tr>
<td>Group-VIII Triton + CMFP Aqueous extract 500 mg/kg</td>
<td></td>
<td>66.37±2.14**</td>
<td>70.45±2.20**</td>
<td>14.09±0.44**</td>
</tr>
<tr>
<td>Group-IX Triton + Atorvastatin 10 mg/kg</td>
<td></td>
<td>58.17±3.06**</td>
<td>61.92±1.38**</td>
<td>12.38±0.28**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M (n=6); #P<0.01 when compared with Group I (Normal control); **P<0.01 when compared with group II (Triton control).
Table 3. Effect of CMFP various extract on LDL-C, HDL-C level and Atherogenic index in serum of control and experimental Rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>Atherogenic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I Normal control</td>
<td></td>
<td>21.85±0.63</td>
<td>20.24±2.41</td>
<td>2.47±0.16</td>
</tr>
<tr>
<td>Group-II Triton control</td>
<td></td>
<td>17.27±0.56##</td>
<td>86.02±2.383##</td>
<td>8.01±0.35##</td>
</tr>
<tr>
<td>Group-III Triton + CMFP Chloroform extract 250 mg/kg</td>
<td></td>
<td>22.18±1.13**</td>
<td>72.19±3.292**</td>
<td>5.16±0.35**</td>
</tr>
<tr>
<td>Group-IV Triton + CMFP Chloroform extract 500 mg/kg</td>
<td></td>
<td>23.22±1.08**</td>
<td>56.55±8.31**</td>
<td>4.183±0.43**</td>
</tr>
<tr>
<td>Group-V Triton + CMFP Methanolic extract 250 mg/kg</td>
<td></td>
<td>25.35±0.650**</td>
<td>34.71±3.79**</td>
<td>2.99±0.22**</td>
</tr>
<tr>
<td>Group-VI Triton + CMFP Methanolic extract 500 mg/kg</td>
<td></td>
<td>25.57±1.23**</td>
<td>20.84±1.35**</td>
<td>2.35±0.05**</td>
</tr>
<tr>
<td>Group-VII Triton + CMFP Aqueous extract 250 mg/kg</td>
<td></td>
<td>25.02±0.61**</td>
<td>39.16±1.85**</td>
<td>3.27±0.08**</td>
</tr>
<tr>
<td>Group-VIII Triton + CMFP Aqueous extract 500 mg/kg</td>
<td></td>
<td>25.35±1.02**</td>
<td>26.91±1.03**</td>
<td>2.62±0.07**</td>
</tr>
<tr>
<td>Group-IX Triton + Atorvastatin 10 mg/kg</td>
<td></td>
<td>26.70±0.77**</td>
<td>19.08±2.19**</td>
<td>2.17±0.06**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M (n=6); ##P< 0.01 when compared with Group I (Normal control); **P<0.01 when compared with group II (Triton control); ns P>0.05, non significant when compared with group II (Triton control).