Effect of crude extract of *Grewia asiatica* L. on lipid profile and coagulation parameters of rats and mice

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The present study was aimed to investigate the effect of aqueous methanol extract of *Grewia asiatica* L. (500 mg/kg; p.o for 28 days) on lipid profile and coagulation parameters of Sprague Dawley rats and Swiss Albino mice. Lipid profile (triglyceride, cholesterol, high density lipoprotein, low density lipoprotein and very low density lipoprotein in mg/dL) and coagulation parameters (thrombin, prothrombin, activated partial thromboplastin and fibrinogen time in s) were studied on 29th day at the completion of treatment period using standard kits. Aqueous methanol extract at the dose of 500 mg/kg showed significant decrease in serum cholesterol, triglyceride and low density lipoprotein level in both rats and mice as compared to control and hyperlipidemic group. Moreover, there was a significant increase in high density lipoprotein, thrombin, prothrombin, activated partial thromboplastin and fibrinogen time both in rats as well as mice. There was no mortality and sign of toxicity observed in the extract treated animals during the study.

**Keywords:** *Grewia asiatica* L., Lipid profile, Coagulation parameters.

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The recent few decades have witnessed a renewed interest in the search of new therapeutic agents derived from natural source due to their low cost and minimum side effects. Indigenous plants have been extensively used to treat various cardiovascular disorders for over a millennium in developing countries, the reason of this could be attributed to lack of modern health facilities and rising cost of synthetic agents. Despite the great effectiveness of medicinal plants, there are few reports describing pharmacological effects and their underlying mechanism of action. Therefore, pharmacological validation of medicinal plants could greatly benefit in the development of new therapeutic agents. *Grewia asiatica* L. is a tree of plain areas bearing fruit in summer. Locally the bark of this plant is used to treat trouble and burning in vagina and its fruit is used for its cooling and digestible effect. It cures inflammation, heart and blood disorders and trouble of throat. Its leaves are reported to have good antibacterial, antiviral, and antifungal activity and fruit has radio protective effect on Swiss albino mice against radiation induced hematological and biochemical alterations.

Many studies have confirmed the relation between hypertension, hypercholesterolemia and ischemic heart diseases. Hypercholesterolemia leads to endothelial dysfunction, which is an early stage of atherogenesis. There is a strong association between serum cholesterol and risks of Coronary heart disease (CHD). Large epidemiologic studies have demonstrated that subjects with hypertension have a marked increase in the prevalence of hypercholesterolemia, diabetes, hypomagnesemia, hypertriglyceridemia. Lipids are major cell membrane components essential for various biological functions including cell growth and division of normal and malignant tissues. They are homogeneous group of compounds related more by physical than chemical properties. Lipoprotein disorders or hyperlipidemia may result from a primary abnormality in lipid metabolism or is a secondary manifestation of some other condition. Saturated fats have a profound hypercholesterolemic effect, being a major risk factor for atherosclerosis and related occlusive vascular diseases. The normal level of blood cholesterol in human beings is 150 - 250 mg/dL but when its level rises to more than 250 mg/dL, it may lead to dangerous consequences like cardiovascular diseases. Increased serum cholesterol levels can be induced when animal fats (saturated fats) and food high in cholesterol are consumed.

Coagulation system has been intended as a possible mechanism of thrombogenesis and atherosclerosis in

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patients with hyperlipidemia. While hyperlipidemia is a risk factor for ischemic heart disease, suggesting that hypercoagulability may perform a role in patients with hyperlipidemia.

The present investigation was undertaken to examine the effect of aqueous methanol extract of *Grewia asiatica* on lipid profile and blood coagulation parameters of rats and mice.

**Material and methods**

**Animal selection**

The present study was carried out on male Sprague-Dawley rats weighing 200 – 250 gm and male Swiss albino mice weighing 30 – 35 gm. The animals were housed in stainless cages under standard laboratory conditions (light period: 8:00 am to 8:00 pm, 21 ± 2 °C, relative humidity 55 %). Animals were provided with balanced diet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee, University of Sargodha. In addition, experiments comply with the declarations of National Research Council.

**Plant material used**

The fruits of *Grewia asiatica* were collected from DI Khan district of Khyber Pakhtoon khwa Pakistan during the month of June, identified and authenticated by Botanist Dr Sarfaraz Khan Marwat, Assistant professor Botany at WENSUM College DI Khan. A voucher specimen has been deposited in the herbarium, Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan for future reference, catalogued under number GA-200E5.

**Preparation of extract**

The fresh fruit of *Grewia asiatica* was macerated in aqueous methanol solution (30:70) and agitated daily for 72 hrs. The mash was filtered through muslin cloth and Whatman filter paper and concentrated in a rotating evaporator under reduced pressure and temperature.

**Experimental design**

Rats and mice were divided into three groups of seven animals in each group. Group I served as control group while animals in group II considered as hyperlipidemic control and received 25 % of fructose in drinking water for 28 days. Group III (treatment group) received the crude extract of *Grewia asiatica* in dose of 500 mg/kg; p.o of body weight for a period of 28 days, while animals of control group were administered normal saline through same route equivalent to the volume of respective doses according to their body weight. Body weights of the animals were measured weekly.

**Estimation of lipid profile**

After 28 days of treatment blood sample of about 5 mL were collected in gel tube. Serum was immediately separated out by centrifugation at 3000 rpm for 15 min in 14K Humax centrifuge. Lipid profile were analyzed on Humalyzer 3000 (semi-automatic chemistry analyzer, Model # 16700) (Human Germany) using standard kits supplied by Human. Cholesterol and low density lipoprotein cholesterol (LDL-C) was estimated by CHOD-PAP method; triglyceride by GPO-PAP methods, and high density lipoprotein cholesterol (HDL-C) following the method of Friedewald et al.

**Estimation of coagulation parameters**

Blood samples of about 3 mL were collected in coagulation tubes containing 3.2 % sodium citrate. Plasma was separated by centrifugation at 3000 rpm for 15 min in 14 K Humax centrifuge. Thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen time (FT) were measured by Humaclot duo, using standard reagent kits supplied by Human.

**Gross toxicities**

The gross toxicities were observed on weekly basis during administration of aqueous methanol extract obtained from fruits of *Grewia asiatica* for a period of 28 days.

**Mortality rate**

Mortality rates were observed in animals treated with crude extract of *Grewia asiatica* during the total period of experiment. The number of animals died was also noted.

**Results**

**Estimation of lipid profile**

To evaluate hypolipidemic potential, aqueous methanol extract obtained from fruits of *Grewia asiatica* was administered in the dose of 500 mg/kg to rats and mice for 28 days. Results showed that crude extract significantly reduced the triglyceride, cholesterol and low density lipoprotein, while level of very low density lipoprotein was not changed significantly. In addition, significant increase in level of high density lipoprotein as compare to control
group and hyperlipidemic group was also observed. As shown in Figs. 1 & 2 Grewia asiatica reduced level of triglyceride by 51.7 ± 6.6 as compare to control group that is 96.5 ± 1.4 in rats and also in mice that is 86.5 ± 0.3 as compare to control that is 95 ± 1.2. Cholesterol level and low density lipoprotein is also profoundly reduced that is 88.6 ± 14.9, 90.5 ± 0.9, as compare to control group that is 151 ± 1.7, 90.5 ± 0.9, in rats and also in mice that is 143 ± 1.2, 86.5 ± 0.3 as compare to control that is 152 ± 1.7, 92.5 ± 0.3, while the level of high density lipoprotein was increased significantly both in rats and mice that is 41.6 ± 3.1, 38 ± 0.6 as compare to control group that is 21 ± 0.6 and 36 ± 1.7. However minor decrease in level of VLDL was observed that was insignificant statistically.

**Coagulation profile**

The effect of aqueous methanol extract of Grewia asiatica in the dose of 500 mg/kg on coagulation parameters of rats and mice after 28 days showed that crude extract significantly increases the thrombin (TT), prothrombin (PT), activated partial thromboplastin (aPTT) and fibrinogen (FT) time as compare to control group (Figs. 3 & 4).

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**Fig. 1**—Effect of Grewia asiatica on lipid profile of rats after 28 days. where, HDL = high density lipoprotein, LDL = low density lipoprotein and VLDL = very low density lipoprotein, n = 7; Mean ± S.E.M; *** = P < 0.001 as compared to control.

**Fig. 2**—Effect of Grewia asiatica on lipid profile of mice after 28 days. where, HDL = high density lipoprotein, LDL = low density lipoprotein and VLDL = very low density lipoprotein, n = 7; Mean ± S.E.M; *** = P < 0.001 as compared to control.

**Fig. 3**—Effect of Grewia asiatica on coagulation profile of rats after 28 days. Where TT = thrombin time, PT = prothrombin time, aPTT = activated partial thromboplastin time and FT = fibrinogen time; n = 7; Mean ± SEM; *** = P < 0.001 as compared to control.

**Fig. 4**—Effect of Grewia asiatica on coagulation profile of mice after 28 days. Where TT = thrombin time, PT = prothrombin time, aPTT = activated partial thromboplastin time and FT = fibrinogen time; n = 7; Mean ± SEM; *** = P < 0.001 as compared to control.
Gross toxicities
No gross toxicities were observed in any group of animals during the total period of experiment.

Mortality rate
Result of present study revealed that no death was observed in the animals of control group and treated groups.

Discussion
In recent years, an increasing percentage of people from industrialized countries have been using complementary and alternative medicines (CAM). Herbal medicine is used by up to 80% of the population in the developing countries\textsuperscript{12}. Total lipid profile of an individual is a contributive principle resulting from blood cholesterol along with its associated varieties of lipoproteins, i.e., high-density lipoproteins (HDL, or α-lipoproteins), low-density lipoproteins (LDL, or β-lipoproteins), very-low density lipoproteins (VLDL, or pre-β-lipoproteins) and triglycerides. Disposition of blood pressure and coronary heart disease has been found to be in strong correlation with lipid profile particularly with blood cholesterol level and also with coagulation parameters. Previously, it has been reported that fructose fed hyperlipidemia has been associated with increased availability of precursor molecule glycerol-3-phosphate and excess free fatty acids while the diminished activity of LPL results in increased content and impaired clearance from circulation. Fructose also causes increase in cholesterol synthesis that is in line with a previous findings, addition of fructose to cultured rat hepatocytes increase HMG-CoA reductase activity 3 folds\textsuperscript{13,14}. Natural product medicines from plant sources of wide diversity have long been used effectively in the treatment of blood pressure and hyperlipidemia\textsuperscript{15}. There is a strong correlation between lipid profile and coagulation parameters, so the alterations in lipid levels influence thrombosis. This modification is due to changing the activity of coagulation proteins, platelets and fibrinolytic factors\textsuperscript{16}. Results showed the effect of aqueous methanol extract at the dose of 500 mg/kg on lipid profile of rats and mice. The result showed that the extract significantly reduced serum cholesterol, triglycerides and LDL-C. High circulating serum cholesterol, low density lipoprotein-cholesterol (LDL-C) and serum triglycerides are major risk factors of cardiovascular diseases. The modification of lipid profile may be important both in the prevention and control of coronary heart disease. Alterations in the lipid profile have also been associated with age\textsuperscript{17}. The lowering of LDL-C of the rats and mice may be due to the presence of flavonoids in the extract which prevent the oxidation of LDL-C which is atherogenic. It may be possible that lipid lowering activity of the crude extract of the fresh fruit may be due to the presence of alkaloids, which are said to be responsible for lowering hyperlipidemia. Furthermore the cholesterol lowering activity of the crude extract may be due to the presence of saponins, which may be present in fresh fruit of plant which is a cholesterol lowering agent\textsuperscript{12}. The HDL was also increased which is one of the most important criteria of an anti-hypercholesterolemic agent. Previous studies show that high level of HDL is associated with a lower incidence of cardiovascular disease. The increase in HDL level might be due to stimulation of pre HDL and reverse cholesterol transport. So, it is suggested that lipid lowering effect of crude extract may be due to combination of the enriched phenolic contents in the extract and enhanced bio synthesis of hepatic HDL resulting in increased plasma HDL concentration\textsuperscript{18}. The process of blood coagulation is an important chemical reaction in the body in response to vascular injuries thrombosis and CVD.

The result shows the effect of crude extract of \textit{Grewia asiatica} on TT, PT, aPTT, and fibrinogen on rats and mice. This study indicated the increase in TT which might be linked with the deficiency of fibrinogen or inhibition of thrombin. This may be due to reduced activity of coagulation factors like IX, X, XI, and XII which are essential for thrombin generation\textsuperscript{14}. Present study also showed that the crude extract of \textit{Grewia asiatica} significantly increased the fibrinogen time, it means that there was decrease in fibrinogen level, which is increased in vascular diseases; thus crude extract could be helpful in combating the risk of vascular diseases by decreasing the fibrinogen level. There was also decrease in PT, so it is possible that this decrease in PT may be due to increase production of coagulation factors like V, VII & X\textsuperscript{11}. The results of present investigation were in line with the treatment of lipid lowering drugs like statins. Which stabilizes atheromatous plaque and has antithrombotic effects\textsuperscript{14}, so it is possible that crude extract of \textit{Grewia asiatica} L. also produce these effects in the similar manner.
Conclusion
From these investigations, it might be concluded that aqueous methanolic extracts of *Grewia asiatica* L. in the dose of 500 mg/kg exhibited hypolipidemic effects. It justifies the traditional use of this plant in the treatment of various types of cardiovascular diseases. However, further studies are required to isolate the compounds from the potent extract and to elucidate the exact mechanism of action.

References