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Original article

Onosma hispidum L. extract reverses hyperlipidemia, hypertension, and associated vascular dysfunction in rats

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ABSTRACT

Onosma hispidum.L (*O. hispidum*) belongs to the family *Boregineacea*. A preliminary study and its medicinal use suggested its role in the management of hyperlipidemia. The present study aimed to assess the effect of methanolic root extract of *O. hispidum* in hyperlipidemia and associated vascular dysfunction. Oral administration of *O. hispidum* crude extract (Oh. Cr) to tyloxopol and high fat diet-induced hyperlipidemic Sprague-Dawley rats for 10 and 28 days significantly reduced total triglycerides and cholesterol ($p < 0.001$), compared to hyperlipidemic rats. Oh. Cr 250 mg/kg orally treated rats significantly ($p < 0.001$) reduced both the total body weight and atherogenic index in tylaxopol and HFD rats. In HMG-CoA assay, the inhibition of the enzyme was significant in Oh.Cr (250 mg/kg) treated group. Histopathological studies indicated that the group treated with Oh.Cr 250 mg/kg/day showed regular morphology of aortic intima, media and adventitia, and improved the endothelial damage. To investigate the vascular dysfunction, isolated rat aorta rings from all groups were pre-contracted with 1 μM phenylephrine (PE), and the effect of acetylcholine (Ach) was monitored. In the aorta isolated from Oh.Cr (50 mg/kg) treated group, Ach completely relaxed the PE-induced contraction with EC_{50} value of 0.05 μg/mL 0.015 (0.01–0.2) compared to the hyperlipidemic control group (<30% relaxation). In atorvastatin (10 mg/kg) treated rat aorta, Ach showed 50% relaxation. The Oh.Cr extract also reduced (105.92 ± 1.14 to 66.63 ± 0.85 mmHg) mean arterial pressure in hyperlipidemic hypertensive rats. These findings suggest that extract of *O. hispidum* is an effective remedy for hypercholesterolemia, and hypertriglyceridemia, which acts through inhibition of HMG-CoA and improving vascular dysfunction.

1. Introduction

Cardiovascular diseases are a major problem worldwide, and foremost cause of mortality. Hyperlipidemia is categorized into high serum total cholesterol (TC), Low-density lipoprotein cholesterol (LDL-c) and decreased high-density lipoprotein cholesterol (HDL-c) levels (Belguith-Hadriche et al., 2016; Hill and Bordoni, 2022), and is associated with an increased risk of hypertension (O'Keefe and Bell, 2007). All lipoproteins carry cholesterol, however, elevated levels of lipoproteins other than HDL, specifically LDL-cholesterol, is associated with increased risk of atherosclerosis and coronary heart disease (Chesebro et al., 1992). An estimated 16% of the normal population and 68% of the obese population of South Asians suffer from hyperlipidemia (Misra and Khurana, 2011). This Prevalence in Pakistan is 39.3% (Basit et al., 2020). The normal functions of the endothelium are impaired in hyperlipidemia and hypertension (Drexler and Hornig, 1999; Felmeden et al., 2003) as a result of shear and oxidative stresses. This leads to increase in the formation of collagen and fibronectin, resulting in less production of nitric oxide and more permeability to lipoproteins. The decrease in nitric oxide, in addition to increased vasoconstriction, results in hypertension associated with hyperlipidemia (Mason and Jacob, 2003; Ross, 1999). One of the main causes of high cholesterol levels in the blood is due to the increasing number of activities in the mevalonate pathway which is controlled by the HMG-CoA reductase known as 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (Nakamura et al., 1999).

Onosma hispidum L. (*O. hispidum)* belongs to the family *Boreginaceae,* locally recognized as Ratanjot and Sra zaila (Kumar et al.,

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2010). *O. hispidum* is traditionally used in the management of hypertension and various blood diseases (Ahmad et al., 2015), and has been reported as an antioxidant (Naz et al., 2006), anti-inflammatory, and inhibitor of lipoxygenase (Kumar and Gupta, 2010). A preliminary study was conducted on *O. hispidum* on diabetes and showed that extract also has a hypolipidemic effect in rabbits without knowing the underlying mechanisms (Hussain et al., 2016). In addition, *O. hispidum* is traditionally used in the management of cardiovascular disorders (Ahmad et al., 2015). These initial findings on *O. hispidum* led to pharmacologically evaluate the antihyperlipidemic and antihypertensive potential of methanolic crude extract and fractions prepared from the roots of *O. hispidum*, in rats.

2. Research methodology

2.1. Plant material

O. hispidum roots were procured from the market and identified by taxonomist Dr. Abdul Nazeer, Associate Professor, Department of Environmental Sciences, COMSATS University Islamabad, CUI, Abbottabad campus.

2.2. Preparation of crude extract of O. Hispidum

O. hispidum roots were shade-dried and macerated in methanol at room temperature three times for 21, 7, and 3 days respectively. Whatmann filter paper number 42 with a pour size of 2 mm and muslin cloth were used to filter the extracted material. Using a rotary evaporator, the filtrate was concentrated at 35–40 °C under reduced pressure of −220 mm Hg (Williamson et al., 1996).

2.3. Fractionation of crude extract

Crude extract from the roots of *O. hispidum* was subjected to fractionation using the solvent–solvent extraction technique. The specific quantity of extract was suspended in distilled water and organic solvents in ascending order of polarity (*n*-hexane, ethyl acetate, and chloroform), were used for the preparation of different fractionations. These fractions were concentrated using a rotary evaporator, as described above. The percent yield (w/w) of each fraction was calculated.

2.4. Phytochemical analysis

Different chemical reagents were used to ensure the presence of different chemical constituents in crude extracts such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, and saponins (Edeoga et al., 2005; Kumar et al., 2013b).

2.5. Drugs and standards

All chemicals such as acetylcholine chloride, potassium chloride, phenylephrine hydrochloride, dimethyl sulfoxide (DMSO), Tween 80, cholesterol, and cholic acid were among the medications and standards purchased from the vendor specified (Sigma Chemical Company, St. Louis, MO). The purest grade of all the chemicals were used. On the day of experiment, physiological salt solutions, stock solutions of all the drugs, and extracts were freshly prepared in the appropriate solvent.

2.6. Experimental animals

The Ethical Committee of the Department of Pharmacy, CUI, Abbottabad Campus, approved the studies following the guidelines and standards of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 2011). Sprague-Dawley (SD) rats of both sex, male and female (200–250 g), were housed and kept at a temperature of 23–25 °C, having free access to food and water in the animal house of the Department of Pharmacy, CUI, Abbottabad.

2.7. High-fat diet (HFD) induced hyperlipidemic model

HFD consisting of butterfat, cholesterol, and cholic acid (5%, 2%, 0.5% w/w) respectively, was added to the normal diet consisting of fish oil (100 mL), wheat and oat flour (5, 4 kg), skimmed milk (500 g), yeast, and multivitamin (100 g) each a total of 10 kg of food material (Buettner et al., 2006).

2.8. Pharmacological investigations

2.8.1. Toxicity study

Different groups of animals (mice) have 5 in each group. The test was carried out by using different doses of *O. hispidum* extract in increasing order, given p.o. Normal saline was administered to another group of mice. The mice were allowed free access to water, and food for 24 hr, and kept under consistent observation for toxic effects such as anorexia, diarrhea, gastrointestinal spasms, lethargy, and also for mortality (Chinedu et al., 2013).

2.9. Assessment of antihyperlipidemic activities

2.9.1. Study on the tyloxapol-induced hyperlipidemia model

SD rats weighing 185–225 g were placed in constant hygienic conditions and fed on a normal diet. Total 6 different groups, having 5 rats each were made (Khanna et al., 2002). Group 1: normal control group. Group 2: hyperlipidemic (untreated). Group 3: hyperlipidemic group + atorvastatin treated (10 mg/kg). Group 4, 5, 6: extract treated groups (normal diet + Oh.Cr (50, 150, and 250 mg/kg p.o). After giving treatment for 10 days, all animals fasted for 12 hr, group 1 received 10 mL/kg (i.p) saline, and groups 2 to 6 were given 500 mg/kg (i.p) tyloxapol. After 24 hr of administration of tyloxapol, rats were anesthetized, and blood was collected via cardiac puncture for the analysis of the complete lipid profile. The liver was collected for HMG-CoA reductase enzyme activity. The rat aorta was dissected from hyperlipidemic and treated groups for the evaluation of vascular dysfunction *in-vitro*.

2.9.2. Study on HFD-induced hyperlipidemia model

Hyperlipidemia was induced using HFD as described previously by Berrougui et al. (2003), with slight modifications. The SD rats (185–225 g) were divided into different groups, each group contains 5 rats. Group 1: normal control. Group 2: HFD (untreated group). Group 3: HFD + atorvastatin (10 mg/kg). Group 4–6: HFD + Oh.Cr (50, 150, and 250 mg/kg p.o).

All rats of different groups were free to access water and food. The consumption of food was observed daily and the increase in weight of the body was notated weekly. After 4 weeks of treatment, rats were fasted for 16 hr and were anesthetized with sodium thiopental 60–90 mg/kg, and blood was obtained via cardiac puncture. Blood serum was investigated for lipid profile.

2.10. Biochemical study

2.10.1. Estimation of a lipid profile

For the calculation of serum TC, HDL, LDL, standard, and blank were taken. These reaction mixtures were incubated at 21–25 °C for 10 min. 0.2 mL from each mixture was transferred into 96 well plates after incubation and the absorbance of each mixture was evaluated against the blank reagent on a microplate reader at

490 nm (Friedewald et al., 1972; Haslam et al., 2020). The atherogenic index was calculated by using the formula described by Friedewald et al. (1972).

$$\text{Atherogenic index} = \text{TC-HDL/HDL} \quad (1)$$

2.10.2. Liver homogenate preparation method

Generally, the tissue homogenate was prepared by the isolation of the liver from the rat. The isolated liver was blotted with chilled saline and weighed. For this assay, 1 g of the liver was taken from the fresh liver of the rat, crushed down into pieces, and added into a 9 mL saline arsenate solution. The next step was the preparation of liver homogenate by using a homogenizer. After the preparation of homogenate, the assay of HMG-CoA was carried out (Rao and Ramakrishnan, 1975; Díaz-Zagoya et al., 2021).

2.10.3. Analysis of HMG-CoA reductase activity via liver homogenate

The functioning of HMG-CoA reductase was determined through an indirect method as described by Rao and Ramakrishnan (1975) . About 1 g of freshly prepared liver tissue homogenate in 9 mL was taken and mixed with 10 mL of 5% perchloric acid and was kept for some time (5–7 min) at 25 °C. The mixture was centrifuged for 10 min through the centrifuge machine at 2000 rpm. To determine the mevalonate activity Solution A freshly prepared 0.5 mL of 1 M aqueous hydroxylamine hydrochloride was added in 1 mL supernatant which was obtained during centrifugation. Then for the estimation of the HMG-CoA Solution B**,** alkaline hydroxylamine hydrochloride 0.5 mL was also added in another tube having 1 mL supernatant of the same rat. The mixture of both tubes was incubated for 5 min at 37 °C and then 1.5 mL of 0.06 M ferric chloride reagent was added to each tube and shaken vigorously. Then after 10 min of incubation, absorbance was read at 450 nm at kinetic mood through IRMECO UV–Vis spectrophotometer model U2020 against a reagent blank and then against each sample.

Enzyme inhibition was calculated by the following formula.

$$\text{Solution B (HMG-CoA)} \div \text{Solution A (mevalonate activity)} = \text{Enzyme inhibition (Iqbal, 2015)} \quad (2)$$

2.10.4. Histopathological examination of hyperlipidemic (HFD) SD rats

Thoracic aortae from all groups were studied for histopathological changes. The thoracic aortae were quickly dissected out and flushed immediately with saline. It was fixed in 10% formalin. Then fixed tissues were inserted in paraffin. The tissues were segmented into 5 μm slices via a rotary microtome. Then these segments were stained with hematoxylin and eosin dye. After fixation, the tissue was examined under a light microscope for tunica intima, tunica media, tunica adventitia, macrophages, vacuolation, and lipid deposition (Mohanta et al., 2016).

2.11. In vitro experimental study

2.11.1. Measurement of endothelium-dependent, and independent effects in rat aorta of hyperlipidemia-induced hypertensive and normotensive rats

Rats were sacrificed by cervical dislocation. The descendant aortae were cut out and shifted instantly into Krebs–Henseleit solution, The composition (mM) of Kreb's solution was NaCl 118.2, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.3, $C_6H_{12}O_6$ 11.7, $NaHCO_3$ 25.0 and $CaCl_2$ 2.5 (pH 7.4), extra tissues were removed from the aortae, and rings of 1–3 mm was made. Each ring was hung in an organ bath which was filled with Krebs–Henseleit solution, aerated with 95% O_2 and 5% CO_2 (carbogen), and coupled to a force transducer attached to a power lab data acquisition system. A resting tension of 2 g was applied to each ring and equilibrated for 60–90 min, with a change of Krebs–Henseleit solution every 15–20 min. Phenylephrine (1 μM) was administered to induce contraction and (1 μM) acetylcholine was added to check endothelium integrity by plotting inhibitory concentration–response curves (CRCs) as described by Shah & Gilani, (2009).

2.11.2. Blood pressure (BP) assessment in anesthetized hyperlipidemia-induced hypertensive (HLIH) and normotensive rats

This study was carried out on both (HLIH) and normotensive rats. Under anesthesia the right carotid artery was cannulated with a PE-50 polyethylene tube, which was linked to a pressure transducer attached to a bridge amplifier, and a power lab data acquisition system, this system was used for blood pressure recording. Similarly, the left side jugular vein was also cannulated for injection of different doses of plant extract and drugs. The temperature of these animals was sustained at 37 °C using an overhead lamp. After 20 min of stability, 0.1 mL saline or an equal volume of the test substance was injected intravenously. Arterial BP was permitted to return to a steady state between injections. Extract of *O. hispidum* was given through intravenous injections, followed by a flush of 0.1 mL saline. Control responses of norepinephrine and acetylcholine (1 μg/kg), each as a standard was acquired before testing the extract, systolic and diastolic pressure were individually calculated, and mean arterial pressure (MAP) was determined (Shah and Gilani, 2011).

$$\text{MAP} = \text{SBP} + 2(\text{DBP})/3 \quad (3)$$

3. Results

3.1. Crude extract

Root parts of *O. hispidum* were dried and homogenized into powder. The dried powder *O. hispidum* (17 kg)) was macerated in

Table 1
Analysis of phytochemicals of *O. hispidum* crude extract.

S/No	Phytochemical constituents	Results
1	Alkaloids	+++
2	Flavonoids	+++
3	Glycosides	+++
4	Phenols	+++
5	Saponins	++
6	Terpenoids	+
7	Tannins	+

+ = Mild, **++** = Moderate, and **+++** = Strong presence of phytochemicals based on intensity of color.

methanol (25 L) for 21, 7, and 3 days, respectively. The yield of crude extract of *O. hispidum* was 2.8% (w/w).

3.2. Fractionation of crude extract

Using solvents in a separating funnel, the crude extract of the plant was fractionated following previously described techniques (Williamson et al., 1996). The yield of the Oh.*n*-hexane (*n*-hexane fraction) was 51.4% (w/w). (Oh. Chlor) with an around 34.8% (w/w) yield. (Oh.EtAc) produced a yield of about 3.1% (w/w). The residual layer, which had a yield of 10.01% (w/w), was classified as aqueous (Oh.Aq).

3.3. ***Phytochemical screening of*** *O. Hispidum*

3.3.1. Preliminary screening

O. hispidum indicated the presence of flavonoids, alkaloids, phenols, glycosides, saponins, terpenoids, and tannins as shown in Table.1.

3.3.2. Total phenolic content

Oh.EtAc fraction represented the highest content. The order of phenolic content was crude, chloroform, n-hexane, and aqueous fraction respectively as shown in Table.2.

3.3.3. Total flavonoid content

The order of flavonoid was crude extract, then ethyl acetate fraction, chloroform, *n*-hexane, and aqueous fraction as shown in Table.2.

3.4. Antihyperlipidemic activities

3.4.1. Serum lipid profile of hyperlipidemic tyloxapol-induced SD rat

In all groups such as normal control, atorvastatin 10 mg/kg group tyloxapol-induced hyperlipidemic group different groups of crude extract 50, 150, and 250 mg/kg, the lipid profile of blood were calculated as shown in Table.3.

3.4.2. Serum lipid profile of HFD-hyperlipidemic SD rat

After treatment with HFD, all groups except normal control, with different doses of crude extract, lipid profile was calculated, as shown in Table.4.

3.4.3. Body weight profile of HFD animals

HFD fed for 4 weeks caused a significant ($p < 0.001$) increase in body weight in the hyperlipidemic control group compared to normal SD rats. The treatment of hyperlipidemic rats with Oh.Cr at the dose of 150 mg/kg caused an approximate 60% decrease in body weight while atorvastatin treatment caused a 64% decrease in body weight. Oh.Cr at the dose of 250 mg/kg markedly reduced the body weight (81%) compared to normal rats (Fig. 1).

Table 2
Total phenolic and flavonoid content of crude extracts.

S/No.	Extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
1.	Crude (*Onosma hispidum*)	64.07 ± 3.2	56.91 ± 1.4
2.	*n*-Hexane (Oh.*n*-Hexane)	62.70 ± 0.4	49.19 ± 1.2
3.	Chloroform (Oh.Chl)	36.85 ± 0.9	27.96 ± 2.1
4.	Ethyl acetate (Oh.EtAc)	126.29 ± 0.3	56.03 ± 0.9
5.	Aqueous (Oh.Aq)	28.37 ± 2.4	21.75 ± 2.6

mg GAE/g: milligram gallic acid equivalent per gram.
mg QCE/g: milligram quercetin equivalent per gram.
Values tabulated as mean ± SEM (n = 3).

3.4.4. HMG-CoA reductase activity in the tyloxapol model of hyperlipidemia

For the determination of HMG-CoA reductase activity in the tyloxapol model of hyperlipidemia all groups received normal diet and water for 10–12 days. The liver was isolated from these.

groups after 18 hr of tyloxapol injection i.p. An assay of HMG-CoA reductase was.

performed which shows the absorbance of HMG-CoA/mevalonate compared with the tyloxapol control group (Table.5).

3.4.5. HMG-CoA reductase activity in the HFD model of hyperlipidemia

An assay of HMG-CoA reductase was performed which shows the absorbance of HMG-CoA/mevalonate as compared with the HFD control group (Table.5).

3.5. Histopathological examination of aorta from HFD hyperlipidemic SD rats

Histopathological studies of the aorta of normal control rats showed the normal structure of three tunics of the aorta; tunica intima, tunica media, and tunica adventitia. Endothelial cells round in shape were present in tunica intima. The endothelium was smooth and regular. Tunica media comprised of smooth muscle cells and elastic lamina. Tunica adventitia is comprised of connective tissues. The thickness of the aorta was normal (Fig. 2A). The aorta from the HFD-induced hyperlipidemic group showed focal discontinuity of tunica intima with infiltration of macrophages, vacuolation in tunica media associated with a large number of foam cells and increase in the matrix (Fig. 2B), while atorvastatin treated rats showed less damage of endothelium and reduced spaces between tunica media and the tunica intima with no macrophages infiltration and reduced lipid deposits (Fig. 2F). Oh. Cr 50 mg/kg orally treated rats showed disruption of the endothelium in the tunica intima and proliferation of smooth muscle cells in the tunica media while lipid accumulation was reduced (Fig. 2C). Aorta from hyperlipidemic rats treated with Oh.Cr 150 mg/kg showed mild disruption of the endothelium in the tunica intima and improvement in the proliferation of smooth muscle cells with reduced lipid accumulation (Fig. 2D). Similarly, a photomicrograph of the aorta from hyperlipidemic rats treated with Oh.Cr 250 mg/kg showed almost regular morphology of the aortic intima, media, and adventitia (Fig. 2E).

3.6. Vascular function study of hyperlipidemic (HFD) SD rats

Isolated rat aorta of the normal control group pre-contracted with phenylephrine (1 μM) showed relaxation to Ach with EC_{50} value 0.08 μg/mL (0.03–0.13). While aortic preparations from HFD hyperlipidemic rats showed < 30% relaxant response to Ach. Atorvastatin-treated rats showed 50% relaxation to Ach with an EC_{50} value of 0.42 μg/mL (0.3 0.54). Aortic ring preparations from 50, 150, and 250 mg/kg (p.o.) treated rats showed significant relaxant response (<48 % 75 %, 100 %), and with their respective EC_{50} values μg/mL of 0.48 (0.3–0.66) and 0.015 (0.01–0.2) (Fig. 3).

3.7. Mean arterial pressure (MAP) in hyperlipidemic rats

A significant rise (92.9 ± 1.45 to 124.96 ± 1.55 mmHg) in MAP was observed in HFD-induced hyperlipidemia in rats. Rats were treated orally for 28 days with Oh. Cr caused a fall (105.92 ± 1.14 to 66.63 ± 0.85 mmHg) in MAP in a dose-dependent manner. Similarly, HFD-induced hyperlipidemic rats treated with atorvastatin causes a significant fall (71.63 ± 2.05 mmHg) in MAP when compared with HFD hypertensive (124.96 ± 1.55 mmHg) rats (Fig. 4).

Table 3
Effect of Oh.Cr at different doses (50,150 and 250 mg/kg) and atorvastatin (10 mg/kg) on serum lipid profile in tylaxapol-induced hyperlipidemic, and atherogenic index SD rats. Values are expressed as mean ± SEM (n = 3–5). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Tylaxapol-induced hyperlipidemic values.

Lipid profile tyloxapol-induced hyperlipidemic model (mg/dL)						
S/No	Groups	TC	TG	LDL	HDL	VLDL
1	Normal control	87 ± 50	82.3 ± 6.60	56.6 ± 4.10	25.3 ± 2.40	48 ± 1.60
2	Hyprlipidimic	274.3 ± 21.80	705.6 ± 11.60	86.6 ± 3.30	27.3 ± 1.60	152.66 ± 17.9
3	Atorvastatin	89.3 ± 180	114.6 ± 10.70	32 ± 2.40	52.3 ± 10.2	56.3 ± 2.80
4	Oh.Cr (50 mg/kg)	159.6 ± 10.50***	635.6 ± 29.10*	75 ± 26.70	29.3 ± 0.90	83 ± 3.20***
5	Oh.Cr (150 mg/kg)	121 ± 2.80***	306.6 ± 32.9***	75 ± 8.80	31 ± 1.60	50.3 ± 7.30***
6	Oh.Cr (250 mg/kg)	74.6 ± 3.80***	251.6 ± 34.7***	47 ± 9.80*	52 ± 10.7**	33.5 ± 3.30***

TC; total cholesterol, TG; triglyceride, HDL; high-density lipoproteins LDL; low-density lipoproteins VLDL; very low-density lipoproteins. Oh.Cr:*Onosma hispidum* crude extract.

Table 4
Effect of Oh.Cr at different doses (50,150, and 250 mg/kg) and atorvastatin (10 mg/kg) on serum lipid profile in HFD-induced hyperlipidemic SD rats. Atherogenic index. Values are expressed as mean ± SEM (n = 3–5). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs hyperlipidemic HFD values.

Lipid profile HFD-induced hyperlipidemic (mg/dL) and atherogenic index							
S/No	Groups	TC	TG	LDL	HDL	VLDL	AI
1	Normal Control	74.75 ± 14.24	76.5 ± 13.5	17.5 ± 1.5	23.7 ± 1.9	14 ± 2.2	2.15 ± 0.02
2	Hyprlipidimic	399.75 ± 30.8	447.5 ± 37.2	261.25 ± 4.2	21.2 ± 2.1	69.2 ± 2.4	13.6 ± 0.30
3	Atorvastatin	79 ± 4	58.5 ± 5.0	48.95 ± 4.5	29.7 ± 2.5	11.3 ± 1.4	1.67 ± 0.07
4	Oh.Cr (50 mg/kg)	104.2 ± 4.9***	88.7 ± 8.4***	48.25 ± 7.1***	23.2 ± 2.8	20.9 ± 2.7***	3.49 ± 0.06***
5	Oh.Cr (150 mg/kg)	91.5 ± 6.8***	74 ± 4.7***	42.25 ± 4.9***	28.5 ± 1.1**	14.2 ± 0.8***	2.21 ± 0.01***
6	Oh.Cr (250 mg/kg)	75.7 ± 3.3***	64 ± 9.2***	37.8 ± 17.1***	30.5 ± 2.6***	17.2 ± 4.40***	1.48 ± 0.04***

TC; total cholesterol, TG; triglyceride. LDL; low-density lipoproteins, HDL; high-density lipoproteins, VLDL; very low-density lipoproteins AI: Atherogenic index. Oh.Cr: *Onosma hispidum* crude extract.

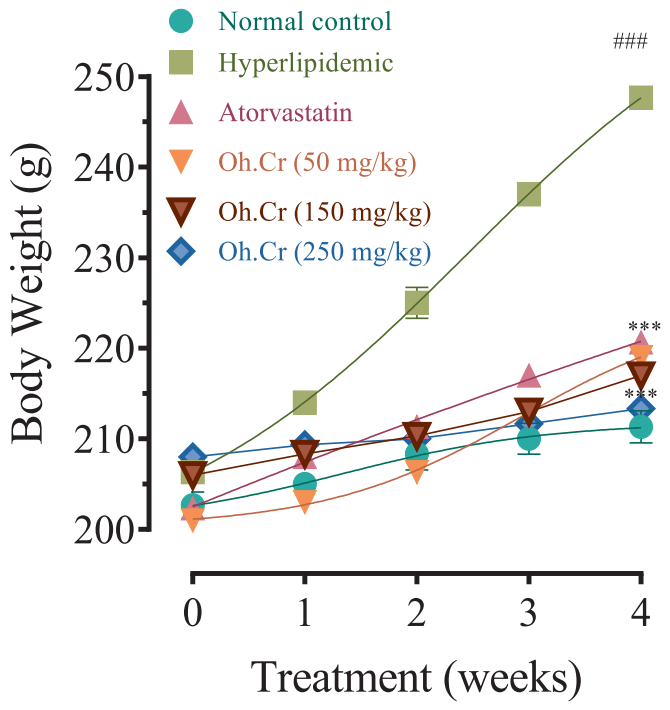

Fig. 1. Shows increase in the body weight treated for four weeks with *Onosma hispidum* crude extract (Oh.Cr) in HFD hyperlipidemic rats, compared with normal, hyperlipidemic and atorvastatin group. Value shows (mean ± SEM, n = 3–5).

3.8. MAP in normotensive rats

I.V. administration of crude extract of *O. hispidum* and its fractions in normal animals, the MAP was measured and calculated as shown in Fig. 5.

Table 5
Comparison of HMG-CoA reductase activity in tyloxapol model and HFDs model, in normal control, atorvastatin (10 mg/kg) treated, tyloxapol control, and Oh.Cr (50 mg, 150 mg, and 250 mg/kg) treated groups, Data was expressed with standard error of means (mean ± SEM) Significance value represents $^{*}p < 0.05$, $^{**}p < 0.01$ and$^{***}p < 0.001$.

S/No	Groups	Tyloxapol-induced	HFD-induced
		HMG CoA/Mevalonate ratio	
1	Normal Control	1.4046 ± 0.08	0.9603 ± 0.09
2	Hyperlipidemic	0.5242 ± 0.11	0.6312 ± 0.06
3	Atorvastatin	1.6535 ± 0.25	1.457912 ± 0.06
4	Oh.Cr (50 mg/kg)	1.1365 ± 0.13 **	1.0109 ± 0.30
5	Oh.Cr (150 mg/kg)	1.5040 ± 0.15 ***	1.4645 ± 0.07 **
6	Oh.Cr (250 mg/kg)	1.8485 ± 0.13 ***	2.1340 ± 0.49***

3.9. Acute oral toxicity study

The crude extract of *O. hispidum* was found safe up to a dose of 300 mg/kg. Following the ingestion of the extract for 3,8,12 and 24 hr, the toxicity sign was initially noted.

4. Discussion

Plants have been used tadionally to cure cardiovascular diseases in human population since ancient times (Ali et al., 2022). The anti-hyperlipidaemic activity of plants plays an important role in the reduction of CVD. Plant parts or plant extract are sometimes even more potent than known hypolipidemic drugs (Asija et al., 2016). The plants like *Garcinia cambogia* (Koshy et al., 2001), *Mangifera indica* (Anila and Vijayalakshmi, 2003), *Hypericum perforatum* L (Zou et al., 2005), and *Asparagus racemosus* (Visavadiya and Narasimhacharya, 2009) that contain flavonoids have been proven to significantly lower the risk of atherosclerosis and CVD (Salvamani et al., 2014). *Onosma hispidum* is traditionally used in the management of hypertension (Kumar et al., 2013a), while some preliminary studies showed that *O. hispidum* reduced the level of total cholesterol in rabbits (Hussain et al., 2017). Crude

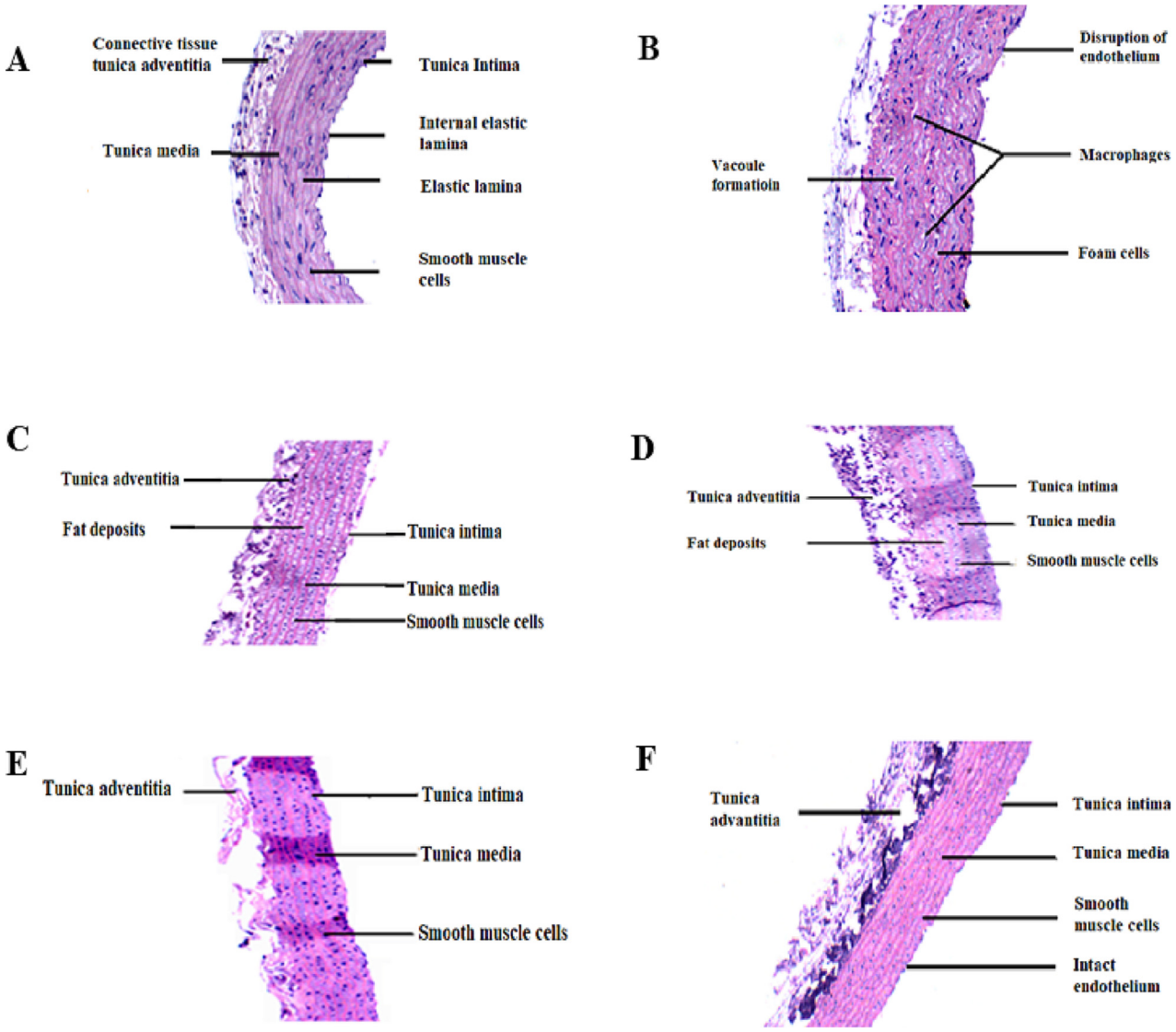

Fig. 2. Histopathological slides shows morphology of **(A)** Normal, **(B)** Hyperlipidemic (HFD), **(C)** Oh.Cr (50 mg/kg) **(D)** Oh.Cr (150 mg/kg) (E) Oh.Cr (250 mg/kg) (F) Atorvastatin (10 mg/kg) treated SD rats.

extract of *O. hispidum* was orally administered to tyloxapol-induced hyperlipidemic rats for 10 days. This treatment protected rats against hyperlipidemia, particularly hypercholesterolemia, and hypertriglyceridemia. The effect of the extract on other lipids such as LDL, VLDL, and HDL was not predictable. Tyloxapol is non-ionic detergent, (an oxyethylated tertiary octyl phenol formaldehyde polymer), is used by several studies to induce hypercholesterolemia in animals (Harnafi et al., 2008). Tyloxapol following intravenous or intra-peritoneal injection that causes the milky serum which last up to 48 hr (Rasouli et al., 2016) as this is the acute model and its effect is abolished after 48 hr so no accumulation of cholesterol was observed and having no significant effect on endothelium (Korolenko et al., 2010). After 48 hr this effect of tyloxapol is reversed that's why it is used as an acute model of hyperlipedimia It causes a significant increase in hepatic cholesterol biosynthesis by stimulating the activity of HMG-CoA reductase (Janicki and Aron, 1962; Bertges et al., 2011). Previously it have been shown that *Hibiscus rosa sinensis* root at the dose of 500 mg/kg/day (Kumar et al., 2009), *Elaeis guineensis* (250 and 500 mg/kg/day) (Owolabi et al., 2013) also exhibit the lipid lowering effect in tylaxapol and olive oil induced hyperlipidemic rat models. The treated normal SD rats with tyloxapol of (500 mg/kg) for one day as a simple prior treated with Oh.Cr. The effect of Oh.Cr on total cholesterol and triglycerides suggests its importance in treating hyperlipidemic conditions. The Tylaxapol model of hyperlipidemia is used for initial screening that predicts the effect on TC and TG in particular (Rasouli et al., 2016). Thus, the findings in the tyloxapol model, suggest the lipid-lowering effect of Oh.Cr was further confirmed through *in-vitro* assay. Blood samples from the extract-treated three groups were investigated and an assay was performed to see the effect on the key enzyme in the biosynthesis of lipids. Enzyme inhibitory activity against HMG-CoA reductase was observed in the blood of three groups treated with different doses of Oh.Cr. This enzyme inhibitory effect of Oh.Cr indicates a decrease in TC and possibly a decrease in TG.

However, for further details high fat diet-induced models are performed. For this purpose, the effect of the extract was further evaluated on lipids in HFD rats. Similar to human metabolic disorders, HFD produces metabolic disorders in rats. Cholesterol, cholic acid, and butter constituents of HFD. In the intestine, cholic acid increases the absorption of butter and cholesterol. According to published studies, long-term HFD feeding causes a considerable

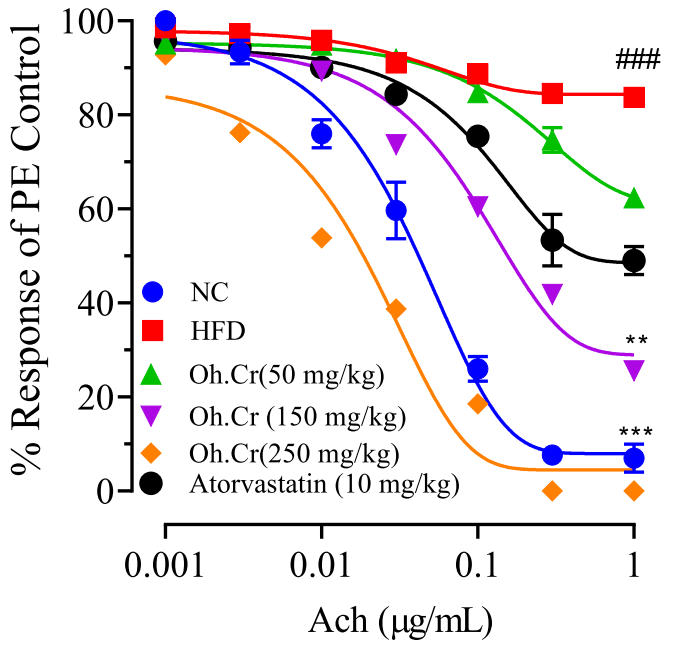

Fig. 3. Acetylcholine response on phenylephrine (PE; 1 µM)-induced contraction in isolated rat aortic rings from normal control (NC), HFD-hyperlipidemic, Oh.Cr treated group at a dose of 50 mg/kg, 150 mg/kg, 250 mg/kg, and atorvastatin 10 mg/kg, treated SD rats. Values are expressed as mean ± SEM (n = 3–5).

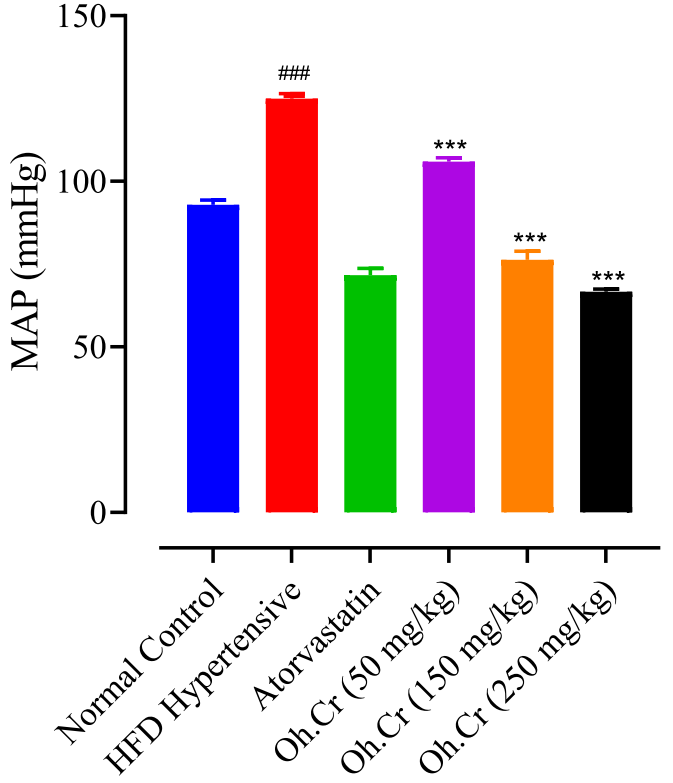

Fig. 4. Bar chart shows percent fall mean arterial pressure (MAP) in response to normal, HFD, atorvastatin 10 mg/kg and Oh.Cr of different doses 50, 150, 250 mg/kg in HFD hyperlipidemic model.

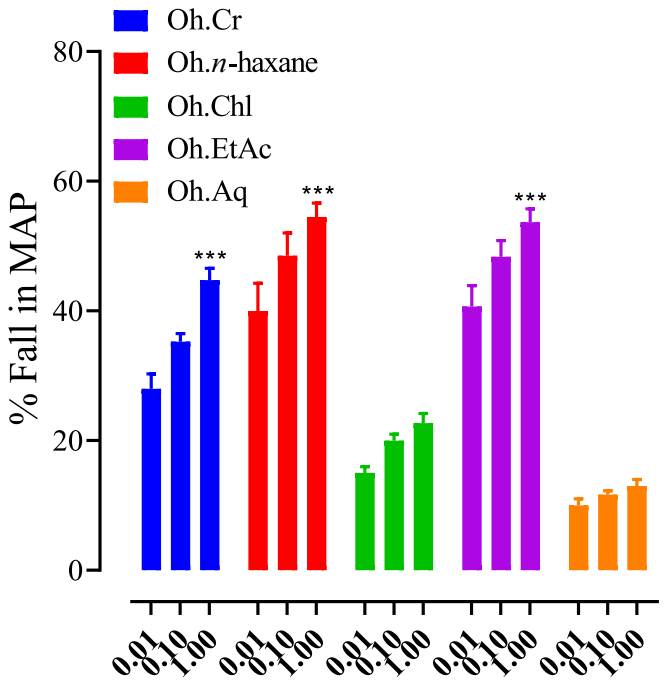

Fig. 5. Bar chart shows the percent fall in mean arterial pressure (MAP) induced by the Oh.Cr and its fractions *n*-hexane, Chloroform, Ethyl acetate, Aqueous (mg/kg) in normotensive rats under anesthesia.

rise in TC, LDL, VLDL, and TG levels when compared to the control group, while HDL levels fall (Li et al., 2018). HFD induces hyperlipidemia by inducing oxidative stress, which eventually results in the generation of reactive oxygen species (ROS). There is evidence in the literature that excessive ROS generation results in cellular damage by oxidising vital cellular elements such membrane lipids and proteins. This model is chronic model having effect for long time (Jain et al., 2010). Our results indicated that all three doses of Oh.Cr extract has decreased TC and TG. According to D'Souza et al. (2007), two processes cholesterol biosynthesis, where HMG-CoA reductase catalyzes the rate-limiting process, and cholesterol absorption maintain cholesterol homeostasis. Dietary cholesterol and cholesterol removed from the liver through biliary secretion are both absorbed. Extract of *O. hispidum* indicated significant depression of enzymatic activity as indicated by an increase in the ratio. A significant increase in the ratio of HMG-CoA/mevalonate was observed at the dose of 250 mg/kg ($p < 0.001$) of Oh.Cr and 10 mg/kg ($p < 0.001$) of standard drug atorvastatin, compared to the HFD group.

In addition to reducing serum lipid levels, *O. hispidum* root extract significantly reduced cholesterol buildup in the aorta of HFD rats. Our findings suggest that oral administration of *O. hispidum* extract to three groups of rats receiving HFD avoided the buildup of lipids in the aorta. Rats fed the extract also showed normal physiology in the aorta. (Fig. 4C, 4D, and 4E). However, the aorta of HFD rats showed spaces within the tunica media and tunica intima. These spaces originally contained fat droplets (Fig. 4B) mainly in the endothelial layer.

A typical symptom of atherosclerosis and persistent hypertension is endothelial dysfunction (Dobrian et al., 2000). Additionally, there is evidence to support the idea that increased ROS production is a significant contributor to vascular dysfunction. Increased ROS activity speeds up the inactivation of nitric oxide (NO), which reduces bioactive NO and increases vascular resistance (Shinozaki et al., 1999; Yoshioka et al., 2000). To assess endothelial dysfunction, acetylcholine (ACh) was used to investigate the effects of HFD on endothelial function in isolated rat aortic rings. Isolated rat aorta of the normal control group, pre-contracted with phenylephrine (1 µM), showed (100%) relaxation to ACh. In HFD hyperlipidemic model, the maximum relaxation induced with

3 μg/mL of acetylcholine was < 30% compared to 100% in the normal control group. Aortic rings of atorvastatin-treated rats showed (50%) relaxation to ACh. Oh.Cr (250 mg/kg) treated group showed complete (100%) relaxation to ACh. In 150 mg/kg treated rats, aortic rings showed 75% relaxation to ACh. HFDs are commonly used to mimic symptoms of metabolic syndrome, including dyslipidemia and hypertension (Taylor et al., 2018).

Our finding confirmed that *O. hispidum* significantly reduced MAP in orally treated rats for 28 days. We observed that hyperlipidemic rats treated with *O. hispidum* prevented the development of hypertension in a dose-dependent manner, associated with the HFD hyperlipidemic control. Maximum preventive effect on hypertension was observed in groups treated with 150 and 250 mg/kg doses, similar to atorvastatin. For comparison, the effect of MAP was also tested in normotensive rats. I.V. administration of different doses of extract and fractions of *O. hispidum* in normotensive rats induced a fall in MAP also. The maximum effect was observed with Oh.Et.Ac fraction that was 55% mmHg at the dose of 1 mg/kg, followed by the crude extract which caused about a 50% fall in MAP. These findings indicate that the antihypertensive constituents of *O. hispidium* are concentrated in the ethyl acetate fraction.

Different classes of constituents particularly, the identification of phenolic and flavonoids have been confirmed, which might be the active antihyperlipidemic and antihypertensive agents.

To assess the safety of the extract, an acute toxicity study was performed in mice, we observed that the extract of *O. hispidium* was found to be safe up to 300 mg/kg dose. Some of the limitations of the current study includes: pharmacokinetics studies have not be carried out to the dose calculation, and the effect should also need to checked in the diverse animal models to compare the effect at different doses.

5. Conclusion

Findings on the pharmacological investigation of *O. hispidium* showed anti-hypercholesterolemic and antihypertriglyceridemic effects, mediated through inhibition of HMG-Co.A reductase. The extract reversed the vascular dysfunction and hypertension secondary to hyperlipidemias and also improved the histopathological changes associated with it. While preliminary phytochemical investigation confirmed the presence of the different classes of secondary metabolites like phenols, flavonoids, alkaloids and glycosides. Future prospects may include nitric oxide pathway study at the molecular level, evaluation of genes involved in the cholesterol regulatory pathways and investigation of inflammatory markers associated with hyperlipidemia and atherosclerosis.

CRediT authorship contribution statement

Nadeem Ullah Wazir: Data curation, Formal analysis, Investigation, Validation, Conceptualization, Methodology. **Irfan Amir Khan:** Data curation, Formal analysis, Investigation. **Adil Javed:** Data curation, Formal analysis, Investigation. **Taous Khan:** Supervision, Project administration. **Abdul Jabbar:** Supervision, Project administration, Data curation, Conceptualization, Methodology, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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