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Antihypertensive and safety studies of *Cydonia oblonga* M.

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Abstract: Antihypertensive studies on aqueous-methanolic extract prepared from seeds of *Cydonia oblonga* M. were carried out. The test extract in 200, 400 and 600 mg/kg doses was investigated in normotensive, high cholesterol and glucose fed hypertensive rats through non-invasive blood pressure measuring technique. Acute and sub-chronic toxicity studies were conducted in mice and rats, respectively. The test extract significantly decreased dose dependently the systolic, diastolic and mean arterial pressures. The test extract in 600mg/kg dose produced maximum effect and prevented rise in blood pressure of high cholesterol diet and glucose fed rats as compare to control in 21 days studies. The extract was found safe up to 4g/kg dose in mice. In sub-chronic toxicity study, no significant alteration in blood chemistry of extract treated rats was observed except reduction in the low density cholesterol levels. It is concluded that *Cydonia oblonga* seeds extract possess antihypertensive effect which supports its use in folklore.

Keywords: *Cydonia oblonga,* hypertensive rats, cholesterol, high glucose, toxicity studies.

INTRODUCTION

Cydonia oblonga M. belongs to family Rosaceae and commonly known as quince. It bears a pome fruit that is similar to pear in appearance and ripe fruit is bright golden-yellow in colour. In sub-continental countries, seeds of *Cydonia oblonga* are commonly known as Bahi Dana. It is used by traditional healers for mucus, skin rashes and ulcerations. Gel prepared from seeds is used for inflammation of throat and vocal cord. Its leaves are used as diuretic, anti-diarrheal, against cystitis and in the treatment of bronchitis (Sezik *et al*., 2001; Kultur, 2007). Leaves and seeds of quince are traditionally used for hypertension, diabetes, fever, diarrhea, cough, nausea, constipation, cystitis and hemorrhoids (Khoubnasabjafari and Jouyban, 2011). It was also found to prolong the clotting and bleeding time (Zhou *et al*., 2014). *Cydonia oblonga* has been reported to contain the phenolic compounds and various organic acids (Ashraf *et al*., 2016). It has shown antioxidant potential comparable to ascorbic acid and quercetin (Khoubnasabjafari and Jouyban, 2011). It has been used for various cardiovascular diseases in folklore, therefore, present study has been planned to evaluate *Cydonia oblonga* seeds for its effects on blood pressure in normotensive and two diet induced hypertensive rats.

MATERIALS AND METHODS

Drugs and chemicals

Analytical grade chemicals were used. Glucose and methanol were obtained from Sigma Chemical Co.

Animals

Sprague-Dawley rats (220-280g) and albino mice (22-30g) of both sexes were used in the study. Animals were kept in stainless steel cages of animal house of University of Sargodha in controlled environment (23-25°C, 12 hours light and dark cycles and relative humidity 55%) and were provided standard diet. Water was provided *ad libitum*. The animals were treated according to guidelines of National Research Council (Clark *et al*., 1997; Alamgeer et al., 2018). Study protocols were approved by Institutional Animal Ethics committee (No. IAEC/UOS/2018/09).

Plant material

Cydonia oblonga dried seeds were obtained from well known herbal market of Lahore, Pakistan and identified from Dr. Amin, a taxonomist of University of Sargodha, voucher sample (CO-205A) was preserved in herbarium of University of Sargodha. An aqueous methanolic (30:70) extract of *Cydonia oblonga* seeds was prepared by following previously described method of Mushtaq *et al*. (2015).

Evaluation of hypotensive activity in normotensive rats

Hypotensive activity of AMECO in normotensive rats was evaluated by following our previously described method (Mushtaq *et al*., 2015; Alamgeer *et al*., 2013). Briefly, rat were assigned randomly into three groups (n = 6). AMECO was orally administered in 200, 400 and 600

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mg/kg doses respectively to groups 1, 2 and 3. Then, blood pressure of rats was determined by non-invasive technique using non-invasive blood pressure (NIBP) measuring apparatus. Briefly, rats were placed in the NIBP restrainers and appropriate cuff with sensor was then mounted on the tails and warmed to about 33-35°C. The tail cuff was inflated to a pressure well above the expected systolic blood pressure, i.e. 250 mm Hg and then slowly released, during which the pulses were recorded by using the Power Lab Data Acquisition system and Lab Chart 5.0 software. Systolic blood pressure (SBP), mean arterial pressure (MAP) and heart rate (HR) were measured directly by using pulse tracing while diastolic blood pressure (DBP) was calculated from the SBP and MAP values by using the formula: DBP= (3MAP-SBP)/2.

Evaluation of acute antihypertensive activity in glucose fed rats

Rats were arbitrarily segregated into three groups (n = 6). Rats were provided with glucose solution (10%) instead of water for a period of 21 days and at day 22, groups 1, 2 and 3 were treated respectively with 200, 400 and 600 mg/kg doses of AMECO orally. Thereafter, their blood pressure was determined at 0, 2, 4 and 8 hours post extract administration (Mushtaq *et al*., 2016a).

Prolong antihypertensive effect in high cholesterol induced hypertensive rats

Rats were arbitrarily divided into two groups of six rats each. Animals in group 1 were provided high cholesterol diet for three weeks. High cholesterol diet was prepared by adding 12 egg yolks into 500g ordinary diet for rats. Rats of group 2 were provided egg-yolk diet plus 600 mg/kg once daily AMECO for three weeks. Blood pressure of animals was measured at 0, 1, 2, and 3weeks as already described by Alamgeer *et al*. (2013).

Prolong antihypertensive effect in high glucose loaded hypertensive rats

Rats were divided arbitrarily into two groups of six rats each. Animals in group 1 were provided glucose solution (10%) for three weeks while those of group 2 were provided glucose solution plus600 mg/kg once daily dose of AMECO for same time duration. Animals were given ordinary diet and blood pressure was determined at 0, 1, 2, and 3 weeks as following the procedure detailed by Saleem *et al*. (2005) and Reaven and Ho (1991).

Acute toxicity testing

Acute toxicity test on the extract was carried out by following method described previously with little modifications. Rats were observed for mortality and general behaviours like excitation, aggressiveness, sedation, skin colour, itching and piloerection for 24 hours in tested animals provided with normal diet and tap water (Iqbal *et al*., 2014).

Sub-chronic toxicity study

Rats were arbitrarily assigned into two groups containing six rats each. Group 1served as control animals and was given normal saline (5ml/kg) while group 2 was administered orally with 600mg/kg once daily dose of AMECO for 29 days. Blood was obtained from rats through cardiac puncture technique at 30th day and serum biochemical analysis was carried out (Mushtaq *et al*., 2016b).

STATISTICAL ANALYSIS

Results were expressed in means ± S.E.M and student t-test was used to analyze data. P<0.05 was considered as statistically significant. GraphPad Prism (5.0) software was used for data analysis.

RESULTS

Hypotensive effect of Cydonia oblonga extract in normotensive rats

Aquesous methanolic extract of *Cydonia oblonga* (AMECO) showed significant decrease (p<0.05) in systolic, diastolic and mean arterial pressures of normotensive rats at tested concentrations in dose dependent fashion. Maximum hypotensive effect was observed at 600 mg/kg dose of AMECO (table 1).

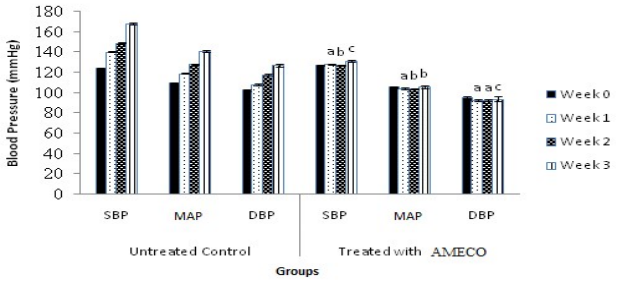

Fig. 1: Antihypertensive activity of *Cydonia oblonga* extract in egg feed treated rats. a = (p<0.05), b = (p<0.01) and c = (p<0.001), compared to control

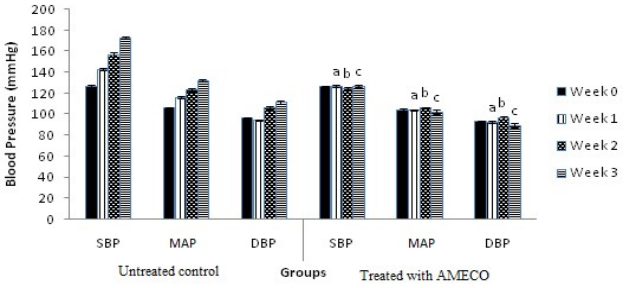

Fig. 2: Antihypertensive activity of *Cydonia oblonga* extract in glucose fed rats. a = (p<0.05), b = (p<0.01) and c = (p<0.001), compared to control

Antihypertensive effect of AMECO in glucose loaded hypertensive rats

Effects of AMECO on SBP, DBP and MAP of glucose loaded rats have been shown in table 2. AMECO dose

Table 1: Effects of *Cydonia oblonga* extract on various parameters of blood pressure in normotensive rats

Time (hours)	200 mg/kg			400 mg/kg			600 mg/kg		
	SBP	MAP	DBP	SBP	MAP	DBP	SBP	MAP	DBP
0	128.2±0.52	104.3±0.8	92.4±1.6	126.8±0.4	106.5 ±0.7	96.4 ±1.3	127.4±0.6	107.6±0.9	97.7±1.4
2	125.1±0.41	101 ±0.9	89± 1.3	121.4 ±2.1	101.4 ±0.4	91.4±0.8	119.4±2.1[a]	91.0 ±1.7[a]	76.7±2.1[a]
4	120.5±0.6	92.6±0.65	87.2±1.0	118± 2.2[a]	91.3 ±1.0[a]	78± 1.5[a]	114.7±2.1[b]	89.5 ±1.3[a]	76.4 ±1.7[a]
6	118.2±1.2[a]	90.5±1.7[a]	78.7±1.8[a]	113.7±1.7[b]	85.0 ±1.8[b]	70.7±1.1[c]	102.2±2.4[c]	83.6 ±1.4[b]	74.3± 1.5[b]
8	124.6±0.48	101± 0.76	89.2± 2.1	122.4 ± 0.7	96.0 ± 2.1	82.8.±2.0[b]	98.5±2.0[c]	81.6 ±2.5[c]	73.2± 1.8[c]

Results are expressed as means ± S.E.M (n = 6), [a] = ($p<0.05$), [b]= ($p<0.01$), [c]= ($p<0.001$) vs. control (0 h).

Table 2: Effects of *Cydonia oblonga* extract on various parameters of blood pressure systolic, in glucose induced hypertensive rats

Time (h)	200 mg/kg			400 mg/kg			600 mg/kg		
	SBP	MAP	DBP	SBP	MAP	DBP	SBP	MAP	DBP
0	166±2.3	148±1.6	139±1.7	172±1.8	157±2.1	150±1.7	165.7 ±2.5	148 ±2.6	139 ±1.8
2	164.3±10	144.9±05	135.2±0.8	160.5±1.2[a]	150±0.87	144± 0.8	152 ±2.2[a]	135± 2.4[a]	126.5±1.3[a]
4	157.1±1.5[a]	132± 1.8[a]	120± 1.8[a]	141± 1.5[b]	133.3±12[b]	129± 2.1[a]	140± 3.6[b]	119± 3.2[c]	108± 2.1[b]
6	148 ± 1.5[b]	130±3.5[a]	119.5±0.7[a]	138± 2.1[a]	130±2.7[b]	126± 1.7[a]	133.4±1.3[c]	107 ±2.2[c]	94± 1.5[b]
8	159± 1.4[a]	134±2.6[a]	121.5±1.9[a]	130± 3.3[b]	123± 3.2[c]	119±2.1[b]	129.4±1.4[c]	101 ±3.0[c]	86.8 ±2.1[c]

Table 3: Effects of *Cydonia oblonga* extract on various other biochemical parameters in rats

Parameters	Untreated (Control group)	Extract treated group(600mg/kg)
ALT(IU/L)	39.4±2.9	37.9±1.9[ns]
AST(IU/L)	92.2±1.0	84.2±1.09[ns]
ALP(IU/L)	71.0±2.1	66.2±1.4[ns]
Triglycerides(mg/dl)	88±1.9	89.0±2.4[ns]
Total Cholesterol(mg/dl)	62.3±2.6	60.0±1.2[ns]
LDL(mg/dl)	26.9±1.07	18.3±1.09[a]
HDL(mg/dl)	31.4±1.18	34.5±2.02[ns]

Values are expressed in means ± SEM (n=6) where [a] = ($p<0.05$),[ns]= non-significant as compare to control

dependently reduced these parameters with maximum effect on pressure was observed at dose 600 mg/kg which consistently lowered blood pressure for 8 hours post drug administration. Hence, further experiments were carried out by using 600 mg/kg dose of AMECO.

Preventive effect of AMECO on blood pressure in high cholesterol diet and glucose fed rats

Effect of AMECO on blood pressure of rats treated with high cholesterol and glucose for three weeks have been shown in fig1 and 2. Treatment with AMECO prevented significantly the SBP, DBP and MAP to rise in high cholesterol and glucose loaded rats when compared with control.

Acute and sub-chronic toxicity

AMECO was found safe up to 4000 mg/kg dose. In sub-chronic toxicity testing, observed parameters viz., ALT, AST, ALP, triglycerides, total cholesterol and HDL levels was not significantly altered in AMECO treated rats as compare to control. However, LDL- level was found to significantly decrease in AMECO treated groups of rats as shown in table 3.

DISCUSSION

Herbal remedies are generally considered safe and easily available mean of treatment for various diseases. The screening of biological properties and bioactive compounds from natural products has become the area of interest to the researchers during the last decades (Mensah *et al*., 2019; Mushtaq *et al*., 2016b). The evaluation of biological effects of the natural products should be at least done prior to the detailed pharmacological analyses, identification and isolation of the active constituents (Rashidi *et al*., 2021; Alamgeer *et al*., 2018). The current study aimed to investigate antihypertensive effect of *Cydonia oblonga*. Crude extract of *Cydonia oblonga* was evaluated in normotensive and two diet induced hypertensive rat models through non-invasive method for the blood pressure lowering effect. The obtained results showed that test extract decreased dose dependently the blood pressure of normotensive and glucose fed hypertensive rats and dose 600mg/kg produced more significant effects ($p<0.001$) and was selected for further experiments. The data showed that AMECO reduced

more significantly the blood pressure in hypertensive rats than that of normotensive rats. These findings agree with our previous investigation (Alamgeer *et al*., 2015).

AMECO also prevented blood pressure to rise in high glucose and cholesterol fed rats in three weeks study. Prolong use of glucose is reported to produce reactive oxygen species (ROS) that increase blood pressure through oxidative stress (Midaoui and Champlain, 2002). *Cydonia oblonga* is reported to contain significant amounts of phytochemicals such as flavonoids, phenolic acids and flavonol, possessing antioxidant properties (Khoubnasabjafari and Jouyban, 2011). These antioxidant phytochemicals counteract the free radical species and prevent their damaging effect on the tissues. The blood pressure lowering effect of tested plant extract may be due to these phytochemical constituents. This is in line with previous investigation (Alamgeer *et al*., 2013). High cholesterol diet induced hypertensive rat model is also used for investigating antihypertensive activity of plant extracts. Increased blood pressure in rats fed with cholesterol is due to high cholesterol level in blood and dyslipidemia leading to hypertension (Hashemzaei *et al*., 2020). AMECO significantly prevented rise in the blood pressure of high cholesterol diet treated rats as compare to untreated control. Our findings are in accordance with the already published data (Alamgeer *et al*., 2015; Mushtaq *et al*., 2015).It has been reported previously that the phytochemical constituents attenuate endothelial dysfunction by decreasing LDL-level and producing nitric oxide with subsequent decrease in blood pressure (Ko *et al*., 2000).

Acute toxicity study showed that AMECO did not produce any toxic sign in mice up to 4000mg/kg dose. AMECO was also studied by administering it orally once daily for 29 days to observe possible adverse effects on hepatic and lipid profiles of AMECO-treated rats. Results showed no significant change in all parameters except the LDLs levels were reduced in AMECO treated rats.

CONCLUSION

Aqueous methanolic extract of *Cydonia oblonga* possesses antihypertensive effect which may be due to phytochemical constituents. Moreover, acute and sub-chronic toxicities testing have shown that plant extract is safe for use. Thus, present investigation calls for further activity directed fractionation study of the test plant extract and to investigate exact antihypertensive mechanism.

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