Antihyperlipidemic activity of chickpea sprouts supplementation in ovariectomy-induced dyslipidemia in rats

Sagili Harini[1], Kaliki Adilaxmamma[2], Emani Madan Mohan[3], Ch. Srilatha[4], Mekapogu Alpha Raj[2]

[1]Departments of Pharmacology and Toxicology and [4]Pathology, College of Veterinary Science, Tirupati, [2]Department of Pharmacology and Toxicology, College of Veterinary Science, Proddatur, [3]Department of Animal Husbandry, Super Specialty Veterinary Hospital, Pulivendula, Andhra Pradesh, India

ABSTRACT

Background: Phytoestrogens are increasingly becoming popular as alternatives for hormone replacement therapy in postmenopausal condition. **Objective:** In this study, the antihyperlipidemic effect of chickpea (*Cicer arientum*) sprouts was evaluated in ovariectomy-induced dyslipidemia in rat model in comparison with standard antihyperlipidemic agent atorvastatin. **Materials and Methods:** A total of 24 female adult Wistar rats were divided into four groups that is, Group I - Control; Group II - Ovariectomized (OVX) rats; Group III - OVX + germinated chickpea sprouts (20% in diet) and Group IV OVX + atorvastatin (1.2 mg/kg b.wt, *p.o.*). Body and organ weights, serum, and liver lipid profile were assessed at the end of 8 weeks. **Results:** The results indicated that ovariectomy significantly ($P < 0.05$) increased total cholesterol, nonhigh-density lipoprotein cholesterol and triglycerides (TGs) in serum and liver. The total lipid and phospholipid content in liver were also significantly ($P < 0.05$) increased. The weights of uterus and heart were significantly ($P < 0.05$) decreased. Dietary supplementation with germinated chickpea normalized the lipid profile in serum and liver. Further, high-density lipoprotein (HDL) cholesterol, body weight, uterine, heart, and spleen weights were significantly ($P < 0.05$) increased. Atorvastatin administration showed similarly normalized lipid profile, but showed no improvement on decreased uterus and heart weights. Histopathological examination revealed fatty changes in liver, uterine atrophy, and subintimal fat accumulation in aorta in OVX group. The changes were mild in chickpea group with no improvement in statin group. **Conclusions:** Germinated seeds of chickpea showed significant antihyperlipidemic activity, which was comparable to atorvastatin. Further, germinated chickpea improved organ weights and helped in the reversal of histopathological changes suggesting its usefulness in postmenopausal condition.

Key words: Antihyperlipidemia, atorvastatin, bengal gram, chickpea, *cicer arientum,* dyslipidemia, ovariectomy

INTRODUCTION

Cardiovascular disease (CVD) is mainly observed in women during later stages of life due to estrogen deficiency consequent to menopausal transition.[1] Hormone replacement is the main line of treatment for preventing coronary heart disease in postmenopausal women, but it is associated with serious side-effects such as breast and endometrial cancers.[2] In this context, phytoestrogenic molecules have received a great deal of attention over the last few years owing to their preventive role in chronic diseases such as CVD, osteoporosis and hormone related cancers.[3] These phytochemicals posses estrogen-like biological activity and provide effective and secure alternative to hormone replacement therapy to adult women.[2]

The inquiry on the pharmacological activity of diverse plants and plant products is growing considerably in the recent past. Novel applications of plants and plant products such as amelioration of heavy metal induced toxicities,[4-6] synthesis of nanoparticles[7-9] including normalization of dyslipidemias[10-12] have been reported. Chickpea or Bengal gram (*Cicer arientum* L)

Address for correspondence:
Dr. Mekapogu Alpha Raj,
Department of Pharmacology and Toxicology, College of Veterinary Science, Proddatur, YSR District - 516 360, Andhra Pradesh, India.
E-mail: alpharajm@gmail.com

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is the second largest legume cultivated in the world providing a rich source of dietary protein.[13] *C. arientum* is known by several names throughout the world such as Bengal gram (Indian), Chickpea (English), Garbanzo (Latin American), Hommes, Hamaz (Arab world), Shimbra (Ethiopian), Nohud, Lablabi (Turkey). Chickpeas were also reported to be a rich source of vitamins, minerals and phytoestrogens.[14] In addition, the process of germination increases the isoflavonoid content in chickpea seeds by 100-fold, mainly due to the increase in formononetin and biochanin A levels.[15]

Chickpea also possess several medicinal properties. In traditionally system of medicine, chickpea seeds were used as tonic, stimulant, aphrodisiac,[16] anthelminthic, appetizer and for relieving burning sensation in stomach.[17] In Ayurveda, the Indian system of medicine, chickpea is considered to be dry (*rooksha*) and light (*laghu*) and hence is used to decrease *Kapha*. Due to these properties, it is indicated in the treatment of obesity and in patients who consume excess oily and heavy foods.[18] Further, it is also used for blood dyscrasias, ear infections, and liver and spleen disorders.[19] In Chinese herbal medicine, chickpea seeds were reported to have been employed for treating hypertension and diabetes mellitus for over the past 2500 years.[20-22] Among the medicinal properties attributed to chick pea, antihyperlipidemic activity has received much attention due to the presence of phytoestrogenic isoflavones biochanin-A and formononetin. The phytoestrogens in *C. arientum* were earlier demonstrated to possess moderate estrogenic effect in ovariectomized (OVX) rat model.[23] Further, several research works have demonstrated the cholesterol lowering effects of *C. arientum* in different types of hyperlipidemias such as induced by diet[24-26] or triton.[27] The sprout extracts of chickpea were able to prevent estrogen deficiency induced bone loss.[23] However, the antihyperlipidemic effect of *C. arientum* was not studied in estrogen deficiency-induced hyperlipidemia. Hence, this study was designed to study the antihyperlipidemic effect of germinated *C. arientum* seeds in ovariectomy-induced hyperlipidemia in rats.

MATERIALS AND METHODS

Germination of chickpea seeds

Chickpea seeds were obtained from local market and were identified by a botanist. The seeds were washed and soaked in water for overnight. The soaked seeds were rinsed and placed in commercially available sprout maker and allowed to sprout for 2 days. Fresh sprouts were used in the preparation of respective experimental diet on a daily basis. The same batch of the seeds was used throughout the experimental period.

Experimental animals

Healthy female adult wistar rats weighing 200-250 g were housed in a solid bottom poly propylene cages (three animals in each) at an ambient temperature of 25°C ± 2°C and 45-55% relative humidity with 12–12 h light and dark cycle. The rats were kept on *ad libitum* feed and water. Permission was obtained from the Institutional Animal Ethics Committee before the start of experiment. Female rats were ovariectomized (OVX)[28-29] and 18 OVX rats were randomly divided into three groups (n = 6) viz., Group II – OVX rats, Group III – Ovariectomy + Chickpea and Group IV – Ovariectomy + atorvastatin (1.2 mg/kg b.wt *p.o.*). Group – I with normal adult females served as sham operated control group. Throughout the experimental period, all the animals in Groups I–IV received isocaloric purified diet with same protein content [Table 1] as per the recommendations of National Research Council[30] with Group III receiving purified diet containing 20% germinated seeds of chickpea for 8 weeks. Body weights were recorded at weekly interval. At the end of experimental period, whole-blood was collected after overnight fasting for 8 h, for the estimation of serum lipid profile. The animals were sacrificed at the end of experimental period and organs were collected and weighed. Liver samples were collected for the estimation of tissue lipids. Liver, uteri, and aorta were collected for histopathological examination in 10% neutral buffered formalin.

Serum lipid profile

Serum lipid profile that is, serum total cholesterol, HDL cholesterol, Non-HDL cholesterol, TGs were determined enzymatically using standard kits obtained from Span diagnostics Pvt. Ltd., Surat, India.

Liver lipid profile

The lipid content of liver was determined using the Folch gravimetric method.[31] The phospholipid content of liver

Table 1: Composition of experimental purified diets

Ingredient	Control (parts/kg)	Test (parts/kg)
Sucrose	0.560	0.400
Cornstarch	0.245	0.247
Casein	0.056	0.018
Germinated chickpea sprouts	-	0.200
Cellulose	0.055	0.050
Corn oil	0.050	0.050
Vitamin mixture	0.010	0.010
Mineral mixture	0.030	0.030
DL- methionine	0.003	0.003
Choline bitartrate	0.002	0.002
Protein (%)	18	18
Energy (kcal/g)	3800	3800

was determined by using Fiske - Subba row method.[32] Liver total cholesterol and TGs were determined using standard kits obtained from Span diagnostics Pvt. Ltd., Surat, India.

Histopathology

Tissue pieces of liver, aorta and uterus were preserved in 10% neutral buffered formalin, later processed and stained by using hematoxylin and eosin and oil red O stains.[33]

Statistical analysis

The data were analyzed using one-way ANOVA followed by Tukey's *post-hoc* test using Statistical Package for Social Sciences (SPSS), Version 17.0. IBM, New York. The value of significance was set at 5% ($P < 0.05$). Means, which were significantly different, were indicated with different superscript alphabets.

RESULTS

Effect on body weights and organ weights

The average body weight of chickpea group was significantly ($P < 0.05$) higher than control and statin groups. The uterine weights were significantly ($P < 0.05$) decreased in OVX and statin groups compared to control and chickpea groups. The spleen weights were significantly ($P < 0.05$) increased in chickpea group compared to OVX and statin groups. The weights of heart in all OVX groups including treatment groups were significantly ($P < 0.05$) lower compared to control group [Table 2].

Effect on serum lipid profile

The mean serum total cholesterol and non-HDL cholesterol was significantly ($P < 0.05$) increased in the OVX group compared to control group. Whereas, both chickpea and statin treatment groups showed significantly ($P < 0.05$) lower values as compared to OVX group. Mean HDL cholesterol was significantly ($P < 0.05$) higher in chickpea and statin groups than control and OVX groups. The mean TG content was significantly ($P < 0.05$) higher in statin group compared to all other groups; whereas, OVX and chickpea had significantly ($P < 0.05$) lower TG levels [Table 3].

Effect on liver lipid profile

Liver total lipids and phospholipids were significantly ($P < 0.05$) increased in the OVX group compared to control group. The total cholesterol content was significantly ($P < 0.05$) higher in the OVX group compared to all other groups. The TG levels were significantly ($P < 0.05$) lower in chickpea group compared to rest of the groups [Table 4].

Effect on histopathology

Histopathological observations revealed fatty degeneration in the liver [Figures 1 and 2], uterine atrophy [Figure 3] and sub-intimal fat accumulation in the aorta [Figure 4] in the OVX group whereas very mild changes were observed in chickpea group. No improvement was observed in statin group.

DISCUSSION

Hormone therapy is the first-line treatment of various vasomotor symptoms in postmenopausal women. However, many women are reluctant to use exogenous hormones for treatment due to concurrent side-effects and are preferring botanicals and dietary supplement products for relief.[34] Despite limited scientific evidence describing efficacy and long-term safety, natural treatments are more appealing with both premenopausal and postmenopausal women being the highest among the users.[34]

Phytoestrogens possess either estrogenic or anti-estrogenic activity. Despite being moderate in their activity[24] compared to endogenous estrogens, the consumption

Table 3: Effect of germinated chickpea sprouts on serum lipid profile

Group	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Non-HDL cholesterol (mg/dL)	Triglycerides (mg/dL)
I	63.15±3.44^{a}	37.87±1.70^{a}	25.28±4.37^{bc}	191.15±2.26^{b}
II	74.45±1.88^{b}	35.47±2.19^{a}	38.98±2.99^{c}	179.43±4.95^{a}
III	63.93±5.38^{a}	51.68±2.53^{b}	13.25±6.98^{a}	169.94±12.81^{a}
IV	57.72±1.58^{a}	51.41±1.85^{b}	04.30±1.82^{a}	251.88±14.45^{b}
Significant	<0.05*	<0.001***	<0.001***	<0.001***

SE=Standard error, HDL=High density lipoprotein. *P<0.05, **P<0.01, ***P<0.001. Values are mean±SE. One-way ANOVA followed by Tukey's *post-hoc* test. Mean with different superscripts are significantly different (P<0.05)

Table 2: Effect of germinated chickpea sprouts on body weight and organ weights

Group	Body (g)	Liver (g)	Spleen (g)	Heart (g)	Kidney (g)	Uterus (g)
I	256.44±3.84^{a}	6.76±0.40	0.68±0.03^{bc}	1.05±0.07^{b}	1.37±0.09	0.56±0.02^{b}
II	265.96±4.74^{ab}	6.04±0.43	0.55±0.05^{ab}	0.84±0.06^{a}	1.39±0.10	0.28±0.04^{a}
III	274.22±4.40^{b}	7.30±0.73	0.73±0.03^{c}	0.79±0.05^{a}	1.46±0.05	0.50±0.04^{b}
IV	256.97±2.91^{a}	6.93±0.33	0.53±0.03^{a}	0.85±0.01^{a}	1.46±0.08	0.25±0.05^{a}
P	<0.01**	NS	<0.01**	<0.05*	NS	<0.001***

SE=Standard error, NS=Non significant. *P<0.05, **P<0.01, ***P<0.001. Values are mean±SE. One-way ANOVA followed by Tukey's *post-hoc* test. Mean with different superscripts are significantly different (P<0.05)

of phytoestrogens has significant clinically significant consequences.[35] Recently, the modulators of estrogen receptors are considered as an important modality for the treatment and prevention of postmenopausal osteoporosis.[36] In the present study, ovariectomy was used to simulate postmenopausal condition. OVX rats are a good model for evaluating estrogen activity in female reproductive and nonreproductive pharmacological areas, including bone and cholesterol related parameters.[37] Further, the model was reported to be an effective predictor of the changes in low density lipoprotein (LDL) cholesterol and is sensitive for monitoring the effects of estrogen on cholesterol.[38] Further, ovariectomy has the advantage of mimicking true menopausal condition minimizing the interference of endogenous estrogen.[39]

Table 4: Effect of germinated chickpea sprouts on liver lipid profile

Group	Total lipids (mg/g)	Total cholesterol (mg/g)	Triglycerides (mg/g)	Phospholipids (mg/g)
I	24.25±1.38^{a}	0.27±0.02^{b}	0.82±0.03^{c}	0.074±0.006^{b}
II	39.16±3.03^{b}	0.30±0.01^{b}	0.53±0.10^{b}	0.094±0.002^{c}
III	23.67±1.33^{a}	0.15±0.02^{a}	0.23±0.01^{a}	0.047±0.002^{a}
IV	23.08±0.98^{a}	0.12±0.01^{a}	0.75±0.04^{bc}	0.049±0.004^{a}
P	<0.001***	<0.001***	<0.001***	<0.001***

SE=Standard error. ***P<0.001. Values are mean±SE. One-way ANOVA followed by Tukey's *post-hoc* test. Mean with different superscripts are significantly different (P<0.05)

Ovariectomy increased body weights of rats, leading to overweight. The increase in body weight is considered as a result of altered energy metabolism caused by estrogen deficiency favoring fat deposition.[40] Contrary to the increase in body weights, the uterine weights were found to be decreased. Such a decrease in uterine weight is a direct consequence of estrogen deficiency, which is required for the normal functioning and maintenance of the uterus.[41]

Ovariectomy also increased the cholesterol content in serum. The deficiency of estrogen is known to increase

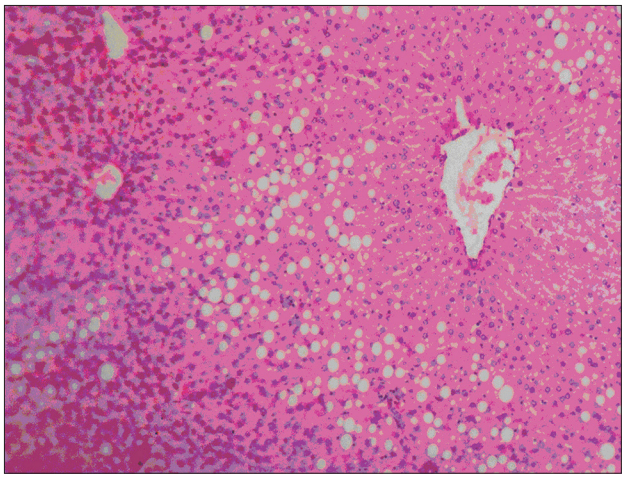

Figure 1: Liver section showing vacuolation in hepatic cells due to fatty changes (H and E, ×70)

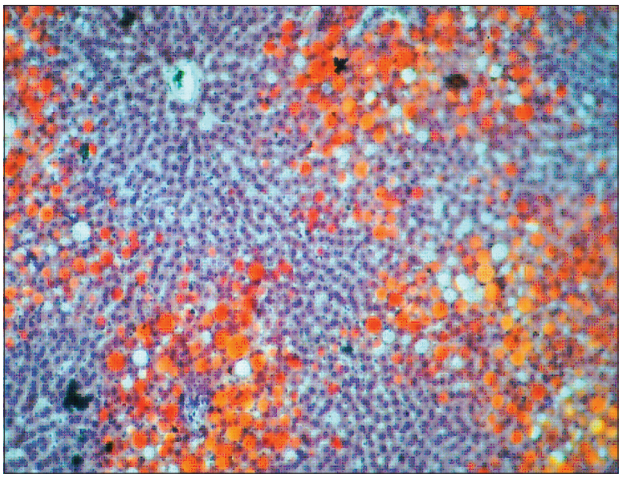

Figure 2: Liver section showing diffuse fatty infiltration (Oil Red 'O', ×70)

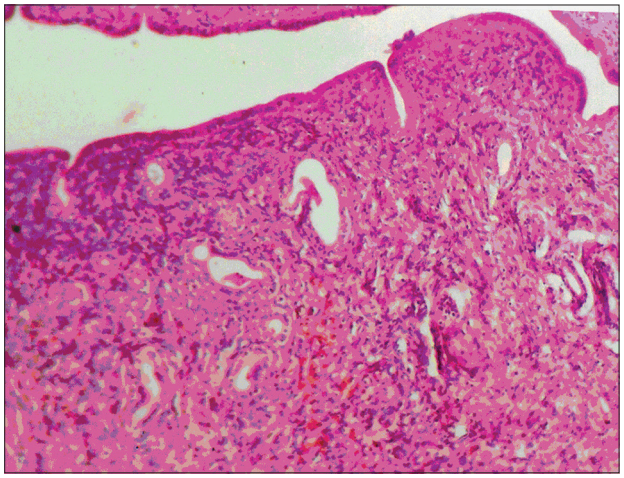

Figure 3: Uterine section showing cystic dilatation and atrophy of endometrial glands (H and E, ×70)

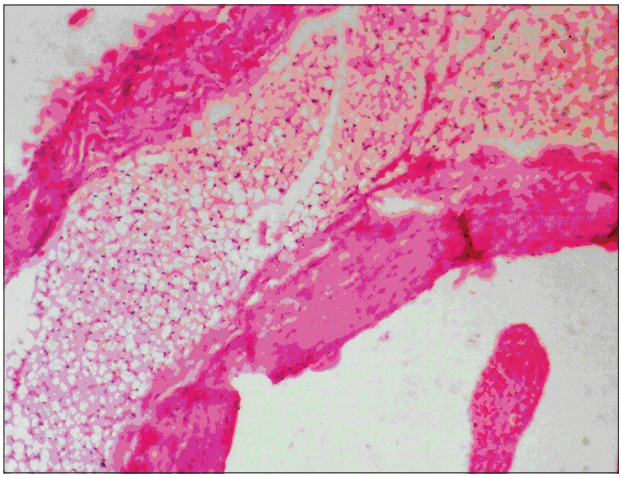

Figure 4: Aorta section showing diffuse sub-intimal fatty infiltration (H and E, ×70)

in cholesterol levels, both in humans[41] and animals.[42-44] subsequent to induction of hepatic HMG CoA reductase, a rate limiting enzyme in the cholesterol synthesis.[45] Further, elevated levels of insulin in OVX rats cause accelerated dephosphorylation of HMG Co-A reductase increasing its activity.[46]

Estrogen is known to have a favorable effect on plasma lipoprotein profile, raising HDL levels.[47,48] In this study, HDL cholesterol was found to be reduced in the OVX group due to estrogen deficiency. Similarly, non-HDL concentration was found to be increased. The increase in non-HDL is a consequence of decreased estrogen concentration causing (1) elimination of LDL clearance sites,[49] (2) diminished LDL receptor activity and concentration[50] (3) directly affects the interconversion of very low density lipoprotein (VLDL) to LDL and (4) direct secretion of LDL by the liver.[51]

A significant decrease in the mean TG concentration was also observed in OVX rats. In normal conditions, estrogen increases TG concentration by enhancing hepatic synthesis and inhibition of lipid uptake by adipose and muscle tissue as a result of decreased lipoprotein lipase (Lp) activity.[52,53] Since ovariectomy increases Lp activity, a significant decrease in TG level was observed in OVX rats.[54] However, an increase in TG concentration was observed in atorvastatin administered group.

Feeding sprouts of chickpea, to OVX rats reversed the changes of ovariectomy in terms of uterine weights, serum and liver lipid profile. The beneficial effects were attributed to the presence of phytoestrogenic flavonoids viz., biochanin A, formononetin, which are found in higher concentration in germinated seeds.[25] Phytoestrogens despite being structurally unrelated to estrogen have the ability to bind to estrogen receptors due to the presence of a phenolic ring[3] and produce similar effects like estrogen on target organs.[24,55] Although a beneficial association between dietary phytoestrogens and lipoproteins is not established clearly, possible antihyperlipidemic mechanism of phytoestrogens could be as a result of increased T_4 level,[56] increased excretion of bile acids, therefore enhancing the removal of LDL-C and altered hepatic metabolism with augmented LDL and VLDL removal by hepatocytes.[57]

CONCLUSION

This study indicated significant antihyperlipidemic activity of germinated seeds of chickpea, which was found to be comparable to atorvastatin. However, due to the presence of phytoestrogens, germinated chickpea was superior in controlling other estrogen deficiency symptoms such as uterine atrophy and fatty changes in liver and aorta. Hence, germinated chickpea could be used as a nutraceutical in postmenopausal condition either alone or in combination with standard antihyperlipidemic drugs. However, the clinical usage of germinated chickpea sprouts would require adequate information on the long-term safety evaluation as per scientific guidelines[58] and checking for possible pharmacodynamics and kinetic herbal-drug interactions.[59]

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