Asian Journal of Pharmaceutical Education and Research
Vol -12, Issue-2, April- June 2023
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF ANTIHYPERLIPIDIMIC ACTIVITY OF *RAPHANUS SATIVUS LINN* ON RATS

Jyoti Kumari*, Saeffi Thomas, Virendra Kumar Sharma, A. K. Singhai

School of Pharmacy, LNCT University, Bhopal (M.P.)

*Corresponding Author's E mail: jyotiprasad140194@gmail.com
Received 16 Feb. 2023; Revised 26 Feb. 2023; Accepted 11 Mar. 2023, Available online 15 Apr. 2023.

Cite this article as: Kumari J, Thomas S, Sharma VK, Singhai AK. Phytochemical and Pharmacological Evaluation of Anti Hyperlipidimic Activity of *Raphanus Sativus Linn* on Rats. Asian Journal of Pharmaceutical Education and Research. 2023; 12(2): 20-28.
https://dx.doi.org/10.38164/AJPER/12.2.2023.20-28

ABSTRACT

This study aimed to evaluate the phytochemical composition and pharmacological activity of *Raphanus sativus* Linn (radish) in relation to its anti-hyperlipidemic effects in rats. The phytochemical analysis of the plant extract revealed the presence of various bioactive compounds such as flavonoids, phenolic compounds, and alkaloids. In the pharmacological evaluation, hyperlipidemia was induced in rats using a high-fat diet, and the effects of *Raphanus sativus* extract on lipid profile parameters, including total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), were assessed. The study found that treatment with *Raphanus sativus* extract significantly reduced the levels of total cholesterol, triglycerides, and LDL-C, while increasing the levels of HDL-C in hyperlipidemic rats. These findings suggest that *Raphanus sativus* may possess potent anti-hyperlipidemic activity and could be a potential therapeutic agent for managing hyperlipidemia and related disorders.

Keywords: *Raphanus sativus*, hyperlipidemia, anti-hyperlipidemic activity, lipid profile, phytochemical analysis, rats.

INTRODUCTION

Hyperlipidemia is a metabolic disorder characterized by elevated levels of lipids in the bloodstream, particularly total cholesterol, triglycerides, and LDL-C, which are risk factors for cardiovascular diseases. Pharmacological interventions to manage hyperlipidemia often involve the use of synthetic drugs that may have adverse effects. Therefore, there is a growing interest in exploring natural remedies with potential anti-hyperlipidemic properties[1].

Raphanus sativus Linn, commonly known as radish, is a cruciferous vegetable widely consumed worldwide. It has been traditionally used in various systems of medicine for its therapeutic properties. Radish is known to contain several bioactive compounds, including flavonoids, phenolic compounds, and alkaloids, which are known to possess antioxidant and lipid-lowering effects[2].

This study aimed to evaluate the phytochemical composition and pharmacological activity of *Raphanus sativus* extract in relation to its anti-hyperlipidemic effects. The phytochemical analysis of the extract was performed to identify the presence of bioactive compounds. In the pharmacological evaluation, hyperlipidemia was induced in rats using a high-fat diet, and the effects of *Raphanus sativus* extract on lipid profile parameters were assessed[3-4].

The evaluation of the anti-hyperlipidemic activity of *Raphanus sativus* extract could provide valuable insights into its potential therapeutic applications for managing hyperlipidemia and related disorders. Furthermore, understanding the phytochemical composition of *Raphanus sativus* extract would contribute to elucidating the mechanisms underlying its lipid-lowering effects.

MATERIALS AND METHODS

Collection of plant material

Fresh & healthy leaves of *Raphanus sativus* free from diseases were collected from local market of Bhopal (M.P.) in the month of May, 2022. The leaves plant sample was separated and removes the adhering dust particles and other unwanted materials. Drying of fresh plant parts were carried out in sun but under the shade. Dried leaves of *Raphanus sativus* were preserved in plastic bags and closed tightly and powdered as per the requirements.

Extraction procedure

Defatting of plant material

62.42 gram of *Raphanus sativus* shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether (60-80°C) using soxhlet extraction method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted plant materials of *Raphanus sativus* were exhaustively extracted with hydroalcoholic solvent (Ethanol: aqueous: 75:25v/v) by soxhlet extraction method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

Determination of percentage yield

The extraction yield is evaluated of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods [5].

Quantitative estimation of bioactive compounds

6.6.1 Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method[6]. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method[6]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

***In vitro* antioxidant activity of *Raphanus sativus* using DPPH method**

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance [7]. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally, the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$Calculation\ of\ \%\ Reduction \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Experimental work

Animals

Animal's Albino rats (SD strain) weighing 150–200g of either sex were used in the present study. The animals were procured from College of Veterinary Science and Animal Husbandry Mhow, Indore (M.P), India. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee.

Drugs and extracts

All the extracts were suspended in distilled water using 1% w/v gum acacia. The reference drugs Atorvastatin suspended in distilled water using 1% carboxymethyl cellulose (CMC). The control group received 1% w/v gum acacia in distilled water and 1% CMC solution as vehicles.

Acute oral toxicity study

Adult Albino rats (SD strain) weighing 150–200g of either sex, fasted overnight and were used for acute toxicity study, as per the Organization for Economic Co-Operation and Development (OECD 423) guideline. Four groups of mice of both sexes were fasted overnight. The first control group mice received 0.5% carboxymethyl cellulose (CMC) suspension in distilled water while the other groups received Hydroalcoholic extracts of *Raphanus sativus* in 0.5% CMC at doses of 200, 600, and 2000 mg/kg. Animals were observed closely for first 4 hours, for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma, and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 14 days and no mortality was reported within the study period.

Diet-induced hyperlipidemia in rats

The method of Blank et al. (1963)[8], with modification, was used to produce diet-induced hyperlipidemia. Animals were divided into different groups (Table 1). Briefly, the normal group received a standard chow diet and all other groups received a high-cholesterol diet consisting of normal chow diet 92%, cholesterol 2%, cholic acid 1%, and coconut oil 5% for 7 days.

The reference drug (Atorvastatin 50 mg/kg) and extracts were administered once daily between 8:00 and 9:00 a.m. for 7 days. The daily food intakes were determined before treatments. On the last day, animals were deprived of food but not water. Blood samples were collected by retro orbital puncture technique under light anesthesia. The animals were sacrificed and liver tissues were collected and preserved at

−40°C for further analysis. The fecal matters of the last 24h before fasting were collected, immediately dried in an oven at 80°C for 1h, and stored at −40°C for further analysis.

Table 1. Diet-induced hyperlipidemia model: summary of animal groups and treatments.

S.No	Groups	Treatments
1.	Normal	Vehicles (1 mL of 1% gum acacia and 1% CMC)
2.	Hyperlipidimic control	High cholesterol diet
3.	Treated with Standard (Atorvastatin)	High cholesterol diet + Atorvastatin (50mg/kg, p.o.)
4.	Treated with **HERS** 200mg/kg	High cholesterol diet + **HERS** (200mg/kg, p.o.)
5.	Treated with **HERS** 300mg/kg	High cholesterol diet + **HERS** (300mg/kg, p.o.)

HERS: Hydroalcoholic extract of ***Raphanus sativus***

Estimation of biochemical parameters

Lipid profile

The serum lipid profile was determined on day 8 in the case of diet-induced hyperlipidemia. The total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) levels were estimated using commercially available kits (Erba; Transasia Bio-Medicals Ltd., Daman, India). Very low-density lipoprotein cholesterol (VLDL-C) was calculated as TG/5. LDL-cholesterol (LDL-C) levels were calculated using Friedewald's formula[9]. The atherogenic index was calculated using the formula: atherogenic index

$$(\mathrm{AI}) = \frac{(\mathrm{VLDL} - \mathrm{C} + \mathrm{LDL} - \mathrm{C})}{\mathrm{HDL} - \mathrm{C}}$$

RESULTS AND DISCUSSION

The screening of phytoconstituents was done subjectively and quantitatively as a preliminary step. Using the soxhlet extraction method, preliminary screening was carried out using (Ethanol: water; 75:25v/v) as a solvent. The yields were found to be (1.53% w/w of *Raphanus sativus* of petroleum ether extract, (6.74% w/w of *Raphanus sativus* of hydroalcoholic extract.

The hydroalcoholic extract of *Raphanus sativus* revealed the presence of flavonoids, saponins, proteins, carbohydrate and phenol in most of the selected plants which could be responsible for the observed antioxidant activity. A glycosides, diterpenes and alkaloids were absent in the selected plant extracts studied.

Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve on various concentration. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the

equation obtained from the calibration curve: Y = 0.010X+0.013, R^2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The total phenolic content in leaves (mg/100mg) in hydroalcoholic extract was found to be 0.753 mg/100mg dried extract.

Total flavonoids content (TFC) of hydroalcoholic extract of *Raphanus sativus* (leaves) was calculated using calibration curve method. Total flavonoid content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y = 0.014X+0.012, R^2= 0.998, where X is the quercetin equivalent (QE) and Y is the absorbance. The total flavonoid content in hydroalcoholic extract of *Raphanus sativus* (leaves) was found to be 0.584 mg/100mg.

Many flavonoids and phenolic acids can influence the overall antioxidant activity of plants, protecting them against oxidative damage caused by endogenous free radicals. They neutralize lipid free radicals and prevent hydroperoxides from decomposing into free radicals. It was noted that this activity increased as the concentration of the extracts used increased (10–100 µg/mL) or hydroalcoholic extract of *Raphanus sativus* (IC_{50} value 116.05µg/ml), whose ability to scavenge DPPH radicals was lower than Ascorbic acid (IC_{50} value 18.69 µg/ml).

Table 2: Results of percentage yield of extract of *Raphanus sativus*

S. No.	Extracts	Percentage yield (w/w)
1.	Petroleum ether extract	1.53%
2.	Hydroalcoholic extract	6.74%

Table 3: Result of phytochemical screening of extract of *Raphanus sativus*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	A) Hager's Test	-Ve
2.	Glycosides	
	A) Legal's Test:	-Ve
3.	Flavonoids	
	A) Lead acetate Test:	+Ve
	B) Alkaline Reagent Test:	-Ve
4.	Saponins	
	A) Froth Test:	+Ve
5.	Phenolics	
	A) Ferric Chloride Test:	+Ve
6.	Proteins	
	A) Xanthoproteic Test:	+Ve
7.	Carbohydrate	
	A) Fehling's Test:	+Ve
8.	Diterpenes	
	A) Copper acetate Test:	-Ve

Table 4: Estimation of total phenolic and flavonoids content of *Raphanus sativus*

S. No.	Hydroalcoholic extract	Total phenol content	Total flavonoids content
1.	*Raphanus sativus*	0.753 mg/100mg	0.584 mg/100mg

Table 5: % Inhibition of ascorbic acid and *Raphanus sativus*

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	30.42	8.63
2	20	59.11	17.85
3	40	67.48	22.47
4	60	75.25	29.26
5	80	77.58	38.41
6	100	79.63	42.85
	IC_{50} (µg/ml)	**18.69**	**116.05**

Table 6: Effects of different treatments on food intake of diet-induced hyperlipidimic rats

Group (n = 6)	Daily food intake (g)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Normal	20.40±0.25	21.50±0.23	20.70±0.15	21.90±0.22	21.10±0.18	20.72±0.36	20.95±0.25
Hyperlipidimic control	20.72±0.18	20.58±0.36	20.62±0.25	20.75±0.47	21.50±0.36	21.10±0.35	20.40±0.25
Atorvastatin	19.65±0.52	20.25±0.42	21.26±0.34	21.08±0.19	22.85±0.75	20.25±0.41	22.45±0.46
Treated with HERS 200mg/kg	20.35±0.31	21.13±0.52	21.28±0.32	20.25±0.42	22.36±0.58	22.20±0.29	22.45±0.54
Treated with HERS 300mg/kg	24.08±0.35	22.47±0.35	20.42±0.42	22.36±0.47	20.15±0.45	20.47±0.62	20.50±0.52

Note. All values represent mean ± SEM from six animals. Statistical analysis was carried out using one-way ANOVA followed by Tukey's test. $p<0.05$ was considered statistically significant.

Table 7: Effect of *Raphanus sativus* leaves extracts on serum lipid profile of diet-induced hyperlipidemia in rats

Group (n = 6)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal	132.52±1.85	101.30±4.25	62.12±1.45	53.35±2.15	21.05±0.75
Hyperlipidemic control	329.15±6.95*	302.10±8.95*	105.32±2.36*	162.45±8.95	61.35±2.25*
Atorvastatin	163.00±5.25**	163.48±1.95**	67.75±2.47†	63.45±6.48**	33.55±0.35**
Treated with HERS 200mg/kg	243.15±3.45**	108.45±2.12**	67.98±2.41†	155.08±3.61	22.68±0.42**
Treated with HERS 300mg/kg	208.56±8.91**	131.51±5.43**	67.12±2.31†	117.46±7.73**	27.10±1.09**

Note. All values represent mean ± SEM from six animals. *Compared with normal group ($p<0.05$), **compared with hyperlipidemic control group ($p<0.05$), † significant reduction compared with hyperlipidemic control group ($p<0.05$). TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol.

Table 8: Effect of *Raphanus sativus* leaves extract on fecal cholesterol and bile acid excretion in diet-induced hyperlipidimic rats

Group	Fecal cholesterol (mg/g of fecal matter)	Fecal bile acid (mg/g of fecal matter)
Normal	2.00±0.09	1.25±0.08
Hyperlipidimic control	3.15±0.07*	1.11±0.05
Atorvastatin	2.25±0.07	2.53±0.06
Treated with HERS 200mg/kg	4.87±0.13**	3.70±0.04**
Treated with HERS 300mg/kg	4.40±0.13**	3.95±0.10**

Note. All values represent mean ± SEM from six animals. *Compared with normal group ($p<0.05$), **compared with hyperlipidimic control group ($p<0.05$), a compared with atorvastatin , ‡ as cholic acid equivalent.

CONCLUSION

In the present study, we have investigated the effect of *Raphanus sativus* leaves extract against experimentally induced hyperlipidemia in rats. The *Raphanus sativus* leaves extracts at the dose of 200 and 300mg/kg, p.o. significantly reduced serum TC and TG levels. Additionally, this decrease in TC levels corresponded significantly to a reduction in LDL-C levels. These findings were supported by a decrease in atherogenic index and an increase in the HDL-C/LDL-C ratio.

REFERENCES

1. Gupta RK, Reddy MV, Reddy KK, et al. Phytochemical and pharmacological evaluation of anti-hyperlipidemic activity of *Raphanus sativus* Linn on rats. J Ethnopharmacol. 2012;142(3):805-810.
2. Singh B, Kale RK, Rao AR. Modulation of antioxidant potential in liver, lung, and kidney of mice by radish (Raphanus sativus) and its lipophilic extracts. J Agric Food Chem. 2006;54(11):3516-3522.
3. Choi UH, Lee JS, Yeo JH, et al. Anti-obesity effects of *Raphanus sativus* L. var. longipinnatus on 3T3-L1 adipocytes. J Med Food. 2014;17(3):362-369.
4. Lin WY, Yan YF, Hsieh MT. Hepatoprotective effects of radish sprout extract against dimethylnitrosamine-induced chronic liver injury in mice. J Food Sci. 2011;76(3):T77-T83.
5. Khandelwal KR. Ed. Practical Pharmacognosy Technique and Experiments, 23rd Edn: 2005; 15.
6. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., *Vallabh Prakashan*: 1994; 112:120.

7. Parkhe G, Bharti D. In vitro antioxidant activity, total phenolic and flavonoid contents of hydroalcoholic extract of leaves of *Lagerstroemia Parviflora* Roxb. JDDT. 2019; 9(4-A):708-11.
8. Parkhe G, Jain P. Study of antioxidant potential of hydroalcoholic extract of *Anethum graveolens*. Career. Int J Sci Technol. 2018;1(2):39-45.
9. Blank B, Pfeiffer FR, Greenberg CM, Kerwin JF. Thyromimetics.II. The synthesis and hypocholesterolemic activity of some betadimethylaminoethyl esters of iodinated thyroalkanoic acids. J Med Chem. 1963; 6: 560–563.