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EFFECT OF ETHANOLIC EXTRACT OF *BRASSICA RAPA SUBSP. RAPA* LEAVES ON *IN VITRO* DISSOLUTION OF CHOLESTEROL GALLSTONES: AN APPROACH TO EVALUATE ANTI-CHOLELITHIATIC PROPERTY

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ABSTRACT

Aim: *Brassica rapa. Subsp. rapa* leaves (Turnip leaf) belonging to family Brassicaceae contains biologically active compounds such as flavonoids including isorhamnetin, kaempferol and quercetin glycosides, phenyl propanoid derivatives, indole alkaloids, and sterol glucosides. Several studies have been reported that polyphenols and flavonoids have beneficial effects. In Perso-Arabic tradition, *Brassica rapa* was employed as a medicine for inflammation of the gall bladder stones. However, the impact of turnip leaf on cholelithiasis has not been elucidated. The present study was aimed to evaluate the anti-cholelithiatic effect of ethanolic extract of turnip leaf. **Materials and Methods:** Cholesterol gall bladder stones and pigment gall bladder stones along with human bile were collected from the hospitals. These gall bladder stones were incubated with two different concentrations of ethanolic extract of *Brassica rapa* (EEBR) leaves (1mg/ml & 2mg/ml) along with human bile for 4 weeks. By the end of every week the dried weight of gall bladder stones and amount of cholesterol released were estimated and compared with initial values measured before the treatment. **Results:** It was found that the amount of cholesterol released from gall stones was increased (35.88mg/l) and the dried weight of gall stones were reduced (8.27mg an 9.62 mg) by the end of 4th week and the results were comparable with that of standard drug ursodiol 2mg/ml. **Conclusion:** The present study has provided an evidence for anti-cholelithiatic activity of EEBR by using *in vitro* model. The study suggests that further research is necessary to confirm the effectiveness of *Brassica rapa* leaves in real biological systems through animal models.

KEYWORDS: Gall bladder stones, turnip leaf, human bile, cholesterol.

INTRODUCTION

Gall bladder stones occurrence is highly prevalent in many of the developed countries and has an incidence rate of 15%.[1] Age, female gender, hyperlipidemia, Diabetes mellitus, greater body mass index and alcohol consumption were major risk factors accounting for occurrence of gall stones.[2-4] Development of gall bladder stones is referred as cholelithiasis and is highly prevalent gastrointestinal disorder that occurs due to disturbance in biliary homeostasis and hepatic cholesterol level.[5] Gall bladder stores bile which is produced in liver. Bile acids play an important role in emulsification of fats and in their absorption from small intestine. In cholelithiasis gall bladder stones are composed of cholesterol or bile pigments formed in the gall bladder. Most of the stones are composed of cholesterol.[6] Oral bile salt therapy may dissolve such gallstones, but was largely abandoned because of low efficacy. Cholecystectomy (removal of the gallbladder) is currently the only practical option for patients with symptomatic or complicated gallstone disease.

Green leaves and roots of *Brassica rapa* commonly calls as turnip has medicinal and nutritional values. The data collected from the phytochemical screening studies performed on brassica rapa leaf extract so far reveals the presence of Glucosinolates, isothiocyanates, flavonoids, volatiles, and other compounds.[7,8] Glucosinolates, isothiocyanates, flavonoids, volatiles and phenylpropanoids are the major active constituents of turnip greens. Flavonoids, mainly kaempferol, quercetin and isorhamnetin derivatives were found to be in turnip greens. Turnip is also used as a traditional medicine for the treatment of headaches, chest complaints,

rheumatisms, oedemas, gonorrhoea, syphilis, and rabies besides being an important vegetable and source of oil. Glucosinolates and isothiocyanates (mainly 2-phenylethyl, 4-pentenyl, and 3-butenyl derivatives) are the main constituents of turnip with diverse bioactivities, especially for the protective effect against cancers.

The isothiocyanates, are reported to have nematicidal,[9,10] fungicidal,[11,12] and bactericidal[13] properties. The roots of turnip possess antibacterial properties and were used in the treatment of common cold.[14] The powder obtained from the seeds of *Brassica rapa* well-known for its anticancer properties especially used in breast cancer and the extracts obtained from roots of *Brassia rapa* has beneficial effects in skin cancer. Turnip has also been a common diet and herbal remedy in Tibet, where high altitude leads to oxygen deficiency and exhaustion. In Perso-Arabic tradition, *Brassica rapa* was employed as a medicine for inflammation of the gall bladder and for gall stones. However, the impact of turnip leaf on cholelithiasis has not been elucidated. The present study was conducted to evaluate the anti-cholelithiatic effect of ethanolic extract of turnip leaf.

MATERIALS AND METHODS

Collection of plant material: Fresh and healthy leaves of *Brassica rapa* belonging to the family Brassicaceae, were collected from the local areas of Talakona forest, Chittor district, Andhra Pradesh. The plant material was authentified by Dr. K. Madhava Chetty, Asst. Prof, Department of Botany, S.V. University, Tirupathi, Andhra Pradesh, India with voucher no 0429. It was preserved for future reference in our Pharmacognosy department. Just after collection, the plant material was washed thoroughly with running tap water. They are kept away from direct sunlight to avoid destruction of active compounds, shade dried at room temperature and ground mechanically into a coarse powder and stored in air tight container for future use.

Preparation of plant extract

Brassica rapa leaves were allowed to dry in shade. Dried leaves were powdered by electric grinder. The experiment is started by building a rig using stands and clamps to support the extraction apparatus. Following this the solvent (ethanol- 500ml) was added to the round bottom flask, which was attached to a Soxhlet extractor and condenser on an isomantle. The plant material was loaded onto the thimble and was placed inside the Soxhlet extractor. The side arm was lagged with glass wool. The solvent was heated using the isomantle and it started to evaporate, moving through the condenser. The condensate then dripped into the reservoir containing the thimble. Once the level of solvent reached the siphon tube it was poured back into the flask and the cycle begins again. The process was runned for 16 hours. The alcohol was evaporated using a rotary evaporator, leaving a small yield of extract (3.78%) in glass bottom flask**.** and the extract was stored at 4-5^0 C until used. Double- distilled water was used to redissolve the extract prior to experimentation to evaluate anti-cholelithiatic activity.[15]

Preliminary Phytochemical Screening

Standard screening test of the Ethanolic extract of *Brassica rapa* leaves (EEBR) was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites using standard procedures.[16]

IN VITRO METHODS

In-vitro model of cholelithiasis

For *in-vitro* activity, some gallstones (cholesterol and pigment stones) along with human bile was collected from hospitals. All gall bladder stones were dried at 45 degrees centigrade in an oven and then their dry weight was noted in an air tight electronic balance. After that, all gallstones were incubated in human bile and were treated with different concentrations of Ethanolic extracts of *Brassica rapa* leaves in different concentrations (1mg/ml and 2mg/ml) and standard drug (ursodiol, 2 mg/ml) separately for 4 weeks at 37 degrees centigrade. During incubation, the gallstones were picked up, dried and then their dry weights were taken weekly. The amount of cholesterol released from stones were measured weekly by using the autoanalyzer. The differences in gall stone weight and amount of cholesterol released before and after treatment were observed to observe the effect of extracts and reference drug on gallstones.[17]

RESULTS

PRELIMINARY PHYTOCHEMICAL SCREENING

The revealed results of the preliminary phytochemical screening of the ethanolic extract of *Brassica rapa* were shown below. The ethanolic extract gave positive results for alkaloids, steroids, flavonoids, flavonones, glycosides, saponins, proteins and tannins.

In-vitro model of cholelithiasis

The effect of ethanolic extract of *Brassica rapa* leaves on the *in vitro* dissolution of cholesterol gall stones and pigment gall stones was observed weekly.

During the experiment the pigment gall stones and cholesterol gall stones (CS-1& CS-2) were incubated with ethanolic extract of *Brassica rapa* leaves of two different concentrations (1mg/ml and 2mg/ml) and standard drug ursodiol (2mg/ml) for 28 days along with the human bile and the results were comparable with that of standard drug ursodiol hence depicted in table-1, 2, 3 and 4.

The cholesterol gall stones which were incubated with standard drug (ursodiol 2mg/ml) has shown complete dissolution by the end of 28th day and no change was observed with the pigment stones.

While the cholesterol gall stones which were incubated with ethanolic extract of *Brassica rapa* leaves (1mg/ml and 2mg/ml) have also shown a weight reduction of

more than 80% by the end of 28th day and no change was observed with pigment stones.

Before treatment the cholesterol stones were too hard and fragility was noticed after treatment with the extracts.

Along with weight reduction the amount of cholesterol content released from gall bladder stones were observed during the experiment when they were incubated with ethanolic extract of *Brassica rapa* leaves of two different concentrations (1mg/ml and 2mg/ml) and standard drug ursodiol (2mg/ml) for 28 days. It was observed that the amount of cholesterol released was increased with increase in concentration of EEBR. The high amount of cholesterol (204.41mg/dl) was released when Cholesterol gall bladder stones were treated with 2mg/ ml concentration of EEBR by the end of 21st day which was comparable with that of standard ursodiol (2mg/ml).

Pigment stones before incubation

Cholesterol stones (CS-1 & CS-2) before incubation

Cholesterol stones after incubation

Table 1: Weight reduction of Pigment stones at different time intervals.

S.no		Weight in mg				
		0 Day	7th Day	14th Day	21th Day	28th Day
1	Control	151.20	150.31	150.12	149.07	150.07
2	Standard Drug(ursodiol 1mg/ml)	148.71	147.62	147.11	146.24	146.02
3	EERB (1mg/ml)	212.03	210.84	209.71	209.01	208.84
4	EERB (2mg/ml)	204.71	204.01	203.12	202.89	201.46

Table 2: Weight reduction of Cholestrol Stones(CS-1) at different time intervals.

S.no		Weight in mg				
		0 Day	7th Day	14th Day	21th Day	28th Day
1	Control	22.21	21.89	21.52	20.21	19.82
2	Standard Drug(ursodiol 2 mg/ml)	31.26	25.89	20.12	13.62	8.21
3	EERB (1mg/ml)	20.12	16.31	10.26	6.87	2.14
4	EERB (2mg/ml)	35.21	29.66	22.87	15.71	8.27

Table 3: Weight reduction of Cholestrol Stones(CS-2) at different time intervals.

S.no		Weight in mg				
		0 Day	7th Day	14th Day	21th Day	28th Day
1	Control	21.27	19.89	19.81	19.02	18.89
2	Standard Drug(ursodiol 2 mg/ml)	32.87	27.62	18.77	10.21	4.26
3	EERB (1mg/ml)	22.12	15.66	10.24	6.21	1.66
4	EERB (2mg/ml)	36.21	29.87	21.21	14.82	9.62

Table 4: Cholestrol released from gall bladder stones at different time intervals.

S.no		Amount of Cholestrol released in mg/dl			
1	Control	7th Day	14th Day	21th Day	28th Day
2	Standard Drug (ursodiol 2mg/ml)	19.89	22.81	28.02	38.11
3	EERB (1mg/ml)	17.62	25.77	30.21	34.22
4	EERB (2mg/ml)	15.66	20.24	28.21	35.88

DISCUSSION

Occurrence of gall bladder stones is one of the major health concerns caused by imbalance between cholic acid and cholesterol. Biliary tract obstruction, cholecystitis, pancreatitis may occur due to gall stones.

Brassica rapa was employed as a medicine for inflammation of gall bladder, gall stones, constipation, hepatic diseases and gastritis in perso-arabic tradition. Turnip has also been a common diet and herbal remedy in Tibet.

The present study was carried out to evaluate the anti-cholelithiatic potential of *Brassica rapa* leaves by an in vitro dissolution study. The cholesterol gall bladder stones and pigment stones were incubated with EEBR at two different concentrations along with human bile. Among two different concentration of EEBR 2mg/ml was most effective in dissolving CS-1 & CS-2 stones. No observable changes were noted with pigment stones which were incubated with EEBR at different concentrations (1mg/ml, 2mg/ml). Further studies were needed to evaluate the activity on pigment stones. The effectiveness of EEBR 2mg/ml was comparatively equal with that of standard ursodiol 2mg/ml. This shows that EEBR was effective in dissolving cholesterol gall bladder stones. The saponins were found to be responsible for the cholesterol dissolving activity.[18,19] The phytochemical screening of EEBR reported the presence of saponins and might have been exhibited their anti-cholelithiatic activity. It was also found from the previous studies that magnesium present in plants was also responsible for anti-cholelithiatic activity.[20]

CONCLUSION

The present study revealed anti-cholelithiatic activity of EEBR leaves against gall bladder stones. The highest conc. of EEBR leaves (2mg/ml) has shown an appreciable degree of dissolution of cholesterol stones (CS-1&CS-2) when they were incubated for 4 weeks but no change was observed with pigment stones. The present study was limited to *in vitro* study. The study suggests that further research is necessary to confirm the effectiveness of *Brassica rapa* leaves in real biological systems through animal models. Furthermore, the mechanism of action for the anti-cholelithiatic activity of *Brassica rapa* leaves should be elucidated.

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