



RESEARCH ARTICLE

ANTI LITHOGENIC EFFECT OF ROHITAKADHYA EXTRACT AND PHALTRIKADI
DECOCTION ON SWISS ALBINO MICE

^{1,*}Gupta Rashmi, ²Gupta Gopal Das, ³Singh Santosh Kumar and ⁴Singh Lakshman

¹M.S., Ph.D. Scholar, Dept. of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Science, Banaras Hindu University-Varanasi 221005

²MD, PhD Dept. of Kayachikitsa, IMS, BHU, Consultant at Jeevak Ayurvedic Medical College Ramnagar, Varanasi

³Senior Research Officer, Centre of Experimental Medicine & Surgery, Institute of Medical Science, Banaras Hindu University-Varanasi 221005

⁴Professor & Head Dept. of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Science, Banaras Hindu University-Varanasi 221005

ARTICLE INFO

Article History:

Received 16th October, 2016

Received in revised form

22nd November, 2016

Accepted 09th December, 2016

Published online 31st January, 2017

Key words:

Lithogenic Diet,
Rohitakadhy Extract and Phaltrikadi
Decoction,
Swiss albino Mice,
Gall Bladder etc.

ABSTRACT

In this study generate gall bladder stone in Swiss albino mice (*Musmusculus*) by given lithogenic diet, then used trial drug (Rohitakadhy extract & Phaltrikadi kwath) to evaluate its effect on gall bladder stone & gall bladder mucosa. Swiss albino mice (*Musmusculus*) were obtained from central, Institute of Medical Sciences, Banaras Hindu University, Varanasi and kept in animal house of Centre of Experimental Medicine & Surgery, IMS, BHU. The experimental Protocol used in this study regarding the use of animals was approved by the Institutional Animal Ethics Committee, Institute of Medical Sciences, Banaras Hindu University (Approval number; CAEC/1145-27/04/2015) and the care of animals was taken as per the CPCSEA guidelines. This experimental study has been divided in four groups & each group having six mice. All mice are operated & gall bladder excised, morphological findings are noted than specimen kept over glass slide to view by microscope. After that the specimen was lay opened & than seen under microscope & findings are noted. Finally histological slides are prepared for histological study. It was found that trial drugs are very effective on gall bladder stone & mucosa. Findings shows- no any sludge's & stone seen over mucosa of gall bladder. Histological findings have been observed that inflammatory changes are very less in number, mucosa becomes intact, edema& congestion are not seen.

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Citation: Gupta Rashmi, Gupta Gopal Das, Singh Santosh Kumar and Singh Lakshman. 2017. "Anti Lithogenic Effect of Rohitakadhy extract and Phaltrikadi decoction on Swiss Albino Mice", *International Journal of Current Research*, 9, (01), 44958-44961.

INTRODUCTION

Gallstones are a major public health problem in all developed countries. Many epidemiological studies have been performed with the aim of establishing gallstone prevalence and incidence rates, and of defining risk factors, amenable to prevention. Cholesterol gallstones constitute more than 80% of stones in the Western world (Monica Acalovschi, 2001). Gallstones constitute a significant health problem in developed societies, affecting 10% to 15% of the adult population, meaning 20 to 25 million Americans have (or will have) gall stones (Shaffer, 2005; Schirmer et al., 2005; Everhart et al., 1999; Tazuma, 2006). The number of surgical procedures for cholelithiasis has risen markedly in developed countries since 1950 (Legorreta et al., 1993).

*Corresponding author: Gupta Rashmi,

M.S., Ph.D. Scholar, Dept. of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Science, Banaras Hindu University-Varanasi 221005.

The introduction of laparoscopic cholecystectomy in 1986 further increased the cholecystectomy rate (Legorreta et al., 1993; Marshall et al., 1994; Kang et al., 2003) from 1990 to 1993, for example, there was a 28% escalation in the number of cholecystectomies performed (Nenner et al., 1994). The change in practice emanated from the laparoscopic surgical approach. Clinical features of Cholecystitis and cholelithiasis are abdominal discomfort and tenderness in hepatic area with or without loss of appetite, nausea, vomiting, early fatigue, weakness and fever. Cholecystitis and cholelithiasis is induced due to alteration of cholesterol and bilirubin metabolism, which take place at liver. There are two ways for treatment of chronic cholecystitis & cholelithiasis; one is conservative & another surgical. No curative & safe medical management has been accepted for chronic Cholecystitis & cholelithiasis as per modern medicine. Despite the efficacy and safety of cholecystectomy medical workers have long pursued and investigated other less invasive method.

Experimental Study

Sr. No.		Group A	Group B	Group C	Group D
1.	No. of Mice	6	6	6	6
2.	Age	2-4 month	2-4 month	2-4 month	2-4 month
3.	Weight	24±2gms	24±2gms	24±2gms	24±2gms
4.	Diet	Standard diet	Lithogenic diet	Lithogenic diet	Lithogenic diet
5.	Duration of Diet	15 Days	15 Days & one month	15 Days	15 Days
6.		After 15 days gall bladder excised	After 15 days, in 50% cases gall bladder excised to access stone	-	-
7.	Trial Drug given	-	Normal Saline water was given for 15 days	<i>Rohitakadhya extract</i> for 15 days	<i>Rohitakadhya extract & Phaltrikadi decoction</i> for 15 days
8.			After one month remaining 50% cases gall bladder excised to access stone.	After one month gall bladder excised to see the effect of trial drug on stone & mucosa of gall bladder mice.	After one month gall bladder excised to evaluate the effect of trial drug on stone & mucosa of mice gall bladder.

Morphologic findings of the Excised Gall bladder in Mice

Group	Case no.	GB Size (in mm)	GB Calculus	
			Sludge's	Stone
Control Negative Group A (standard diet)	6	2-3mm	0%	0%
Control Positive Group B Two week (lithogenic diet)	3	4-5mm	25%	75%
Four week	3	5-7mm	-	100%
Group C (treated with Rohitakadhya Extract)	6	4-5mm	25%	0%
Group D (treated with Rohitakadhya Extract & Phaltrikadi Kwath)	6	4-5mm	0%	0%

Microscopic and Histological Findings in All Groups

	Group A (Standard diet)	GroupB (lithogenic diet)	GroupC (lithogenic diet) + <i>Rohitakadhya extract</i>	GroupD (lithogenic diet) + <i>Rohitakadhya extract</i> + <i>Phaltrikadi Kwath</i>
1.GB microscopic findings	Excised gall bladder shows yellowish color transparent gall bladder seen & no any sludge or stone.	Findings are-distended gall bladder having blackish spot & 50% cases shows whole gall bladder occupied by large stone.	In group C microscopic findings shows- transparent gall bladder & no any black spot or stone seen.	In group D microscopic findings are- transparent gall bladder & no any black spot seen.
2.GB Transverse section findings	Findings Shows- normal mucosa no any sludge's or stone.	Findings are- black sludge's & stone seen over mucosa of gall bladder.	Findings are- 4% cases shows black sludge's seen very small in number over mucosa of gall bladder.	Findings shows- no any sludge's & stone seen over mucosa of gall bladder.
5.Gall bladder bile findings	Greenish yellowish color bile seen small in amount, not possible to aspirate for analysis	Greenish yellowish color bile present slightly more in amount as compare to group A but not possible to aspirate for analysis	Greenish yellowish color bile present very less in amount not possible to aspirate for analysis	Greenish yellowish color bile present very less in amount not possible to aspirate for analysis
6.GB histological findings	Group A shows normal intact single line mucosa, no proliferation seen in mucosa, muscles layer & lamina propria are normal.	In group B histology of excised gall bladder of mice shows mucosa becomes proliferated & showing villi projection. In epithelial zone inflammation with chronic inflammatory cells & edema present.	In group C histology of excised gall bladder shows as compare to group A reduction in inflammation however proliferation less marked. There is reduced edema & congestion in mucosa layer.	In group D histological slides has been observed that inflammatory changes are very less in number, mucosa becomes intact, edema & congestion are not seen.

So, no such drug is available which can dissolve gall bladder stones safe, economically & without any untoward effect. Finally the treatment of choice is at present only surgical management that is open, minimal access surgery or laparoscopic cholecystectomy. The pattern of trauma has drastically decreased in laparoscopic cholecystectomy, but even then no patient is ready to get surgery if alternative of treatment is available. Govind Das Sen described in his treatise that *Rohitakadhya* Compound in *yakritpleeha roga chikitsa* (Bha, Ra. 41/10, 11) and *Phaltrikadi* decoction in reference to *Pandurogadhikar* (Bha, Ra. 12/22). The clinical manifestation of chronic cholecystitis and cholelithiasis resembles the manifestation of *Shakhashrita kamala*, *Jwar*, *Pandu*, *Aruchifor*

which *Rohitakadhya* Extract & *Phaltrikadi* decoction are indicated. In *Ayurvedic* text the manifestation of hepatobiliary diseases are described under heading of *Kamala* with its complication, *Pittodar*, *PittajUdarShool*, and *Yakritdadulyodar*. *Kamala* and *Yakritdadulyodar* are medical diseases and manifest as complication of extra hepatic biliary lithiasis. Both conditions can be treated with medical treatment. Most of the *UdarRog* especially *Pittodar* and *Pittaj Udar Shool* are well described in *Sushruta samhitawhich* is considered a text book belonging to surgical discipline. After *Samhitaperiod* there is increasing trend of oral herbal drugs & kshar, which became very effective for the medical management for manifestation of cholecystitis and

cholelithiasis, one of them was *Rohitakadhya* compound & *Phaltrikadi* decoction. Concept of management of a disease as per *Ayurveda* is that "*SthanVishesh Chikitsa*" (origin place of disease is treated first). In this concept many herbal drugs acting on hepatobiliary system are described for the manifestation similar to Chronic Cholecystitis and cholelithiasis. Many research works have been conducted by earlier workers on Hepatobiliary system but there are many lacunae. *Rohitakadhya extract & Phaltrikadi decoction* has been studied earlier by various researchers & got significant good result.

MATERIALS AND METHODS

This experimental study divided in four groups

Group A: Control Negative Group - given standard diet throughout the study.

Group B: Control Positive Group – Initially given lithogenic diet for 15 days then standard diet.

Group C: *Rohitakadhya extract* Treated group- Initially given lithogenic diet for 15 day then standard diet.

Group D: *Rohitakadhya extract & Phaltrikadi kwath* Treated group- Initially given lithogenic diet for 15 day then standard diet.

Dose of Standard diet - Freely access to mice.

Duration - For 15 days

Contents Standard Diet (Korean, 1999) (Wheat flour-2kg, Milk powder-250gm, Yeast-1%, Salt-1/2 tsf, Water-according to need)

Dose of Lithogenic diet- Freely access to mice.

Duration- For 15 days

ContentsLithogenic diet (Korean, 1999; By Eva Reihner, 1996)

(Wheat flour -2kg, Milk powder- 250gms, Saturated oil (dalda ghee)-500gm, Cholesterolpowder-2%, Yeast - 1%, Salt-1/2tsf)

Dose of Drug* *Rohitakadhya extract* - 5.148mg in twice a day

Phaltrikadi decoction -.208ml in twice a day

Dose fixation

Dose of the drug has been calculated by human therapeutic dose to mice on the basis of body surface area ratio (conversion factor 0.0026 for mice) by referring to the table of Paget and Barnes (1964) also referring to the internet shodhganga.inflibnet.ac.in by KJ Gohil 2011 as follows-

Rohitakadhya extract: Human dose*: 1.98gm/day, Mice dose: $1980\text{mg} \times 0.0026 = 5.148\text{mg}$

Phaltrikadi kwath: Human dose- 80ml/day, Mice dose-80ml $\times 0.0026 = .208\text{ml}$

Duration - 15 days

Route of drug administration - The drug was administered through oral route by rubber catheter sleeved on to disposable syringe.

The present study was taken to evaluate the effect of Ayurvedic Compound (*Rohitakadhya extract & Phaltrikadi decoction*) in gall bladder stone of Swiss albino mice on the basis of morphological, microscopical& histological character of gall bladder & its mucosa.

Conclusion

It was found that anti lithogenic effect of Ayurvedic formulation on resected Swiss albino mice gall bladder. In view of histological study resected mice's gall bladder showed there were no inflammatory changes and stones. Although the group who received only *Rohitakadhya Extract* showed few of sludge's particles and inflammatory cells. The forth group where both the trial drug was given showed complete absence of sludge particles or stones and devoid of inflammatory cells. Overall findings suggest that trial drug (*Rohitakadhya Extract and Phaltrikadi decoction*) are effective to the great extent and may prevent the stone formation as well as sludge's & inflammatory cells.

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