

Evaluation of Oxidative Stress Status Following Polyherbal Formulation Therapy In Patients of Cholelithiasis with Choledocholithiasis

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ABSTRACT:

Free radicals produce persistent oxidative stress in biological system and are highly reactive molecules produced as a byproduct of metabolism. A reactive free radical generated in the body reacts with non-radical molecules and results in free radical chain reaction leading to formation of new free radicals. If the defense mechanism of body fails to combat them or they are not properly utilized in the body –these silent killers pose a threat by injuring tissues, their proteins and fat contents. Lipids in the cell membrane undergo degradation to form hydroperoxides^(1, 2, 3). Polyunsaturated fatty acids, PUFA, are especially liable to lipid peroxidation. Lipid hydroperoxids decompose to form a variety of products including malondialdehyde (MDA) which is used as an indicator of oxidative damage of cells and tissues⁽⁴⁾.

The present investigations involve the study of oxidative stress in the bile juice from the patients of cholecystitis/cholelithiasis with choledocholithiasis treated by cholecystectomy with choledochotomy (CBD exploration) with T-tube drainage. Results of malondialdehyde status in the bile juice of these patients pre-operatively and following polyherbal formulation therapy from 3rd to 10th post operative day are discussed.

Key Words: Oxidative stress, MDA status, Bile, Polyherbal drug therapy.

INTRODUCTION

Phaltrikadi Kwath is a well known and commonly used drug in the management of (Jaundice) kamala roga. This decoction comprises of eight different plants viz. Haritaki, Vibhitak, Amalaki, Guduchi, Kutki, Chirayita, Vasa and Neem. The chemical constituents and pharmacological actions of these drugs are well established. These are potent cholagogue, immunomodulator, antiallergic, antioxidant, bitter, anti-inflammatory, antiallergic, antioxidant, antipyretic and hepatoprotective

drugs. Therefore the present study was carried out to evaluate the efficacy of “Phaltrikadi Kwath” in the patients before and after administration of the drug following the cholecystectomy.

MATERIAL AND METHODS

Selection of Cases

Thirty cases of cholelithiasis with choledocholithiasis were selected from the

patients attending to shalya OPD/IPD of University Hospital, BHU. Each patient was selected on the basis of the diagnostic criteria which included a detailed history in the form of chief complaint-occurrence of abdominal pain, its site, character, duration, associated features Viz. nausea, vomiting, fever, chills, jaundice and flatulence. Apart from this, history of systemic disorders like tuberculosis, hypertension and diabetes mellitus was taken. History of previous treatment particularly previous surgery (type of operation), family history, occupation and dietary habits was recorded. Blood, urine, stool and radiological investigations were done for each patient.

Selection Clinical Model

The selection of clinical model was done in such a way so that it should be easy, non-invasive, less expensive and patient friendly to meet our the needs of the research work. Therefore, we selected the patients (age group 30-60 years) of cholecystitis/cholelithiasis with choledocholithiasis treated by cholecystectomy with choledochotomy (CBD exploration) with T-tube drainage.

In these patients T-tube drainage of bile was the usual procedure and the bile was collected either directly from the T-tube or collection bag without doing anything specific to the patients for at least 10 days. In routine, postoperative bile samples were taken by T-tube whereas intra-operative samples were taken by direct aspiration of common bile duct.

Thirty cases of cholelithiasis with choledocholithiasis were selected for the present study and they were divided into two groups of fifteen each. Group B was the drug treated whereas group A was without any drug and served as control.

Drug and Dosage

Table 1 illustrates the composition of drug material of polyherbal formulation known as "Phaltrikadi K wath" depicting the name of ingredients, part taken along with its properties for preparation of decoction. All the ingredients were dried in shade, taken in equal proportion and chopped into small pieces. All materials were thoroughly mixed and soaked into water for 3-4 hours and then boiled in the stainless steel container at 70°-80° c till only one-fourth (1/4th) of the initial volume remained. During this period intermittent stirring of the contents was done. The decoction was finally filtered through muslin cloth and then allowed to cool at room temperature. For preparation of 100ml of drug (decoction) approximately 22 gms of crude drug mixture was taken.

Freshly prepared decoction was given orally to each patient of group (B) at the dosage of 30ml twice daily and the duration was 3rd to 10th post operation day whereas no drug (A) cases during this period.

Physico-chemical studies of the drug (decoction) were made to record its absorption spectrum by using a recording Beckman spectrophotometer, pH with pH meter, viscosity in relation to water with an Ostwald's type of viscometer, relative density with a specific gravity bottle, refractive index with a refractometer, conductivity with a conductometer, Osmolarity with an Osmometer and polarity with a polarimeter.

Assay of Lipid peroxidation (LPO):

The assay of LPO in the serum was performed by the technique of Philpot⁽⁵⁾ with the suggested modifications⁽⁶⁾. A fresh stock reagent of TBA-TCA-HCl was

prepared at the time of assay. 0.01% butylated hydroxy toluene was added to the stock reagent to abolish the metal catalysed autoxidation of lipids⁽⁷⁾. Standard malondialdehyde bis (dimethyl) acetal solution obtained from Aldrich chemical Co., USA. 2ml of stock reagent was added separately to 1ml test sample, standard and blank respectively. All the samples of test, standard and blank were heated for 20 minutes at 80°C and then allowed to cool at room temperature. Thereafter, the pink pigment from all the samples was extracted with 4ml of n-butanol and their optical absorbance was recorded at 530 nm in a SICO spectrophotometer.

RESULTS

All the patients in both the groups were age and sex matched. The patients were in the age group of 30-60 years. The patients were distributed equally in both the groups according to their habits viz., vegetarian or non vegetarian, occupation, socio-economic and bowel habits. Maximum number of the patients were tobacco chewers followed by smoking and alcohol whereas 27% of the patients had no addiction. The presenting features of the patients varied from upper abdominal pain with nausea and fever. Jaundice was observed in more than 90% of cases in both the groups.

Assessment of preoperative hematological parameters of patients showed routine TLC, DLC, hemogram, fasting blood sugar level, blood urea and serum creatinine were within normal range with lower levels of hemoglobin values. Liver function tests were also performed in all patients. Serum bilirubin levels were higher in group in group B cases.

Physico-chemical characteristics of drug "Phaltrikadi K wath" shown in the table 2

points that it contains approximately 5% total solid content, reducing sugar and tannins were present in all samples whereas polysaccharides were absent; with mean values of pH 3.52 ± 0.14 , viscosity 1.465 ± 0.024 centipoise, specific gravity 1.008 ± 0.0013 , refractive index 1.333 ± 0.0007 , conductivity 2.406 ± 0.234 m Mhos/cm and Osmolarity 139.65 ± 4.0688 mOsm/kg. The decoction (Kwath) is optically inactive.

The effect of Phaltrikadi Kwath (decoction) was assessed in the randomly selected 30 patients. The drug was given orally in the form of freshly prepared decoction to all the patients of treated cases (group B from 3rd post-operative day to 10th post operative day whereas no drug was given to the control cases (group A) during this period.

Lipid peroxidation levels in the bile juice of patients of cholecystitis/cholelithiasis with choledocholithiasis treated by cholecystectomy with choledochotomy (CBD exploration) and Kehr's T-tube drainage were assessed in all the treated patients immediately on day 0 (sample I), on 3rd day (sample II) and on 10th day (sample III). Bile samples were taken from all the patients irrespective of the size, number and type of stones. Bile samples were analysed for malondialdehyde levels immediately after operation or were stored at 20°C until the time of analysis. The results were compared with their control cases (table 3).

The observed lipid peroxidation levels show.

Difference between the levels of sample I (day 0) and sample II (day 3) in both the groups was significant ($p < 0.001$)

Difference between the levels of sample II (day 3) and sample III (day 10) in both the groups was significant ($p < 0.0001$)

Difference between the levels of sample I (day 0) and sample III (day 10) in control and treated groups was also significant at $P < 0.01$ and $p < 0.001$ respectively.

Observed increase in lipid peroxidation levels in sample I and II followed by a decrease in MDA levels in sample III on day 10 of drug therapy with respect to control cases points that the drug “Phaltrikadi Kwath” has antioxidant potential.

DISCUSSION

This study was designed to evaluate the oxidative stress status following the polyherbal drug formulation therapy in patients of cholelithiasis with choledocholithiasis. In Ayurveda “Phaltrikadi Kwath” is described for management of obstructive jaundice shakhashrita kamala roga⁸. Phaltrikadi Kwath contains eight herbal drugs which are having predominantly kamalahara properties like pittkapha shamaka, yakriduttejaka, shothahara, pandurogahar, rechan, and Deepan etc.

The objective of the present study therefore was to evaluate antioxidant potential of polyherbal formulation “Phaltrikadi Kwath” in biliary stone diseases.

To assess the effect of phaltrikadi Kwath, bile samples were analyzed at different intervals and the study points that the drug significantly lowers the oxidative stress in bile. As free radical injury is proved to be implicated in gall stone formation, reduction of free radical formation (oxidative stress) improves the biochemistry of bile and thus prevents the stone formation.

CONCLUSION

- ✓ Surgical trauma exerts significant oxidative stress in the body as it is seen in bile of both the groups.
- ✓ Free radical injury in gall bladder alone is not responsible for gallstone formation; this is the liver bile which may contribute to the gallstone formation.
- ✓ The evaluated polyherbal formulation (PHALTRIKADI KWATH) has got antioxidant potential as it decreases oxidative stress more as compared to control group.
- ✓ The drug can be used prophylactically to prevent the recurrence of stones and oxidative stress in bile.
- ✓ Drug is cheap, freely available, easily prepared and cost –effective.

Table 1: Composition of DRUG MATERIAL

In Ayurvedic classics (Sarangdhar Samhita – Madhyama Khan; 257) the polyherbal Formulation is known as “PHALTRIKADIKWATH (=KASHAYA)”, decoction (water extract) of eight different plants taken in equal quantity.

S. No	Name of Ingredients	Part Taken	Properties
1.	<i>Haritaki [Terminalia chebula]</i>	Fruit without seed	Antiseptic Antispasmodic, Anti-inflammatory

2	<i>Vibhitaki [Terminalia bellerica]</i>	Fruit without seed	Choleretic Antipyretic, Antiemetic, Anti-inflammatory
3	<i>Amalaki [Embllica officinalis]</i>	Fruit without seed	Antipyretic, Antispasmodic, Antioxidant
4	<i>Guduchi [Tinospora cordifolia]</i>	Stem	Anticomplementary, Antispasmodic, Antioxidant Immunomodulator
5	<i>Kutki [picrorrhiza kurroa]</i>	Rhizomes	Choleretic, Anticholestatic, Antiallergic, Antianaphylactic, Anti-inflammatory, Immunostimulant Potent Antioxidant
6	<i>Chirayita [Swertia chirayita]</i>	Whole plant	Tonic, Antispasmodic, Antipyretic, anti-inflammatory
7	<i>Vasa [Adhatoda vasica]</i>	Whole plant	Astringent, Antispasmodic, Tonic, Styptic
8	<i>Neem[azdirechta indica]</i>	Stem bark	Atipyretic, Anti-inflammatory, Liver tonic

Table 2: Physico – chemical parameter of Phaltrikadi Kwath:

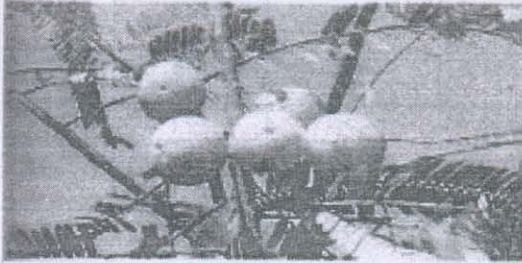
S.No	pH	Viscosity Centipoise at 20oc	Specific gravity	Refractive Index	Conductivity mMhos/cm	Osmolarity mOsm/kg	Polarity
1	3.4	1.458	1.008	1.334	2.38	135	NP
2.	3.4	1.446	1.008	1.333	2.38	135	NP
3.	3.8	1.459	1.006	1.334	2.40	138	NP
4.	3.6	1.462	1.008	1.334	2.42	142	NP
5.	3.4	1.462	1.006	1.334	2.44	144	NP
6.	3.5	1.458	1.008	1.332	2.42	145	NP
7.	3.4	1.458	1.010	1.334	2.42	135	NP
8.	3.4	1.466	1.006	1.334	2.44	145	NP
9.	3.5	1.462	1.008	1.332	2.38	138	NP
10.	3.4	1.462	1.010	1.334	2.40	144	NP
11.	3.6	1.466	1.010	1.334	2.38	138	NP
12	3.4	1.458	1.008	1.333	2.38	142	NP
13	3.4	1.459	1.006	1.333	2.38	145	NP
14	3.5	1.460	1.010	1.334	2.40	142	NP
15	3.6	1.462	1.008	1.334	2.42	136	NP
16	3.8	1.460	1.008	1.334	2.38	135	NP
17	3.6	1.467	1.010	1.334	2.44	138	NP

18	3.4	1.462	1.008	1.334	2.44	136	NP
19	3.6	1.458	1.008	1.332	2.42	135	NP
20	3.8	1.467	1.008	1.334	2.40	145	NP
Mean	3.525	1.465	1.008	1.333	2.406	139.65	-
SD	0.1446	0.024	0.0013	0.0007	0.234	4.0688	

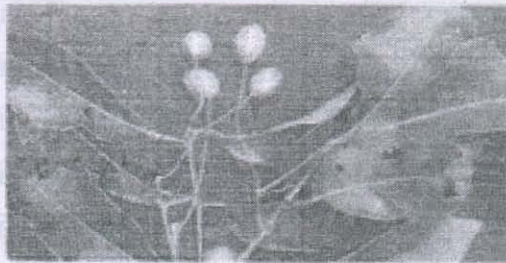
Table 3: Lipid peroxidation levels, MDA in mmol/L, in bile juice at different intervals:

Group	Sample I (Day 0) Mean ±SD	Sample II (Day 3) Mean ±SD	Sample III (Day 10) Mean ±SD	I vs II	I vs III	II vs III
				P-value		
Group A Control (n=15)	3.0240±1.008	3.4941±1.01109	2.66601±0.9016	<0.001	<0.01	<0.001
Group B Treated (n=15)	3.5252±1.5677	4.4702±1.5059	2.4855±0.9020	<0.001	<0.001	<0.001

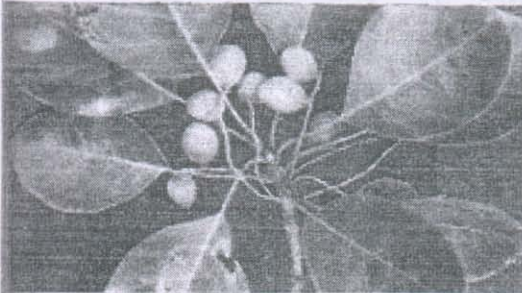
INGREDIENTS OF PHALTRIKADI KWATH



AMALKI [*Emblica officinalis*]



HARITAKI [*Terminalia chebula*]



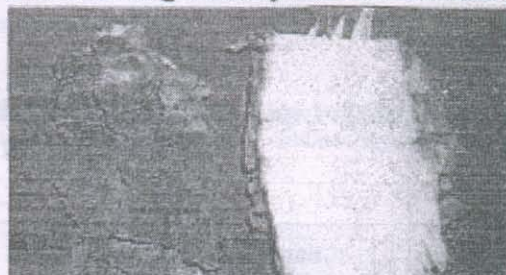
VIBHITAK [*Terminalia bellerica*]



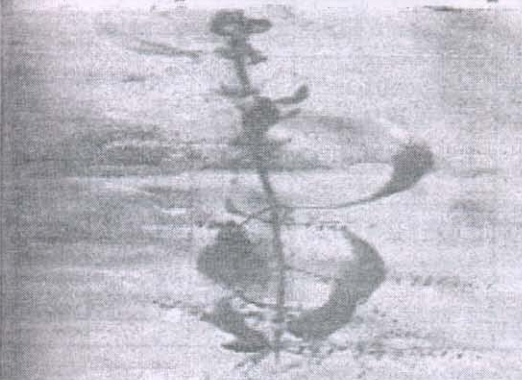
GUDUCHI [*Tinospora cordifolia*]



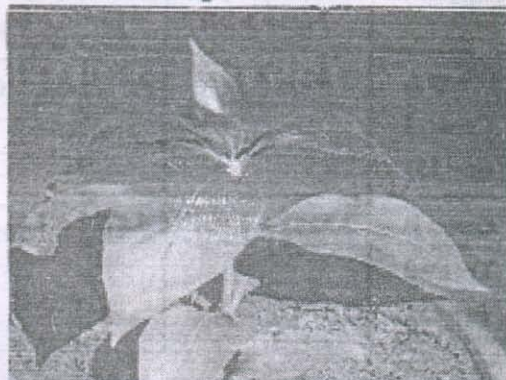
KATUKA [*Picrorrhiza kurroa*]



NIMBA BARK [*Azadirachta indica*]



CHIRAYITA [*Swertia chirayita*]



VASA [*Adhatoda vasica*]

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