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In Vivo Study of Unani Herbal Formulation Beikh-E-Karafs (Apium graveolens) Revandchini (Rheum Emodi) Namak-E-Turb (Raphanus sativus) with Lithogenic diet (Cholesterol Cholic acid & Fatty diet) Induced Hasat-ul-Mirarah (Cholelithiasis) in Mice

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Abstract: ***Cholelithiasis is a chronic and recurrent hepato-biliary disease resulting from the impaired metabolism of cholesterol, bilirubin, and bile acids. This condition is characterized by the formation of gallstones in the gallbladder and common bile duct. The development of gallstones is a complex process involving various factors such as cholesterol supersaturation, nucleation, and crystal growth. The interaction of genetic, environmental, and lifestyle factors contributes to the pathogenesis of cholelithiasis. Management strategies for cholelithiasis include lifestyle modifications, pharmacological interventions and in some cases, surgical procedures such as cholecystectomy. Understanding the underlying mechanisms of gallstone formation is crucial for the development of effective preventive and therapeutic approaches. The disease was mentioned by Hippocrates as Biliary obstructive disease. Accordingto Unani physicians the Hasath-ul-mirarah is due to imbalanced humours in the body Safra (Bile) and Phlegm are the main factors to produce a "Hasat-ul-mirarah." Aim: "In Vivo study of Unani herbal formulation Beikh-e-Karafs (Apium graveolens) Revandchini (Rheum emodi) Namak-e-Turb (Raphanus sativus) with lithogenic diet (cholesterol, cholic acid & fatty diet) induced Hasat-ul-Mirarah (Cholelithiasis) in Mice". To evaluate the Physiochemical analysis and Standardization of Unani Herbal formulation. To manage the disease and its complication with safe, cost effective and easily available drug in the Unani system of medicine. Material & Methods: Swiss Albino Mice were randomly divided into Control group, Disease Control group, Ethanolic Extract Test Group (Low dose & High dose), Crude Test Group (Low dose & High dose) 6 animals in each group. Gall Stones were induced in mice by feeding lithogenic diet for 8 weeks. After 8 weeks, Disease control group was sacrificed sampled to calculate the incidence of stone formation and biochemical parameters (Serum Cholesterol, triglycerides HDL, SGPT, SGOT, ALP, Bilirubin, Albumin and Total proteins, Serium amylase) and Histopathological assessment was done. The test drug was given for 4 weeks to Ethanolic Extract Test Group (Low dose & High dose) Crude Test Group (Low dose & High dose), after 4week animals was sacrificed biochemical parameters and Histopathological assessment was done. Result: There is significant decreased in biochemical parameters (P<0.05***) & histopathological changes were reduced in liver and the inflammatory changes were less in gall bladder in Ethanolic Extract Test Group (High dose) (0.014mg/kg). The results were less in Crude Test Group (Low dose & High dose) Ethanolic Extract Test Group (Low dose) when compared to Ethanolic Extract Test Group (High dose) Conclusion: Unani herbal formulation given in Ethanolic extract form can decrease the incidences of stone formation and improve the biochemical parameters, which may be one of the mechanisms in the treatment and prevention of gall stone formation.***

Keywords: Cholelithiasis, Unani herbal formulation, biochemical parameters, ethanolic extract & crude test drug

1. Introduction

Gallstones are a common biliary pathology, with an estimated prevalence of 10-15% in the adult population of developing countries. The majority of gallstones are asymptomatic, with more than 80% of patients not experiencing any symptoms. However, approximately 1-2% of asymptomatic patients will develop symptoms that may require cholecystectomy. [1]

Pathogenesis: In Unani literature, *Hasat-ul-mirarah* Gallstones in the gallbladder form as a result of an increase in bile concentration in the gallbladder, which is also known as a bile stone or *Safrawai pathriya*. *[*2] Gallstones are formed by the following method**: (**1) Before the bile solidifies, a small crystal is formed, upon which layers are getting deposited in succession. (2) There is a specific substance in the bile (*Jamad-e-Safrawi: Hajr-e-Safrawi*), which becomes precipitated under special conditions. (3) Layers are formed over the solidified fine thread / balgham. (4) In the composition of *safra*, when the alkaline elements are reduced and the acidic elements are more, or when lime elements (*Ajza-e-arziya)* are more stones are formed. [3] [4] The major kind of abnormal safra is that in which the *kayfiyat (*quality-composition) of bile is itself altered eg. bile pigments specially bilirubin, calcium and cholesterol are altered with respect to their quantity and as such their proper ratio is disbalanced and thereby the *kayfiyat of safra* is altered, which develops gall stones. Therefore, bilirubin stone is formed in those persons whose bilirubin production is increased. The cause of bilirubin stone in the patients of anaemia due to excessive haemolysis of red blood

corpuscles is the excessive production of bilirubin. Similarly, when the ratio of bile salts is disturbed, cholesterol begins to precipitate, which produces cholesterol stones. Likewise, when the quantity of *balgham (*mucous) in the safra is increased, it begins to cause precipitation of bilirubin and calcium, which develops gall stones. [5] According to *Jalinoos,* sometimes it happens when gall bladder congestion occurs, therefore the process of absorption of bile from liver stops, sometimes due to bile duct obstruction or due to weakens of *Quwat-e-jazeba* of gallbladder. *(Kitab al hawi)* [6]According to *Ibn rushd,* the causes of *Sue mizaj* of liver are generally the same as those of *Amraz hadda* maddi and Qair maddi (material and non-material disease), except the cause specific to liver or gall bladder, which is the suspension of the function of gallbladder, which is caused either by blockage between the liver and gallbladder or *Sudda* develop in canal or the absorption power *Quwat-e-Jaziba* of gallbladder is weak, or the obstruction occur in the canal between gallbladder and intestine, due to the weaken of *Quwat-e-Dafiya* of gallbladder. [7]

In contemporary medicine, the use of non-surgical procedures or medications to dissolve gallstones is still regarded as experimental. Occasionally, oral urodeoxycholic acid can dissolve cholesterol gallstones, but the patient may need to take this medicine for one or two years; once the medication is stopped, gallstones may recur. Most of the times, two thirds of the individuals undergo Cholecystectomy. [11] [12] [13] [14] Overall, it is evident that the treatment for Cholelithiasis recommended by modern medicine is far from effective and frequently accompanied by a number of negative effects. So, there is large scope in Unani medicine in the field of management of Cholelithiasis.

To reduce the risk of surgery and to prove the efficacy of Unani medicine, an Experimental study was conducted in animal model Mice, in which gall stones were induced in mice using Lithogenic diet (**Cholesterol 0.1%, Cholic acid 0.5% & Fatty diet)** [8] [9] [10] which is purchased from **National Institute of Nutrition, ICMR, Tarnaka.** and the test drug or *Unani herbal Formulation* was made with *Beikh-e-Karafs (*Apium graveolens) *Revandhchini (*Rheum emodi) *Namak-e-Turb (*Raphanus sativus Linn) was given along with normal diet. It assumed to be useful in management of Cholelithiasis, and choosing these drugs are based on their *Mufattit-e-hasat (*lithotriptic) [15] *Mufateh-sudad (*Deobstruent) [16] *Muhallil (*Resolvent) [17] *Mulateef* (Demulcent), *Mudir* (Diuretics), *Muhalil-e-awram* (Anti-inflammatory) *Muqavi-e-jigar* (hepatotonic), *Mullaiyain at* (mildlaxative), Antidyslipidemic effects. [18] [19]. The present work is expected to contribute substantially to the fulfilment of this requirement.

2. Material & Methods

This research was approved by IAEC (Institutional Animal Ethical Committee GNTC HYD) Reg. No 1070/ac/07CPCSEA. Both Male and Female Swiss Albino Mice, with weight 25-30gms, 10-12 weeks of age, were purchased from the Mahaveera Enterprises Laboratory agency (CPCSEA-NO.1665/P0/Bt/S/12CPCSEA), Ghatkesar, Dist. Medchal-Malkajgiri, Hyderabad, Telangana.

Collection of Test drug Material: Test drug Beikh-e-Karafs (Apium graveolens), Revandchini (Rheum emodi), Namak-e-turb (Raphanus sativus linn) were provided and identified by the department of Ilmul Advia, Govt. Nizamia Tibbi College, Charminar, Hyderabad. These drugs were submitted for Botanical Identification and Authentication to Survey of Medicinal Plant Unit (SMPU), NRIUM-SD. Identification and authentication was done by Dr. Mohd Kashif Hussain (Botanist) of SMPU of NRIUM-SD, Hyderabad.

Test drug: The test drugs used for experimental studies are in the form of Ethanolic extract (Low dose & High dose) and Crude test drug (Low dose & High dose) of Beikh-e-karafs (Apium graveolens) & Revandchini (Rheum emodi), Namak-e-turb (Raphanus sativus linn) with Oral route. Extraction was prepared through the process of Soxhlet apparatus. **Extraction of Salt from Raphanus sativus:** 5kgs of whole Raphanus sativus was taken and cut into small pieces and dried well (358gm). The pieces are put in earthen pot and burnt into ash (114gm). then the ash is allowed to cool. Water is added to the ash and mixed well, then strained through filter paper. This process of straining may be done two or three times till a clear liquid is obtained. This liquid is then put in an iron or earthen vessel and heated over a moderate fire till the water evaporates, leaving a solid salty white substance which is collected as salt (6gm).

Dose of the test drugs: The doses of the test drugs for Swiss Albino mice were calculated accordingly using the human equivalent dose as described in Unani literature multiply by the conversion factor of mice 12.3. The dose of the test drug "Revandchini" in Unani literatures mentioned as 1.5-2gm [20], dose of "Beikh-e-karafs"as 5-7gm [21] and dose of Namak-e-turb as 0.5-1gm [22] [23]

Formula for dose conversion based on body Surface Area:
Human Equivalent Dose (HED) (mg/kg) =Animal dose (mg/kg) multiplied by Animal Km/Human Km. Combined Crude low dose of mice-1438.4mg/kg & Combined Crude high dose of mice-2054.91mg/kg. Dose of Crude drug of animal divide by yield of extract, will give the Extract dose, i. e Combined Crude low dose of animal is 1438.4mg/kg and yield is 30%, gives Combined Extract low dose of mice-48.4mg/kg & Combined Extract high dose of mice-69.4mg/kg.

Experimental Procedure: Swiss Albino mice were divided into 6 groups for Ethanolic extract test group (Low dose & High dose) & for Crude test group (Low dose & High dose) of each group having 6 animals as mentioned below:

Group I: Animals received chow (feed) and normal saline orally for 90 days and was considered as Plane control group.

Group II: Animals received normal saline and Lithogenic diet, for 60 days and was considered as Disease control group, and sacrificed after 60days, and sampled to calculate the incidence of stone formation and biochemical parameters

(Serum Cholesterol, triglycerides, HDL, SGPT, SGOT, ALP, Bilirubin, Albumin and Total proteins, Serium amylase) and Histopathological assessment was done.

Group III: Animals received chow (feed), normal saline, Lithogenic diet and Ethanolic extract of 1.62 mg/kg body weight (Low dose) test drug was given after 60days of induced for 30 days.

Group IV: Animals received chow (feed), normal saline, Lithogenic diet and Ethanolic extract of 3.69 mg/kg body weight (High dose) test drug was given after 60days of induced for 30 days.

Group V: Animals received chow (feed), normal saline, Lithogenic diet and Crude drug of 40.05 mg/kg body weight (Low dose) test drug was given after 60 days of induced for 30 days.

Group VI: Animals received chow (feed), normal saline, Lithogenic diet and Crude drug of 55.4 mg/kg body weight (High dose) test drug was given after 60 days of induced for 30 days.

At the end of experiment /study (on 90th day) all the animals from each group, either sex of Ethanolic extract and Crude test drug from low and high dose groups were sacrificed by Euthanizing the animals. Before sacrifice blood sample was collected by retro orbital for biochemical assay (CBC, LFT, KFT, LIPID PROFILE, Sr. AMYLASE). Blood was collected in labelled test tubes and Brain, Liver, Gall bladder and Kidney of animals from each group were dissected out, washed with normal saline, and preserved in Formalin 10% for the purpose of Histopathological examination and send to the Veterinary pathology lab by maintaining cold chain.

Statistical analysis: Parameters mentioned above were assessed in all groups and finding were expressed as Mean ± SEM. The different values determined were compared with each other and comparison was made using One way ANOVA test. The difference of mean was considered significant at p value of 0.05 or less.

3. Observation & Results

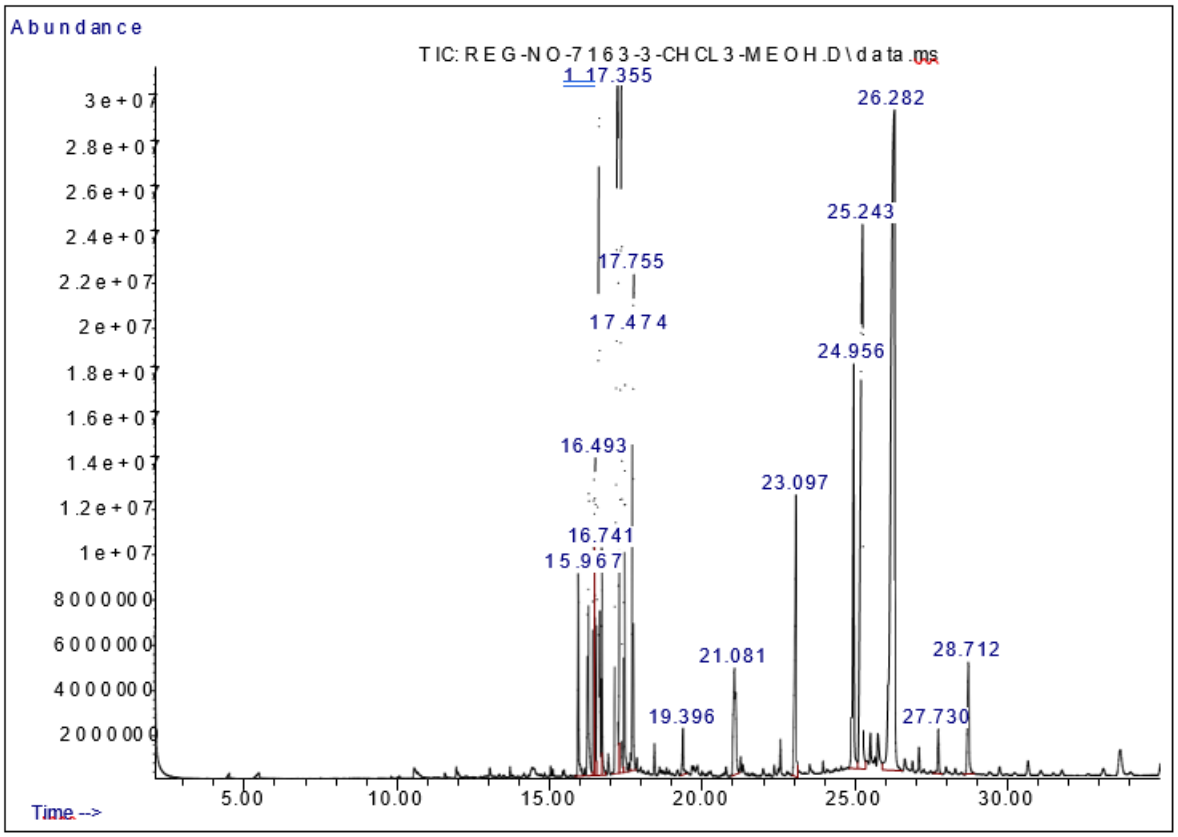
Thin Layer Chromatography done at CSIR-IICT, Uppal Road, Tarnaka: Thin layer chromatography is one of the important parameters used for detecting the adulteration for determining the standard of the drugs. The resolution of different kinds of chemical components are separated by using TLC and calculating the Rf values after detecting the spots in order to standardise the drug for its identity, purity and strength. TLC studies of compound formulation obtained in different organic solvent was conducted and Rf value of various spots appeared in different solvent were noted.

Rf =Distance travelled by the spot /Distance travelled by the solvent.

Form	Solvent System	Spray or Treatment	No. of Spots	Rf Values
Crude	Ethyl Acetate and Toulene (80: 20)	Iodine 10% per chloric acid	3	0.56 0.63 0.90
Ethanolic Extract	Ethyl Acetate and Toulene (80: 20)	Iodine 10% per chloric acid	3	0.48 0.52 0.74

GC-MS Analysis: GC-MS Analysis of Polyherbal formulation Research drugs done at CSIR-IICT, Uppal Road, Tarnaka. Gas Chromatography (GC) and Mass Spectroscopy (MS) works a powerful tool to have a deeper insight by identifying the various compounds present in the sample. Present study was focus on identification of compounds by using GC-MS technique and biological activities of Unani Compound formulation using Methanol and Chloroform as a solvent.

Peak no.	Compoud identified	Retention time	Area	Peak Area %
8	2-Methoxy-4-vinyl-phenol	11.971min	12340243	0.09
12	Phenol, 2, 4, 6-trimethyl-	13.733min	9127965	0.07
15	Cyclohexane, 1-ethenyl-1 Methyl	14.333min	3716585	0.03
17	Cyclohexene, 4-[(1E)-1, 5-Dimethyl	14.508min	30382952	0.23
19	Cyclohexane, 1-ethenyl-1-Methyl	15.058min	10633288	0.08
19	Cyclohexanol, 2-methyl-5-(1-Methyl	15.058min	10633288	0.08
28	Cyclohexene, 1-methyl-4-(1-Methyl	16.745min	212204877	1.59
45	n-Hexadecanoic acid	19.394min	65913035	0.49
55	9, 12-Octadecadienoic acid	21.081min	293814897	2.2
56	Stearic Acid	21.281min	25320733	0.19
68	Cyclohexane, 1, 1-bis (5-methyl-2	24.118min	15209930	0.11
77	9, 10-Anthracenedione, 1, 8-Dihydro	25.405min	23488072	0.18
83	Emodin	26.642min	65713373	0.49
85	Phthalicacid, isobutyl3-methox	27.105min	49134926	0.37
92	Phenol, 2-(1, 1-dimethylethyl) 3	29.729min	19089303	0.14
94	Chrysophanol	30.653min	32807118	0.25
97	Physcion	31.766min	13641349	0.1
98	4-(methylthio)-3-butenyl Isothiocyanate	33.115min	17810244	0.13
99	Dimethyl trisulfide	33.665min	89085465	0.67



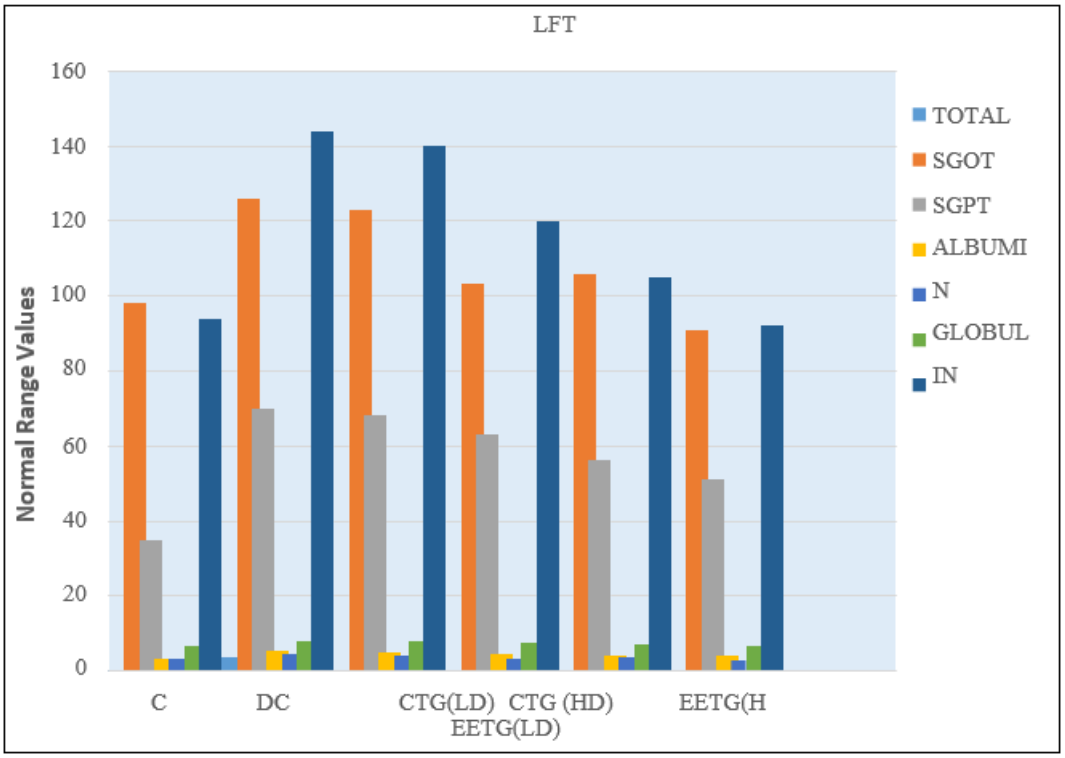
Acute toxicity study:
The animals were observed continuously for 1-2 hrs for gross behavioural changes and intermittently once every 2 hrs and finally at 24 and 72 hours to note any signs of toxicity including death of animal. All the animals were safe at this dose and No LD 50 was recorded.

Mortality: There were no deaths either on the day of treatment or throughout the 14-days post-treatment observation period.

Necroscopy: All the animals survived until the scheduled necroscopy on Day-15. No abnormalities were noted and all organs of mice proved to be free of Combined drug dose treatment related gross pathological changes.

Haematological Parameters: Liver Function Test

S. N O	Parameters	Control	Disease Control	CD (Low)	CD (High)	EE (Low)	EE (High)
1	TOTAL BILIRUBIN	SEM 0.4167 ± 0.06009	SEM 3.700 ± 0.05774	SEM 0.4667 ± 0.03333***	SEM 0.3500 ± 0.04282***	SEM 0.4167 ±0.04773***	SEM 0.3167 ±0.04773***
		SD 0.1472	SD 0.1414	SD 0.08165	SD 0.1049	SD 0.1169	SD 0.1169
2	ALKALINE PHOSPHATE	SEM 94.00 ± 2.696 SD 6.603	SEM 144.0 ± 2.921 SD 7.155	SEM 140.2 ± 2.056 SD 5.037	SEM 120.3 ± 3.739***SD 9.158	SEM 105.0 ±2.160*** SD 5.292	SEM 92.50 ±2.094*** SD 5.128
3	SGOT/AST	SEM 97.67 ± 2.929	SEM 126.0 ± 2.366	SEM 123.3 ± 1.994	SEM 102.8 ± 1.579***	SEM 104.5 ±2.446***	SEM 91.33 ±2.390***
		SD 7.174	SD 5.797	SD 4.885	SD 3.869	SD 5.992	SD 5.854
4	SGPT/ALT	SEM 34.67 ± 2.894	SEM 69.50 ± 1.727	SEM 67.50 ± 1.544	SEM 62.83 ± 2.272	SEM 55.67 ±1.054***	SEM 50.67 ±1.542***
		SD 7.090	SD 4.231	SD 3.782	SD 5.565	SD 2.582	SD 3.777
5	TOTAL PROTIEN	SEM 6.533 ± 0.1022 SD 0.2503	SEM 8.100 ± 0.1155 SD 0.2828	SEM 7.917 ± 0.07032 SD 0.1722	SEM 7.467 ±0.08819*** SD 0.2160	SEM 7.183 ±0.07032*** SD 0.1722	SEM 6.483 ±0.09458*** SD 0.2317
6	ALBUMIN	SEM 3.400 ± 0.09661	SEM 5.383 ± 0.1167	SEM 5.117 ± 0.1195	SEM 4.550 ±0.07638***	SEM 4.350 ±0.07638***	SEM 3.967 ±0.2076***
		SD 0.2366	SD 0.2858	SD 0.2927	SD 0.1871	SD 0.1871	SD 0.5086
7	GLOBULIN	SEM 3.200 ± 0.1065	SEM 4.450 ± 0.1500	SEM 4.217 ± 0.1195	SEM 3.467 ±0.1022***	SEM 3.700 ±0.05774**	SEM 2.950 ±0.2141***
		SD 0.2608	SD 0.3674	SD 0.2927	SD 0.2503	SD 0.1414	SD 0.5244
8	P Value			P≤0.001	P≤0.001	P≤0.001	P≤0.001

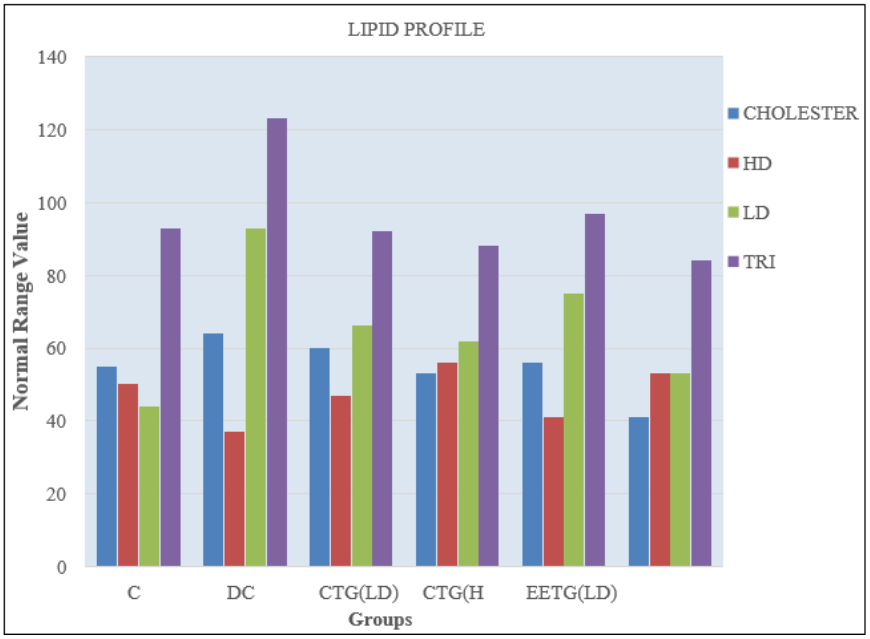


In Turkey's Multiple comparison test there is significant difference in Liver Function Test between EETG (HD) and other groups. The p-value is significant (***P<0.05) indicating that the difference is statistically significant.

Lipid Profile

S. No	Parameters	Control	Disease Control	CD (Low)	CD (High)	EE (Low)	EE (High)
1	CHOLESTEROL	SEM 55.00 ± 1.673	SEM 63.67 ± 1.116	SEM 59.67 ± 0.8819	SEM 52.50 ± 1.408***	SEM 56.00 ±1.065**	SEM 41.17 ±0.9458***
		SD 4.099	SD 2.733	SD 2.160	SD 3.450	SD 2.608	SD 2.317
2	HDL	SEM 49.83 ± 1.376	SEM 36.67 ± 0.8819	SEM 46.50 ± 1.057***	SEM 55.83 ± 0.9458***	SEM 41.17 ±0.9458	SEM 52.50 ±1.335***
		SD 3.371	SD 2.160	SD 2.588	SD 2.317	SD 2.317	SD 3.271
3	LDL	SEM 43.50 ± 2.553	SEM 92.67 ± 1.926	SEM 66.17 ± 1.905***	SEM 62.33 ± 1.820***	SEM 74.67 ±0.8819***	SEM 52.50 ±0.7638***
		SD 6.253	SD 4.719	SD 4.665	SD 4.457	SD 2.160	SD 1.871
4	TRIGLYCERIDES	SEM 93.33 ± 1.706	SEM 123.7 ± 1.116	SEM 91.83 ± 2.136***	SEM 87.67 ± 0.6667***	SEM 97.00 ±0.9661***	SEM 83.50 ±0.8466***
		SD 4.179	SD 2.733	SD 5.231	SD 1.633	SD 2.366	SD 2.074
5	P Value			P≥0.05	P≤0.0001	P≤0.001	P≤0.0001

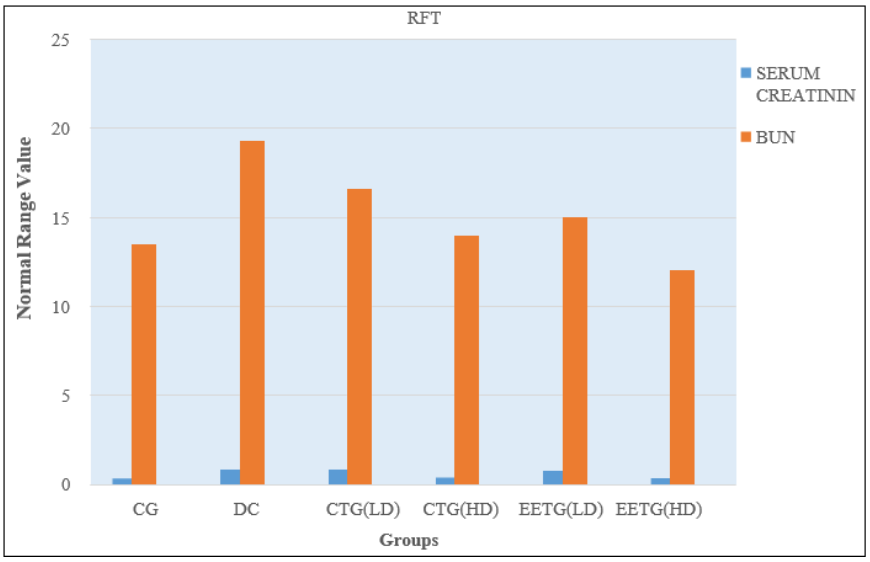
In Turkey's Multiple comparison test there is significant difference in Liver Function Test between EETG (HD) and other groups. The p-value is significant (***P<0.05) indicating that the difference is statistically significant.



Renal Function Test

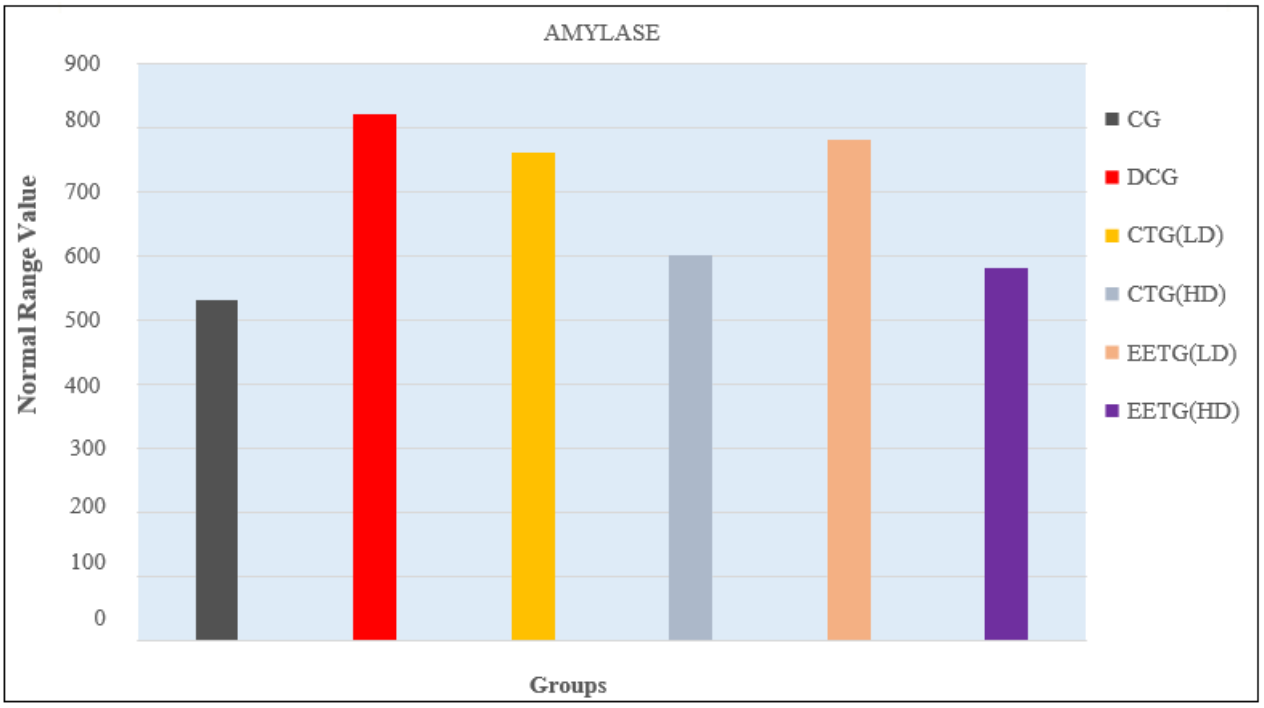
S. NO	Parameters	Control	Disease Control	CD (LOW)	CD (High)	EE (Low)	EE (High)
1	SERUM CREATININE	SEM 0.3333 ± 0.04944	SEM 0.8333 ± 0.03333	SEM 0.8333 ± 0.03333	SEM 0.3833 ± 0.06009***	SEM 0.7500 ±0.04282	SEM 0.3667 ±0.05578***
		SD 0.1211	SD 0.08165	SD 0.08165	SD 0.1472	SD 0.1049	SD 0.1366
2	BUN	SEM 13.50 ± 0.5627	SEM 19.33 ± 0.8819	SEM 16.67 ± 0.8028	SEM 14.00 ± 0.5774***	SEM 15.00 ±1.000**	SEM 12.00 ±0.5774***
		SD 1.378	SD 2.160	SD 1.966	SD 1.414	SD 2.449	SD 1.414
3	P Value			P≥0.05	P≤0.0001	P≥0.05	P≤0.0001

In Turkey's Multiple comparison test there is significant difference in Renal Function Test between EETG (HD) and other groups. The p-value is significant (***P<0.05) indicating that the difference is statistically significant.

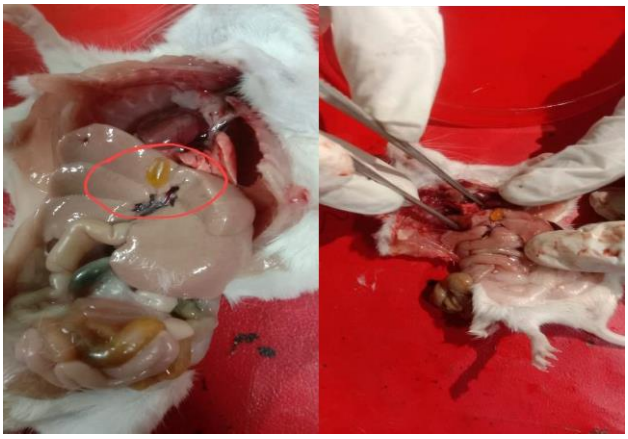


Serum Amylase

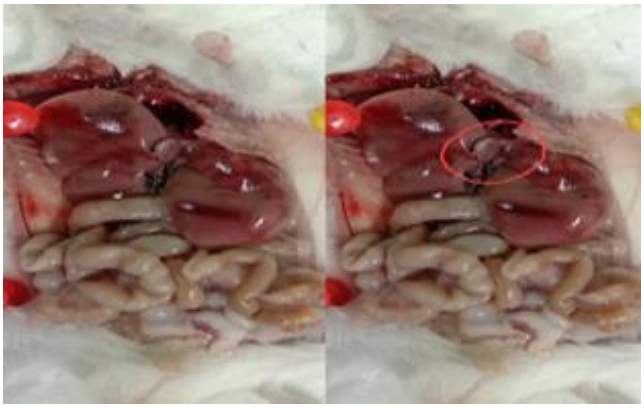
AMYLASE CONTROL	AMYLASE DISEASE CONTROL GROUP	AMYLASE CTG (LD)	AMYLASE CTG (HD)	AMYLASE EETG (LD)	AMYLASE EETG (HD)
530.	820.	760.	600.	780.	580.



In Turkey's Multiple comparison test there is significant difference in Serum Amylase Test between EETG (HD) and other groups. The p-value is significant (***P<0.05) indicating that the difference is statistically significant.



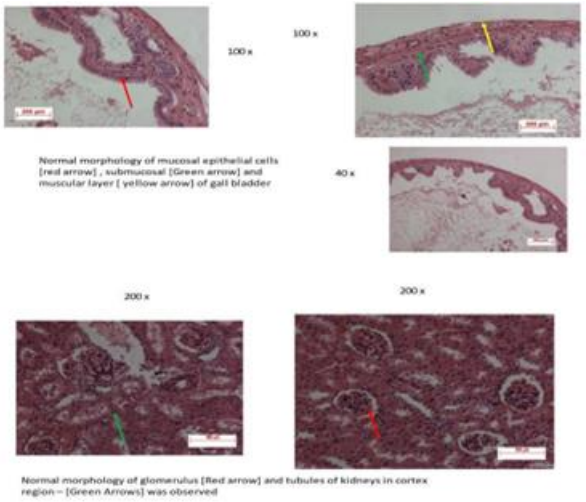
After induced with Lithogenic diet

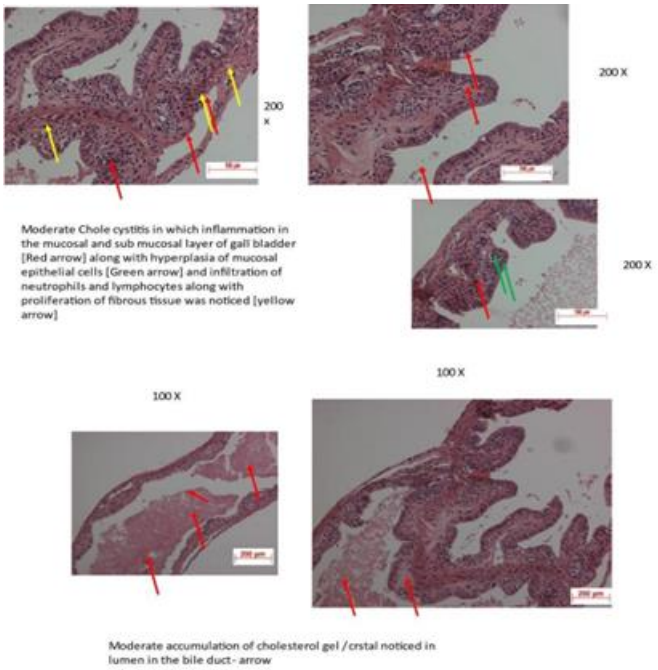


After Ethanolic Extract test drug (high dose)

Histopathology: Histopathological report of **Control group** gallbladder shows normal morphology of mucosal epithelial cells, sub mucosal and muscular layer of gall bladder and normal morphological of glomerulus and tubules of kidney in cortex region was observed. Report of **Disease Control group** of gall bladder shows moderate accumulation of cholesterol gel and crystals noticed in lumen in the bile duct along with moderate cholecystitis in which inflammation in the mucosal, sub mucosal layer of gall bladder, along with hyperplasia of mucosal epithelial cells observed and multifocal mild interstitial/tubular inflammation with infiltration of inflammatory cells seen in kidney. Mild few foci of micro/macro vesicular fatty/vacuolar degeneration of hepatocytes and foci of infiltration of lymphocytes were observed in centri lobular region of liver. Reports of **Crude Low dose** form shows moderate micro and macro vesicular fatty degeneration along with ballooning of hepatocytes was observed in lobular pattern throughout the liver and multi focal peri vascular infiltration of mononuclear cells in kidneys were observed. Reports of **Crude High dose** form shows mild diffuse spread of macrovesicular fatty degeneration of hepatocytes were noticed through out the liver, partial replacement cholecystitis. The mucosal layer appeared normal and mild tubular degeneration (proximal tubules and distal tubules) ducts with loss of brush borders, normal morphology of glomerulus tubules of kidney in cortex region was observed. Reports of **Ehanolic extract low dose** form shows mild macrovesicular fatty degeneration along with ballooning hepatocytes was noticed in peri portal region of liver. multi focal peri portal infiltration of inflammatory cells were observed. Foci of perivascular infiltration of inflammatory cells were observed in kidneys, normal morphology of glomerulus tubules of kidney in cortex region was observed. Reports of **Ehanolic extract high dose** form shows Mild cholecystitis with replacement of fibrous tissue (healing process). The mucosal layer appeared normal, no hyperplasia of mucosal epithelial cells. Submucosal inflammation with infiltration of inflammatory cells were replaced with fibrous tissue (positive response). Normal morphology of glomerulus and tubules of kidney in cortex region was observed.

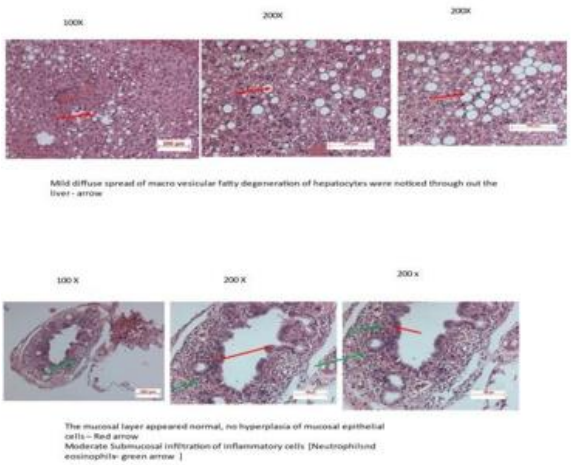
Histopathological Report of control group

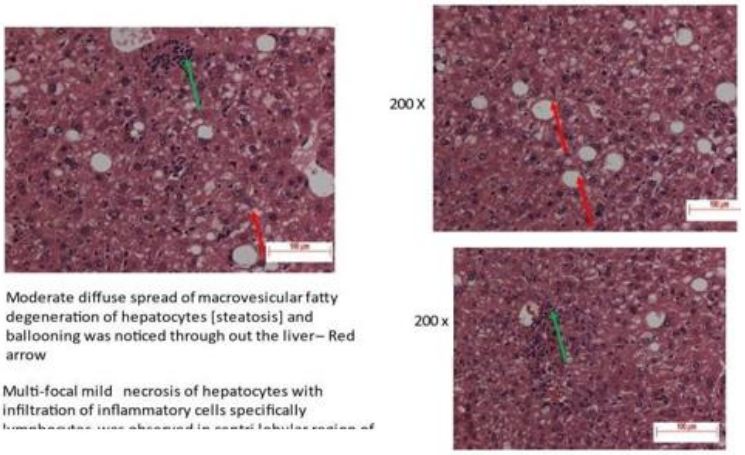




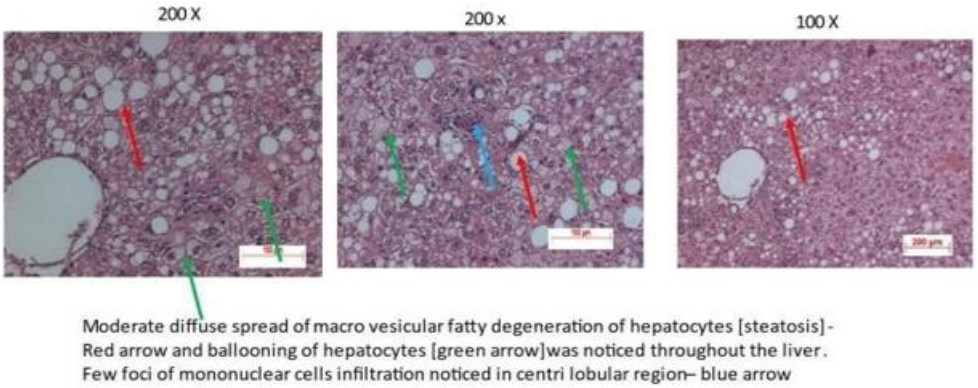
Histopathological Report of Disease control group

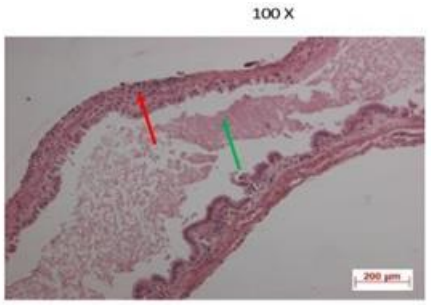
Histopathological Report of CTG (LD) group

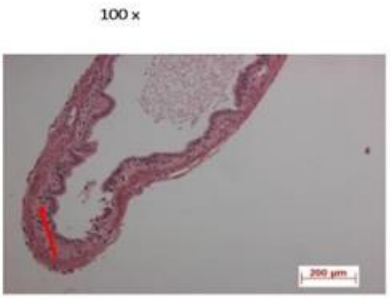




Histopathological Report of CTG (HD) group

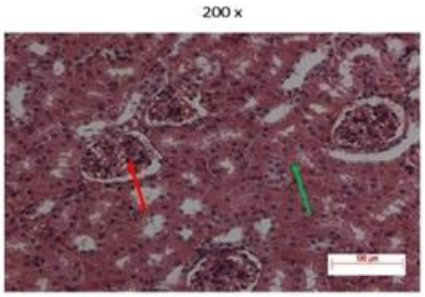


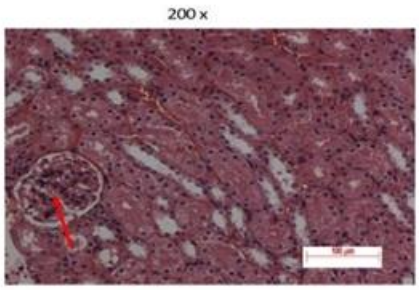




Mild cholecystitis with sub mucosal infiltration of inflammatory cells– red arrow with mild connective tissue proliferation

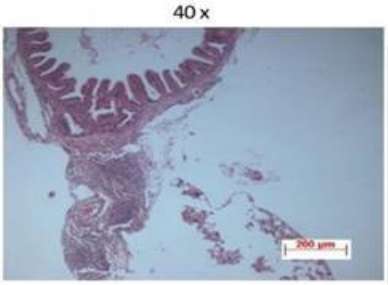
Mild accumulation of cholesterol gel noticed in lumen in the bile duct - green arrow

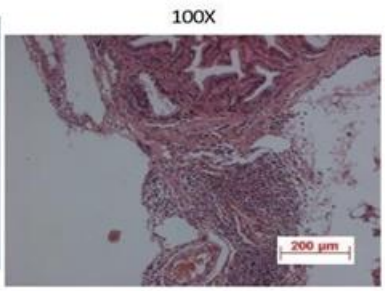


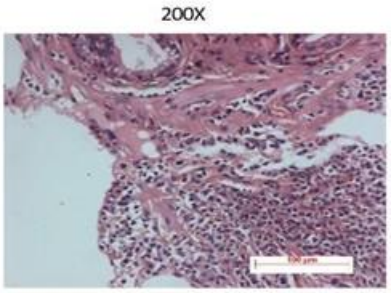


Normal morphology of glomerulus [Red arrow] and tubules of kidneys in cortex region– [Green Arrows] was observed

Histopathological report of EETD (LD) group

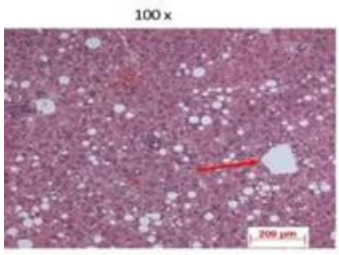


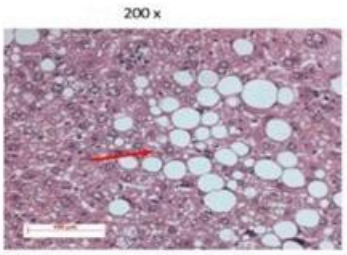


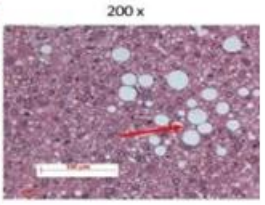


The mucosal layer appeared normal, no hyperplasia of mucosal epithelial cells – Red arrow

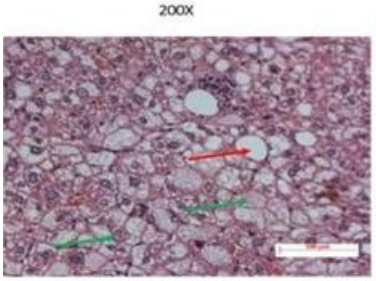
Moderate Submucosal inflammation with infiltration of inflammatory cells [Neutrophils and eosinophils – green arrow]

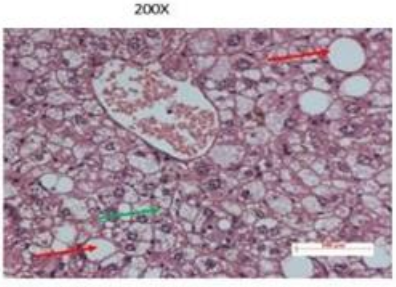






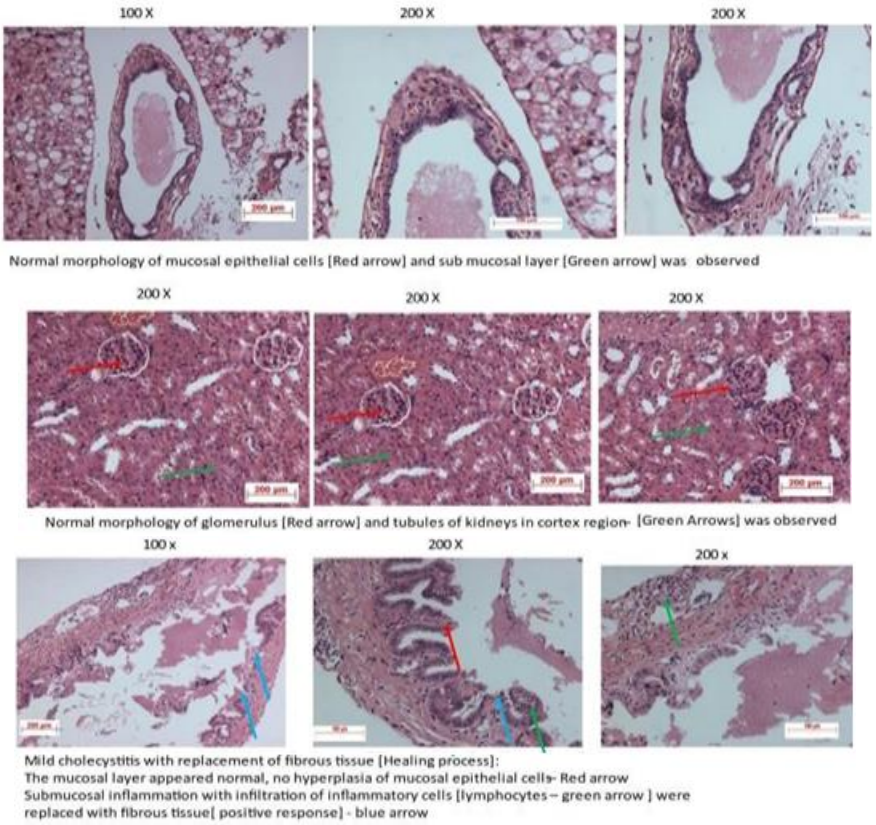
Mild diffuse spread of macro vesicular fatty degeneration of hepatocytes were noticed through out the liver - arrow





Severe micro and macro vesicular fatty degeneration (red arrow) along with ballooning of hepatocytes [Green arrow] was observed in lobular patterns throughout the liver-arrow

Histopathological report of EETD (HD) group



4. Discussion

Comparative analysis at 8th week: Group 1 (Control) with Group 2 (Disease control): On comparison with values of biochemical parameters of Liver function and Lipid of Control group with lithogenic diet induced (Disease control group), it was observed that the values of group 2 increased significantly ($p<0.05$) due to lithogenic diet resulting in derangements of liver function test leading to formation of abnormal constituents, increased biliary secretion of cholesterol and decreased synthesis of bile salts resulting in formation of gall stones and significant changes in histopathology of gallbladder and liver with cholesterol gel, crystal formation with moderate cholecystitis along with hyperplasia of mucosal epithelial cells was observed as compared to group 1, which was normal.

Comparative analysis at 12th week: Group 2 (DCG) with Group 3, 4, 5, 6 [CTG (LD, HD), EETG (LD, HD)]: On comparison with the values of group 3, 4, 5, 6 with group 2 the biochemical parameters of liver and lipids decreased significantly ($p<0.05$). Attributing this credit to the antilithogenic and hepatoprotective potential of Unani Compound formulation which improved the values indicating near to normalization but not to the extent of control range of bile constituents leading to dissolution of gall stones which were formed due to effect of lithogenic diet and in histopathology partial replacement cholecystitis, the mucosal layer appeared normal, moderate micro and macro vesicular fatty degeneration along with ballooning of hepatocytes in lobular pattern throughout the liver and mild tubular degeneration (proximal tubules and distal tubules) ducts with loss of brush borders in kidneys was observed.

Comparative analysis of CTG (LD, HD) with EETG (LD, HD): After 8weeks, the test drug, Unani Compound formulation was given for 4 weeks to EETG (LD, HD) (n=6) (CTG (LD) (HD) (n=6) and after 4 weeks of test drug i. e at the end of 12 weeks mice were sacrificed and biochemical parameters (Serum Cholesterol, triglycerides, HDL, SGPT, SGOT, ALP, Bilirubin, Albumin and Total proteins, Serium amylase) and Histopathological assessment was done. The result of biochemical parameters (LFT, LIPID PROFILE, Sr, AMYLASE, CBP, RFT) of **EETG (HD)** were normal and decreased significantly ($p<0.05$***) when compared to EETG (LD) & CTG (LD, HD). Histopathological reports of EETG (HD) shows Mild cholecystitis with replacement of fibrous tissue (healing process). The mucosal layer appeared normal, no hyperplasia of mucosal epithelial cells. Submucosal inflammation with infiltration of inflammatory cells were replaced with fibrous tissue (positive response). Normal morphology of glomerulus and tubules of kidney in cortex region was observed. The incidences of stone formation were 72.2% in lithogenic group (Disease control groups), 0% in normal control group and 40% in LD groups i. e (EETG (LD) (n=6), EETG (HD) (n=6), CTG (LD) (n=6), CTG (HD). Contents of serum cholesterol, triglyceride, ALP, SGOT, SGPT, Bilirubin in the UCF group was significantly decreased ($p<0.05$***) than that in the lithogenic diet group and serum total protein, HDL, Albumin in the UCF group was significantly increased in the lithogenic diet group ($p<0.005$). The result was less in CTG (LD, HD) and EETG (LD) when compared to EETG (HD) and the p-value is significant ($P<0.05$***), indicating that the difference is statistically significant. Thus, Unani Compound formulation when given in Ethanolic high dose form ((0.014mg/kg) can decrease the incidence of stone

formation and improve the biochemical parameters, which may be one of the mechanisms in the treatment and prevention of gall stones. Further, an elaborate clinical study can be conducted to establish the physio-chemical and pharmacological effects, so that the test drug formulation can be ensured for future studies.

5. Summary

The present pre-clinical study was conducted for screening of **Mufattit-e-Hasat (anti-lithotriptic**) activity of Unani Compound formulation Rewandchini, Beikh-e-Karafs Namak-e-Turb, for a period of 12 week by induction of gall stones in Swiss Albino Miceby feeding lithogenic diet (Cholesterol-1%, Cholic acid-0.5% and dietary fat-10%) 3-4gm/kg/day for 8weeks, animals are divided into 6 groups, Control group (n=6), Disease control group (n=6), EETG (Low dose) & (High dose) (n=6), CTG (Low dose) & (High dose) (n=6). At the end of 8 weeks mice were sacrificed from Disease control group and sampled to calculate the incidence of stone formation and biochemical parameters (Serum Cholesterol, triglycerides, HDL, SGPT, SGOT, ALP, Bilirubin, Albumin and Total proteins, Serium amylase) and Histopathological assessment was done. After 8weeks, the test drug/UCF was given for 4 weeks, to EETG (Low dose) & (High dose) (n=6), CTG (Low dose) & (High dose) (n=6) groups and after 4 weeks of test drug/UCF i. e at the end of 12 weeks mice were sacrificed and biochemical parameters & Histopathological assessment was done.

Control group which was fed with normal diet and water, biochemical parameters are Serum Cholesterol 55.00±1.673, Triglycerides 93.33±1.706, HDL 49.83±1.376 Bilirubin 0.4167±0.06009, SGOT/AST97.67±2.929, SGPT/ALT34.67±2.894, Alkaline phosphatase 94.00±2.696, Total protein 6.533± 0.1022, Albumin 3.400±0.09661 were found and histopathological report of gallbladder shows normal morphology of mucosal epithelial cells, sub mucosal and muscular layer of gall bladder and normal morphological of glomerulus and tubules of kidney in cortex region was observed. In Disease control group the Serum Cholesterol 63.67±1.116, Triglycerides 123.7±1.116, HDL36.67±0.8819, Bilirubin 3.700±0.05774, SGOT/AST 126.0±2.366, SGPT/ALT69.50±1.727, Alkaline phosphatase 144.0±2.921, Total protein 8.100± 0.1155, Albumin 5.383±0.1167 were found, which were increase significantly ($p<0.05$). Thus lithogenic diet found to increase all biochemical parameters of liver function and lipids, significantly as compared to Control group and Histopathological report of gall bladder shows moderate accumulation of cholesterol gel and crystals noticed in lumen in the bile duct along with moderate cholecystitis in which inflammation in the mucosal, sub mucosal layer of gall bladder and multifocal mild interstitial/tubular inflammation with infiltration of inflammatory cells seen in kidney. Mild few foci of micro/macro vesicular fatty/vacuolar degeneration of hepatocytes and foci of infiltration of lymphocytes were observed in centri lobular region of liver. Crude Low dose form had the Serum cholesterol 59.67±0.8819, Triglyceride91.83± 2.136, HDL 46.50±1.057, Bilirubin 0.4667 ±0.03333, SGOT/AST123±1.994, SGPT/ALT 67.50 ±1.544, Alkaline phosphatase 140.2±2.056, Total protein7.917± 0.07032, Albumin 5.117± 0.1195, thus the UCF was not found to decrease all biochemical parameters of liver function not significantly ($p<0.05$). Histopathogical reports shows moderate micro and macro vesicular fatty degeneration along with ballooning of hepatocytes was observed in lobular pattern throughout the liver. In Crude High dose form had the Serum cholesterol 52.50±1.408, Triglyceride 87.67±0.6667, HDL 55.83±0.9458, Bilirubin 0.3500±0.04282, SGOT/AST 102.8±1.579, SGPT/ALT 62.83±2.272, Alkaline phosphatase 120.3±3.739, Total protein 7.467± 0.08819, Albumin 4.550 ± 0.07638, in histopathology mild diffuse spread of macro vesicular fatty degeneration of hepatocytes were noticed throughout the liver, partial replacement cholecystitis. The mucosal layer appeared normal. Ehanolic extract low dose form had the Serum cholesterol 56.00±1.065, Triglyceride 97.00±0.9661, HDL 41.17±0.9458, Bilirubin 0.1467±0.04773, SGOT/AST 104.5±2.446 SGPT/ALT 55.67±1.054, Alkaline phosphatase 105.0 ± 2.160, Total protein 7.183± 0.07032, Albumin 4.350 ± 0.07638, and histopathological reports shows ballooning hepatocytes was noticed in peri portal region of liver. multi focal peri portal infiltration of inflammatory cells were observed. Ehanolic extract high dose form had the Serum cholesterol 41.17±0.9458, Triglyceride 83.50±0.8466, HDL52.50±1.335, Bilirubin 0.3167±0.04773, SGOT/AST 91.33±2.390, SGPT/ALT 50.67±1.542, Alkaline phosphatase 92.50±2.094, Totalprotein 6.483±0.09458, Albumin 3.967 ± 0.2076, thus the UCF was found to decrease all biochemical parameters of liver function and lipids significantly ($p<0.05$) as compare to all other groups (CTG (Low dose & High dose), EETG (Low dose), and all the parameters in group 6 were similar to that of Control group. Histopathological reports show Mild cholecystitis with replacement of fibrous tissue (healing process). The mucosal layer appeared normal, no hyperplasia of mucosal epithelial cells. After 4 weeks of Unani compound formulation to EETG (Low dose & High dose), CTG (Low dose & High dose) there is significantly decreased in biochemical parameters & histopathological changes were reduced in liver and the inflammatory changes were less in gall bladder in Ethanolic Extract Test Group (High dose). The result was less in Crude Test Group (Low dose & High dose), Ethanolic Extract Test Group (Low dose) when compared to Ethanolic Extract Test Group (High dose). All the parameters in Ethanlic Extract Test Drug (HD) were similar to that of control group ($p<0.05$) which is statically significant. Thus, UCF when given in Ethanolic high dose form was found to improve all biochemical parameters of Liver function and Lipids. Therefore, the study shows that Unani Compound formulation possess potential to normalize the liver function with antilithogenic activity.

6. Conclusion

The Unani compound formulation (UCF) has demonstrated several significant effects in various studies. Firstly, it has shown anti-lithogenic effects against induced gallstones. Additionally, the UCF has exhibited hepatoprotective potential, as evidenced by improved liver function tests Moreover, it has proven to have anti-inflammatory effects, as indicated by significant changes in histopathological studies. The UCF has also been found to reduce serum cholesterol and triglyceride levels while increasing HDL

levels, demonstrating its cardioprotective potential. Furthermore, it has shown an improvement in various biochemical parameters of liver function and a reduction in serum bilirubin levels. The cited study provides evidence of the UCF's effectiveness in addressing the aforementioned health concerns. The findings support the potential of the UCF as a therapeutic agent for gallstone-related issues, liver function & inflammation.

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