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Pharmacological Study

Evaluation of anti-hyperlipidemic activity of *Lekhana Basti* in albino rats

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Abstract

Lekhana Basti (medicated enema) is a *Tikshna* formulation which is basically aimed for *Apatarpana* of the body. The present study was undertaken to evaluate its anti-hyperlipidemic activity in diet-induced hyperlipidemia animals. Parameters like changes in body weight, organ weight, serum total cholesterol, serum triglyceride, serum HDL cholesterol, and serum (LDL + VLDL) cholesterol were studied to assess the effect of therapy in comparison to the control groups. *Lekhana Basti* was found to be ineffective in producing anti-hyperlipidemic action potently, but still found to have cytoprotective activity against hyperlipidemia induced organ damage, which was also confirmed by attenuation of cell infiltration and microfatty changes on histopathological examination.

Key words: *Apatarpana*, cholesterol, cytoprotective, hyperlipidemia, *Lekhana Basti*

Introduction

Hyperlipidemia is highly prevalent and is closely related to coronary heart disease which is the most common cause of death.[1] Raised cholesterol is estimated to be responsible for 18% of cerebrovascular diseases and 56% of ischemic heart diseases. Overall, these diseases account for about 4.4 million deaths (7.9% of the total).[2] Based upon the etiological factors and symptom complexes, hyperlipidemia can be stated under broad umbrella of "*Santarpanjanya Vyadhi*,"[3] (diseases caused due to over nutrition and sedentary lifestyle). *Apatarpana* is the remedy for *Santarpanjanya Vyadhi*.[4] Taking into consideration the treatment modalities in *Ayurveda*, "*BASTI*" seems to be the best treatment in *Santarpanjanya Vyadhi* as it is the fastest *Apatarpana*, when prepared with *Apatarpaka* drugs.[5] In *Apatarpana* also being more specific "*Lekhana*" is the treatment which can remove abnormally increased *Sneha*.[6] *Lekhana* is nothing but a process of emaciation.[7]

Thus, *Basti* prepared with *Lekhana* drugs, seems to be the proper treatment modality for hyperlipidemia, but the experimental data regarding anti-hyperlipidemic potential of *Lekhana Basti* is lacking. Considering this, the study attempts to evaluate the effect of *Lekhana Basti* on lipid profile in experimental animals.

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Materials and Methods

Test formulation

The raw materials [Table 1] of *Lekhana Basti* were procured from pharmacy attached to Gujarat Ayurved University, Jamnagar, and authenticated at Pharmacognosy laboratory, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar. *Triphaladya Taila*[8] was used as *Sneha*. *Putoyavanyadi Kalka*[9] was used in formulation as it is a commonly used *Kalka* (paste of herbs) while preparing *Basti*.[10] *Lekhana Basti* was prepared freshly just before administration by classical method.[11]

Animals

Wistar strain albino rats of either sex weighing 190 ± 20 g were obtained from animal house attached to the institute. Animals were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature (22 ± 2°C) and humidity (50-60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given *ad libitum*. All procedures and experiments were conducted in day time according to specification of the Indian National Science Academy (INSA). The experiments were carried out after obtaining the permission of Institutional Animal Ethics Committee (Approval number; IAEC/07/2010/09/MD).

Dose fixation

The quantities of ingredients in liquid form were determined in milliliters (ml) by considering the specific gravities of the basic component of the formulation and converting the grams

Table 1: Contents of the *Lekhana basti* (for preparing *12 Prasruta,* i.e. approximately 1000 ml of *Niruha*)

Content name	Quantity
Makshika (honey)	210 ml
Saindhava (Rock salt)	12 g
Triphaladya Taila	160 ml
Putoyavanyadi Kalka	96 g
Triphala Kwatha	480 ml
Gomutra (Cow urine)	120 ml
Yavakshara (Potassium carbonate), *Shuddha Shilajit* (Black bitumen), *Kasisa* (Ferrous sulfate), *Hingu* (*Ferula alliacea*), *Tuttha* (Copper sulfate)	12 g

to ml. Thus, the finally prepared 12 *Prasruta Basti* comes to be of 1000 ml. For present trial, half of the standard dose, i.e. *Ardhamatrika Basti* was considered as the adult human dose, i.e. 500 ml for *Niruha*. For *Anuvasan Basti*, *Triphaladya Taila* was used and the classical dose is 3 *Pala* (i.e. 1.5 *Prasruta* = 160 ml). The dose of *Lekhana Basti* and *Anuvasana Basti* were calculated by extrapolating the therapeutic dose to rat dose on the basis of body surface area ratio by referring to the table of Paget and Barnes.[12] Accordingly, the rat dose for *Niruha Basti* became 45 ml/kg and *Anuvasana Basti* was 14.4 ml/kg.

Prior to the experiment proper, to rule out any possible practical difficulty while administering this particular dose of *Niruha Basti* and to know, up to what region of colon this dose of *Basti* reaches, a pilot study was done in which two rats were used. First rat was administered with above mentioned dose of *Niruha Basti* and observed for any ill effect. Further, the animal was sacrificed about 5 minutes after the *Basti* administration with overdose of ether anesthesia and its intestinal tract was observed for presence of *Basti* material. The *Basti* contents were found to reach up to cecum. Second animal was first sacrificed with overdose of ether anesthesia and the abdomen was opened by midline incision and the large intestine was carefully exposed. Then, the *Niruha Basti* was given through rectum. It was confirmed that it reaches up to the ileocecal junction. Thus, the dose decided for the animals was fixed for the present study.

Lekhana Basti is used for *Kaphapradhana Vikara*. *Acharya Charaka* has quoted to give *Anuvasana* on every third or fifth day in "*AnativriddhaVata*" and "*PravriddhaKapha-Pitta Avastha*," (*Kapha* dominant disease) respectively.[13] As hyperlipidemia is a *Kaphapradhana* condition, schedule of *Basti* is fixed as one *Anuvasana* followed by three *Niruha* and so on with one *Anuvasana* on last day for 21 days.

Experimental procedure

The selected animals were grouped randomly irrespective of sex into three groups. To first group, only tap water was administered without hyperlipidemic diet, whereas second and third groups received hyperlipidemic diet. The hyperlipidemic diet included hydrogenated vegetable oil (Vanaspati Ghee - 'Raag' brand, Batch No. (AO) VA05, Adani Wilmar Ltd., Gujarat) and cholesterol extra pure powder (Batch No. 5593 Suvidhanath Laboratories, Baroda) made into suspension in 20% coconut oil (Parachute coconut oil, Batch No. PH001 Mumbai). The suspension was administered in the dose of 0.5 ml/100 g body weight of rats, respectively, daily for 21 days (at evening hours between 4 pm-5 pm) to the rats of second and third groups. Concomitantly, second group was treated with water enema (Vehicle) whereas third group was treated with *Lekhana Basti*. The enema administration was done at morning hours for 21 consecutive days. *Lekhana Basti* was administered to the animals after doing local *Abhyanga* (oil massage) at lower abdomen and lower back with lukewarm *Triphaladya Taila* and *Swedana* (sudation) with hot water filled in a polythene bag, i.e. the classical enema procedure which is generally adopted clinically, was followed in experimental animals. To administer the test drug, simple rubber catheter (no. 4) attached to 20 cc plastic syringe was used in case of *Niruha* while the same catheter attached to 3-ml plastic syringe was used in case of *Anuvasana Basti*. Water enema control group was given tap water enema by using same instrument as of *Niruha Basti*.

On 22nd day, after overnight fasting, animals were weighed again and anesthetized with diethyl ether. Blood was drawn from supraorbital plexus by puncturing and sent to biochemical laboratory for biochemical estimations. Then, the rats were sacrificed by overdose of anesthesia and important organs like liver, kidney, aorta, and heart were dissected out. Liver, kidney, and heart were weighed and they were (including aorta) transferred to fixing solution (10% formalin) for histopathological examinations. Biochemical parameters like serum total cholesterol,[14] serum triglyceride,[15] serum HDL cholesterol,[16] and serum (LDL + VLDL) cholesterol were studied. Serum (LDL + VLDL) was calculated by subtracting HDL cholesterol value from total cholesterol instead using both values separately, as in rats whose serum cholesterol is <100 mg/dl Friedewald formula overestimates LDL levels.[17] Atherogenic Index (AI) was calculated by using the formula; AI = (Total Cholesterol/HDL-C).[18]

Statistical analysis

The data generated during the study were subjected to student's '*t*' test for unpaired data to assess the statistical significance. '*P*' value less than 0.05 was considered as statistically significant.

Results

Data related to effect of *Lekhana Basti* on body weight of albino rats in different experimental groups have been provided in Table 2. In normal control rats, a marginal decrease in body weight was observed in comparison to their initial values. In contrast, significant increase in body weight was observed in water enema + Cholesterol control rats in comparison to both initial values as well as water control group. In *Lekhana Basti*-treated group, a marginal increase in body weight was observed in comparison to its initial value; however, in comparison to water enema + cholesterol group significant decrease in body weight was observed.

The data related to the effect of *Lekhana Basti* on weight of different organs have been shown in Table 3. Marginal increase of relative weight of liver was found in water enema + cholesterol control group in comparison to water control group which was found to be statistically

non-significant. However, increase in *Lekhana Basti* group was statistically significant when compared to the water enema + cholesterol group.

Marginal decrease of relative weight of heart was found in water enema + cholesterol control group in comparison to water control group which was found to be statistically non-significant. In *Lekhana Basti*-treated group, a marginal and statistically non-significant increase in relative weight of the heart was observed in comparison to water enema + cholesterol control group. Significant decrease in relative weight of kidney was observed in water enema + cholesterol control group in comparison to water control group. Administration of *Lekhana Basti* significantly reversed relative weight of kidney in comparison to water enema + cholesterol control group.

The data related to the effect of *Lekhana Basti* on serum lipid profile have been shown in Table 4. Statistically non-significant increase in serum cholesterol was seen in water enema + cholesterol control group in comparison to water control group. *Lekhana Basti* group also have shown marginal increase in serum cholesterol in comparison to water enema + cholesterol control group which was found to be statistically non-significant. Statistically significant increase in serum triglyceride was seen in water enema + cholesterol control group in comparison to water control group. Treatment with *Lekhana Basti* apparently decreased serum triglyceride level in comparison to water enema + cholesterol; however, the observed change was found to be statistically non-significant.

HDL was seen to be decreased in water enema + cholesterol control group when compared to water control group. Treatment with *Lekhana Basti* apparently increased serum HDL level in comparison to water enema + cholesterol; however, the observed change was found to be statistically non-significant. Administration of cholesterol and treatment with *Lekhana Basti* did not affect the serum (LDL + VLDL) and AI to significant extent.

Table 2: Effect of *Lekhana Basti* on the body weight in albino rats

Group	Initial body weight	Final body weight	Actual change in body weight
Water control	203.00±6.38	199.67±7.22	−3.33±4.18
Water enema+ Cholesterol	189.67±7.42	223.67±10.42	57.67±15.88##
Lekhana Basti+ Cholesterol	192.00±6.28	206.67±14.01	14.67±11.63*

##*P*<0.01 (Compared with water control), **P*<0.05 (Compared with water enema+ cholesterol)

Table 3: Effect of *Lekhana Basti* on organ weight in albino rats

Group	Relative weight (g/100 g)		
	Liver	Heart	Kidney
Water control	3.06±0.10	0.34±0.01	0.76±0.02
Water enema+ Cholesterol	3.30±0.16	0.32±0.01	0.67±0.02#
Lekhana Basti+ Cholesterol	3.89±0.15*	0.34±0.01	0.79±0.03*

#*P*<0.05 (Compared with water control), **P*<0.05 (Compared with water enema+ cholesterol)

Discussion

Administration of hyperlipidemic diet led to significant increase in body weight in albino rats. *Lekhana Basti* was found to be effective in weight reduction as seen from the significant decrease in body weight in *Lekhana Basti*-treated group as compared to the water enema + cholesterol control group. This indicates that the test drug has the antagonizing effect against hyperlipidemic diet-induced changes in the body weight. This effect may be attributed to the *Shodhana* property of the *Lekhana Basti* that removes excess *Kleda* from the body. Moreover, the *Tikta, Katu, Kashaya Rasa; Ushna Virya, (purificatory)* and *Katu Vipaka* of the *Lekhana Basti* has an additional action through *Rasapanchaka*.

Decrease in the organ weight is indicative of degenerative changes or loss of tissue of that particular organ, increase in the weight may be due to hyper functioning of that organ or edematous changes. In present study, administration of hyperlipidemic diet did not affect the weight of heart and liver to significant extent in comparison to normal rats; however, it caused significant decrease in weight of kidney. Furthermore, histopathological studies of the kidney sections from cholesterol control group show fatty degenerative changes, this may be the reason for observed decrease in kidney weight. Administration of *Lekhana Basti* significantly decreased the weight of kidney and histopathological studies of the kidney sections of this group show almost normal cytoarchitecture. This indicates cytoprotective property of *Lekhana Basti*.

Lipid profile is the objective measurement of body lipids and AI specifies the cardiac risk. In present experimental trial, serum triglycerides were increased significantly in water enema + cholesterol control group; however, the marginal non-significant decrease was seen in *Lekhana Basti* group. HDL was found to have non-significant increase as compared

Table 4: Effect of *Lekhana Basti* on lipid profile in albino rats

Group	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL cholesterol (mg/dl)	Serum (LDL+VLDL) (mg/dl)	Atherogenic index
Water control	66.33±3.77	76.67±6.91	40.33±5.27	26±5.10	1.78±0.21
Water enema+cholesterol	72.5±6.02	145.83±15.60##	39.83±3.03	32.67±3.57	1.82±0.06
Lekhana Basti+cholesterol	77.67±2.60	120.33±21.33	43.83±3.22	33.83±2.36	1.81±0.10

##*P*<0.01 (compared with water control). HDL: High density lipoproteins, LDL: Low density lipoproteins, VLDL: Very low density lipoproteins

to water enema + cholesterol control group which was having non-significant decrease in serum HDL as compared to water control group. But these marginal non-significant changes are not sufficient to prove protective role of *Lekhana Basti*. This data regarding lipid profile prove *Lekhana Basti* ineffective in producing anti-hyperlipidemic action more as compared to water enema + cholesterol control group. However, the non-significant increase in serum cholesterol and (LDL + VLDL) complex in water enema + cholesterol control group which was expected to be significant suggests lukewarm water enemas effect on these parameters which can be justified by the colon cleansing leading to protection from autointoxication which is a cause of many metabolic syndromes including lipid disorders.[19] Just a marginal decrease in AI in *Lekhana Basti* group found as compared to water enema cholesterol control group proves it ineffective in prevention of cardiac risk profile more than the water enema + cholesterol control group.

Histopathological sections from control group show normal cytoarchitecture [Figures 1, 4, and 7]. In contrast, hyperlipidemic diet produced perivascular cell infiltration and microfatty changes in liver, cell infiltration and fatty changes in kidney, and cell infiltration and fatty changes in majority of sections of heart of animals in water enema + cholesterol control group [Figures 2, 5, and 8]. These changes were markedly attenuated by treatment with *Lekhana Basti* [Figures 3, 6, and 9].

Triphala Kashaya is the main ingredient of this formulation. *Triphala* is proven hypolipidemic combination in experimentally induced hypercholesteremic rats.[20] Moreover, contents of *Triphaladya Taila* like sesame,[21] *Haridra*,[22] *Guggulu*,[23] etc., are also known anti-hyperlipidemic agents. Thus, the moderate anti-hyperlipidemic changes and protection from the organ damage due to hyperlipidemia can be attributed to the combined effect of all the components of *Lekhana Basti*. Also, it is hypothesized that *Basti* has action on enteric nervous system (ENS). It is recognized that the ENS has a unique ability to mediate reflex activity independently of input from the brain or spinal cord.[24] The ENS contains sensory receptors, primary afferent neurons, interneuron's, and motor neurons. The events that are controlled, at least in part, by the ENS are multiple and include motor activity, secretion, absorption, blood flow, and interaction with other organs such as the gall bladder or pancreas.[25] *Basti* has the prime function of colon cleansing. The colon cleansing has the effect on whole body that can be compared to the *Srotoshodhana*. The rationale for colon cleansing is the concept of "auto-intoxication," the idea that food enters the intestine and rots.[26] Thus, cytoprotective activity of the *Lekhana Basti* can also be attributed to the colon cleansing and ENS stimulation action of *Bastikarma*.

Conclusion

On the basis of this study, it can be concluded that *Lekhana Basti* is ineffective in producing anti-hyperlipidemic action significantly, but still it has cytoprotective activity which prevented hyperlipidemia-induced organ damages. Also, significantly lower the values of body weight as compared to water enema + cholesterol control group which suggests

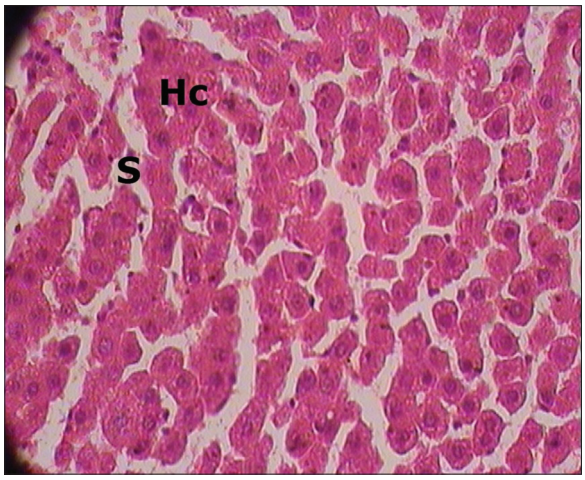

Figure 1: Photomicrograph of representative sections of Liver of albino rat from water control group. (1 × 400 magnification). Hc-Hepatocytes, S: Sinusoid. Note-Normal cytoarchitecture

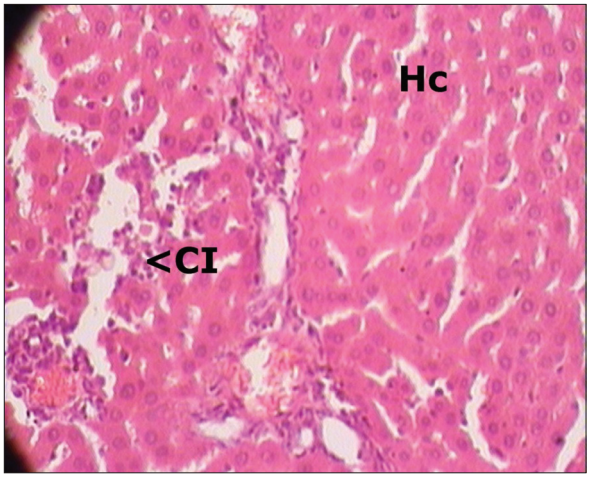

Figure 2: Photomicrograph of representative sections of Liver of albino rat from Water Enema + Cholesterol control group. (1 ×400 magnification). Hc: Hepatocytes; CI: Cell infiltration. Note-Diffused microfatty changes and perivascular cell infiltration

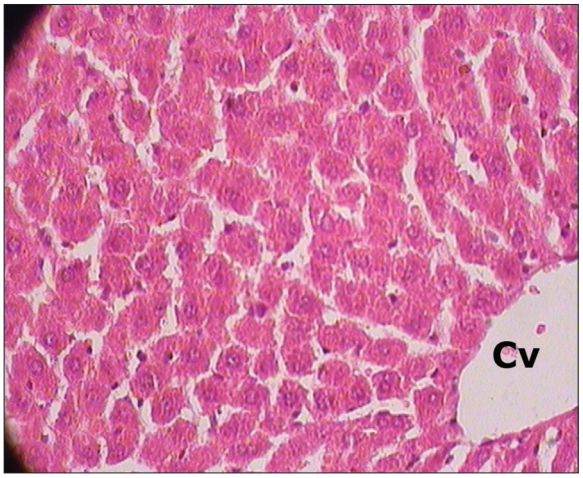

Figure 3: Photomicrograph of representative sections of Liver of albino rat from *Lekhana Basti* group. (1 ×400 magnification). Cv: Central vein. Note-Almost normal cytoarchitecture

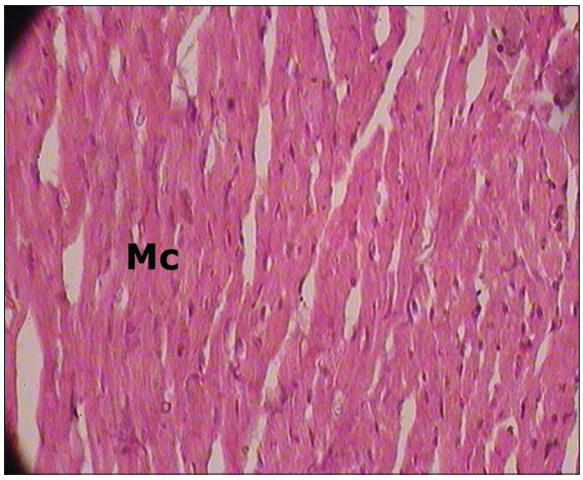

Figure 4: Photomicrograph of representative sections of Heart of albino rat from water control group. (1 ×400 magnification). Mc: Myocardium. Note-Normal cytoarchitecture

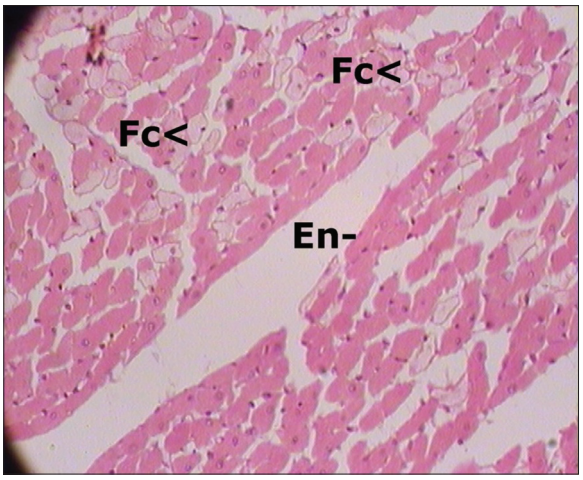

Figure 5: Photomicrograph of representative section of Heart of albino rat from Water Enema + Cholesterol control group. (1 ×400 magnification). Fc: Fatty changes; En-Endocardium. Note-Fatty changes

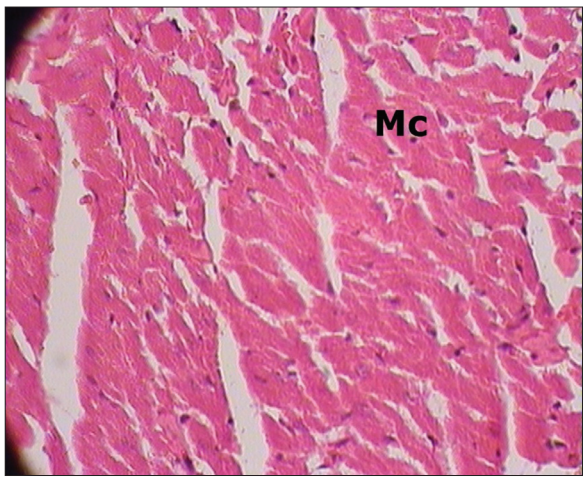

Figure 6: Photomicrograph of representative section of Heart of albino rat from *Lekhana Basti* group (1 ×400 magnification). Mc: Myocardium. Note-Almost normal cytoarchitecture

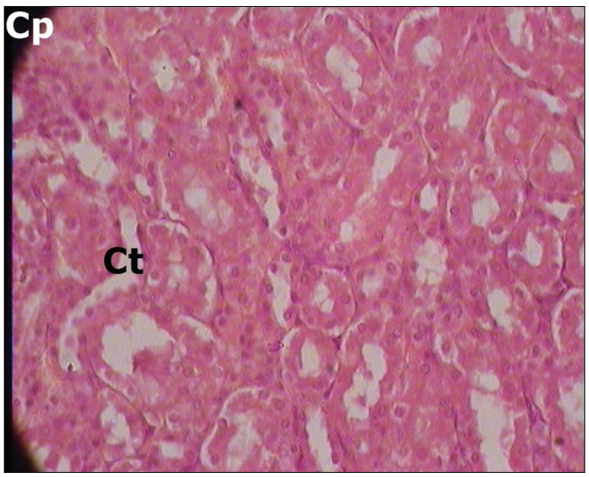

Figure 7: Photomicrograph of representative sections of kidney of albino rat from water control group. (1 ×400 magnification). Cp: Capsule; Ct: Convoluted tubule. Note-Normal cytoarchitecture

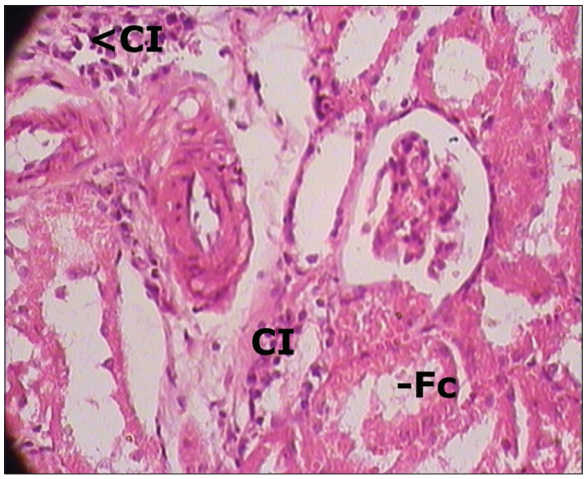

Figure 8: Photomicrograph of representative section of kidney of albino rat from Water Enema + Cholesterol control group. (1 ×400 magnification). CI: Cell infiltration, Fc: Fatty changes. Note-Cell infiltration and fatty changes

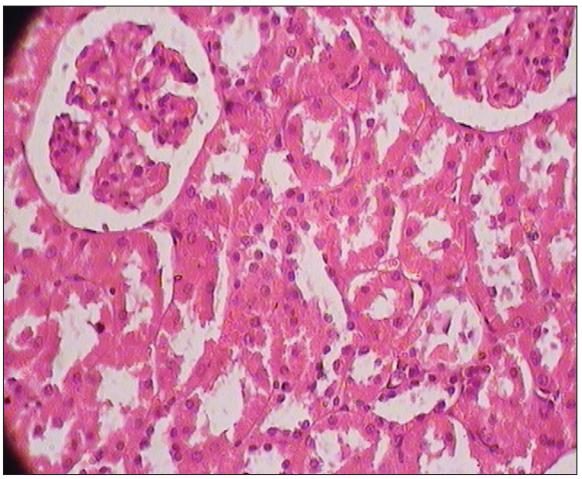

Figure 9: Photomicrograph of representative section of kidney of albino rat from *Lekhana Basti* group (1 ×400 magnification). Note-Almost normal cytoarchitecture

its anti-obesity activity in comparison to ant-hyperlipidemic activity.

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हिन्दी सारांश

लेखन बस्ति का चूहों पर हायपरलिपिडिमिया विरोध प्रयोगशालीय अध्ययन

औटि स्वप्नील, अनुप ठाकर, वि. जे. शुक्ल, अशोक बी. के., बी. रविशंकर

लेखन बस्ति की हायपरलिपिडिमिया विरोध क्रिया का अध्ययन करने हेतु प्रस्तुत प्रयोगशालीय परीक्षण चूहों पर किया गया । चूंकी, लेखन बस्ति एक तीक्ष्ण कल्प है, जो मुख्यतः शरीर के अपतर्पण के लिये प्रयुक्त होता है, चिकित्साविधी की उपयोगिता जानने हेतु शरीरभार, कोष्ठांगो का भार, कोलेस्टेरोल, एच.डी.एल.,(एल.डी.एल.+व्हि.एल.डी.एल.) आदि भावो का परीक्षण किया गया । लेखन बस्ति में कुछ हद तक हायपरलिपिडिमिया विरोध क्रिया पायी गयी । इसके अतिरिक्त हिस्टोपैथोलोजिकल परीक्षण मे जैवपेशीसंरक्षक क्रिया भी प्राप्त हुई।