AMELEORATIVE EFFECT OF CRUDE EXTRACT OF HARIDRA ON EMPTY BLADDER OF INDUCED DIABETIC MODEL SWISS ALBINO MOUSE

Maurya D. K.1*, Singh Royana2 and Singh K. N.3

1M.D. Scholar Department of Sharira Rachana, Faculty of Ayurveda, Banaras Hindu University, Varanasi, UP, 221005.
2Associate Professor, Department of Anatomy, IMS BHU, Varanasi.
3Reader, Department of rachana sharir, faculty of Ayurveda IMS BHU, Varanasi.

ABSTRACT

Background: The literatures of Ayurveda describe that Premeha (Diabetes Mellitus) is a disease of urinary System, which respond well to dried and cured rhizome of the Curcuma longa. The extensive perennial herb Haridra shows tremenous effect on Prameha when it’s crude extract is applied orally in combination with Kshaudra (Honey) and Amalki rasa (Emblica officinalis). Aims and Objectives: The current study aimed to show as an ameliorative effect crude extract of Haridra cause anti-proliferative activity on the bladder wall of Streptozotacin induced Diabetic Swiss albino mouse of Charles Foster variety Strain code 022. Material and Methods: 50 days aged sixteen healthy mature Swiss albino mice were included in this study for total duration of 6 weeks divided into four groups namely Non diabetic carboxy methyl cellulose vehicle control group, Positive control treated with turmeric extract, Diabetes induced group treated with vehicle and same treated with CMC & turmeric extract. Histological changes were observed in the urothelium and muscle layer after cutting six-micron thick slides which stained by Haematoxylin & Eosin staining. Result: The first group Mice Bladders showed not any progressive changes in the urothelium & muscle layer, the second group mouse bladder urothelium showed apparently not any changes where as third group showed significant proliferative activity in the muscle and urothelium layer in contrast to it the fourth group showed histologically significant anti-inflammatory activity in the same. Conclusion: Crude ethanolic extract of Curcuma longa, which is suitable therapeutic agent for Prameha
(Diabetes mellitus) cause significant anti-inflammatory effect in the muscle and urothelium of bladder.

KEYWORDS: Urothelial Distortion, Detrusor muscle Inflammation, Zinziberaceae.

INTRODUCTION

Haridrā consists of the dried and cured rhizome of the Curcuma longa (Family: Zingiberaceae). It is a perennial herb extensively cultivated in all the states of the India. Crop is harvested after 9-10 months when lower leaves turn yellow rhizomes are dug up carefully with hand-picks between October-April and cured by boiling and drying. In the domestic sector crude extract of Haridra is used for multiple purposes in huge quantities. Transverse section of rhizome of Haridra shows epidermis with thick-walled, cubical cells of various dimensions; cortex characterized by the presence of mostly thin-walled, rounded parenchyma cells scattered collateral vascular bundles; a few layer of cork developed under epidermis and scattered oleo-resin cells with brownish contents; cork generally composed of 4-6 layers of thin-walled, brich shaped parenchyma; cells of the ground tissue contain starch grains of 4-15 micron in diameter; oil cell with suberised walls containing either orange-yellow globules of volatile oil or amorphous resinous matter, vessels mainly spirally thickened, a few reticulate and annular.[1] According to its morphology and therapeutic application Acarya Bhavmishra have given following synonyms- Kaecani, Pita, Nida, Varavaraeni, Kemighni, Haladi, Yooitpriya and Haooavilasini.[2] “Haridra Prameha haranam” As per the view of Acarya Vagbhatta, Haridra is the main classical drug which cure and kills Prameha (Diabetes mellitus).[3] Powder or juice of Àmalaki given orally with Haridra is highly useful in diabetes mellitus.[4] “Marute pragune vastou mutarm samyak pravartate. Vikara vividhaschipratiolome bhavanti hi. Mutraghatah pramehasch shukra dosh tathaiv cha. Mutradosha ye kechid Bastavev bhavanti hi.” As per Acarya Susruta view apan vayu is the vayu which terbulated in the vasti (urinary bladder) due to which urine produced and terbulated in the same place. Urinarry badder is the site where different types of abnormalities and diseases take place those diseases are retention of urine (Mutraghata), Prameha(Diabetes Mellitus) and some extra bladder disease also evokes in the bladder as an foreign disease.[5] For an example akinesia bladder and different types of sperm diseases like oligospermia with associated bladder complication. “Hairdra katuka tikta rukhosna kapkapittanuta. Varnyatwagamehadosha panduvranapaha.”As per view of Bahlvprakash 6/198, crude extract of Haridra, which is tikta in taste and rukhosna in properties, exhibits healing properties to
distorted effect in prameha of bladder along with healing effect against extrabladder diseases like skin diseases, anemia and carbuncles. Therefore, the present study indicates that Haridra is the agent, which heals Prameha from the situ level also act as a predominant antagonistic agent, which prevents invitro risk assessment against bladder complications executed by Prameha.

AIMS AND OBJECTIVE
The present study is aimed to estimate the diseased and normal, urothelial layer and muscular layer of urinary bladder in Swiss Albino mice of non- Diabetic and Streptozotacin induced Diabetes mellitus group. Objective of this present study is to measure total bladder thickness, which includes urothelium and muscle layer histologically in the above-mentioned four groups.

MATERIALS AND METHOD
Material- 50 day’s aged sixteen healthy heterozygous mature Swiss albino mice weighing 25-35 gram of average weight, from different breeding colonies were selected for experimentation from Central Animal House, BHU. They were divided into four groups and named as Group-I, Group-II, Group-III and Group-IV. The total duration of study was 6 weeks.

Group-I: Negative control group treated with carboxy methylcellulose (4 Non-Diabetic mice)
Group-II: Positive control treated with turmeric extract dissolved in carboxy methylcellulose. (Four Non-Diabetic mice).
Group-III: Streptozotacin induced Diabetic group treated with carboxy methylcellulose (Four Diabetic mice).
Group-IV: Streptozotacin induced Diabetic group treated with turmeric extract dissolved in carboxy methylcellulose. (Four Diabetic mice).

Materials used during study were, Ethanol extract of turmeric (Curcuma longa), Instrument to measure the blood glucose and weight of mice, Instruments and equipments required for histological study.

METHODS
Procedure for ethanolic extraction of Haridra: The methodology part of study was conducted strictly in accordance with institution care and ethics. The alcoholic extraction
from crude turmeric powder was done in the lab of Department of Pharmacology IMS, BHU. First, the raw turmeric rhizome (wt 3 kg) was obtained from the market, and then it was washed with normal water to remove the soil, mud and rootlets attached from it. The rhizome were cut into small pieces and dried into hot air oven at 40°C for 72 hours. The coarse powder (wt 500gm) was formed in a mixer. This powder was mixed with 3 Liter of ethyl alcohol in a flask and kept for three days at room temperature and then this mixture was filtered by filter paper. The residue was removed away and a crimson red color solution was obtained. This solution filled in a glass bowel and kept in a hot air oven at 30°C for five days for evaporation of solvent (ethyl alcohol). Finally, a dark brown paste of turmeric extract (wt 30 gm) was obtained.

**Procedure for diabetes induction:** The diabetes was induced in the animals of group III & IV. The animals were kept overnight fasting (about 16 hrs) and weight and fasting sugar level (tail prick method) was recorded before diabetes induction. Mean weight of both the group was calculated. For identification marking on tail was done. At zero day fresh citrate buffer
(0.1M) was prepared in the morning by combining 20 ml of 0.1 M Sodium citrate (1.47g in 50ml distil water) with 30 ml of 0.1M Citric acid (1.05g in 50ml distil water). The pH was adjusted to 4.0 by using 1N NaOH. The intraperitoneal injection of Streptozotacin in a dose of 40 mg/kg body weight was given by dissolving it into citrate buffer solution (0.5ml/mice) for five consecutive days. Fasting sugar level and weight was recorded 72 hours after the fifth dose of STZ. 0.5 ml of citrate buffer was injected to all animals of control group also. Mice were feeded with 10% sucrose solution in place of water on the first day after injection of STZ to counteract hypoglycemia.

Fig. 5.

Procedure of giving oral gavage: In the first week beginning, up to end the Diabetic group mice were injected with streptozotacin continuously. From the beginning of the second week till end the Non-Diabetic and induced Diabetic mice were fed on lab approved diet, content of 10 kg mixture given to animal are as follows.

- Wheat flour: 6 kg.
- Maize flour: 3 kg.
- Fiber husk: 500 gm.
- Sugar: 500 gm.
- Salt: 100 gm.
- Mineral powder 1000gm. (Agrimin forte, Galaxo, India.) and were added with tap water libidum.

From the beginning of the third week till the end of six week the mice of group II & IV were treated with turmeric extract (ethanolic) in a dose of 100mg/kg body weight by oral route, dissolved in distilled water with help of carboxy-methyl cellulose (1% of total solution) vehicle. The 1ml solution of CMC was also given to each animal of control group (Group-I)

Fig. 6.
and plane diabetic group (Group-III) in a dose of 1mg/100 ml of distilled water from beginning of third week to end of six week.

Collection of specimen: After the completion of four-week treatment of turmeric extract the animals were sacrificed by using the method of cervical dislocation. In next step, a midline incision was made in the abdomen, to expose the entire abdominal organs. The organs of urinary system, kidney, ureter and bladder were collected by excising from its attachment points and preserved in 10% neutral formalin solution for ten days.

Procedure for doing organ histology: The proposed organ urinary bladders from four groups of this study were processed. Six-micron thick sections were cut by rotator microtome and were stained by Hematoxylin and Eosin for histological evaluation.

OBSERVATIONS AND RESULT

Table-1: Weight changes observed in non diabetic & induced diabetic study group.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Mean±Std. Deviation</th>
<th>Between the group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group-I</td>
<td>Group-II</td>
</tr>
<tr>
<td>Zero</td>
<td>34.45±1.21</td>
<td>28.37±2.66</td>
</tr>
<tr>
<td>One</td>
<td>34.57±1.65</td>
<td>29.27±2.72</td>
</tr>
<tr>
<td>Two</td>
<td>35.07±1.51</td>
<td>29.20±2.69</td>
</tr>
<tr>
<td>Three</td>
<td>35.47±1.44</td>
<td>29.75±2.75</td>
</tr>
<tr>
<td>Four</td>
<td>35.62±1.25</td>
<td>29.87±2.90</td>
</tr>
<tr>
<td>Five</td>
<td>36.05±1.19</td>
<td>30.85±2.93</td>
</tr>
<tr>
<td>Six</td>
<td>36.62±1.10</td>
<td>31.65±2.78</td>
</tr>
</tbody>
</table>

In this study, it was observed that mean weight of mice in group I & II (Non-diabetic) was gradually increased throughout study, while in group III & IV (diabetic) weight was decreased throughout study. In group IV (Diabetic +treated) throughout study the rate of decrease in weight is less than rate of weight decrease in group III (Diabetic) up to the six week.
In this study, it was observed that mean of fasting blood sugar level of mice in-group I & II was in normal range throughout the study. In-group III (diabetic) mean of fasting blood sugar level was highly increased up to six week than the normal fasting blood sugar level. In-group IV (diabetic + treated) it was observed that fasting blood sugar level was increased up to third week and then gradually decreased towards normal fasting blood sugar level up to six week.
Histomorphometrical evaluation

**Fig. 9**: Normal Urinary bladder 10X magnification.

**Fig. 10**: Turmeric treated Urinary bladder 10X magnification.

**Fig. 11**: Diabetic Urinary bladder 10X magnification.

**Fig. 12**: Turmeric treated diabetic Urinary bladder 10X magnification.

**Group. I**: Sections stained by H&E stain, showed that the wall of the bladder was formed of three layers: mucosa, muscle layer (musculosa) and adventitia. The mucosa of the bladder was thrown into numerous folds. It was composed of urinary epithelium (urothelium), and the underlying lamina propria. The urothelium was transitional epithelium where the basal cells were cuboidal and the intermediate cells were polygonal while the superficial cells (umbrella-like cells) were large rounded cells bulging into the lumen, which were found absolutely
normal. The lamina propria was formed of connective tissue containing small blood capillaries. Muscle coat of the bladder was composed of three loosely arranged layers of normal smooth muscle fibers.

**Group: II-** This group contains normal healthy mice treated with turmeric extract dissolved in CMC. Sections stained by H&E stain, showed the same microstructure as control group whereas the urothelium of the same showed mild distortion. There is no change seen in the thickness of the muscle layer.

**Group: III-** The animals of this group were diabetic not treated with turmeric extract. Examination of H&E stained section of this group, showed that after six week from induction of diabetes the wall of bladder and its corresponding muscle layer with mucosa apparently increase in thickness when compared with control.

**Group: IV-** The animals of this group after one week of induction of diabetes were treated with ethanolic extract of turmeric for last four weeks. Examination of H&E stained section of this group, showed the remarkable decrease in thickness of bladder wall along with its muscle layer, mucosa and adventitia histologically when compared with diabetic group (group III). The decrease in the thickness of above layers was found significant. (P <0.005).

**The Morphometric & statistical results Analysis:** Total thickness of the wall of urinary bladder from ten different sites was measured from 10X magnification micrograph. Results were represented in millimeter. Photomicrograph of various study groups were placed in 4 quadrants and were compared. Thickness of wall of urinary bladder were measured which were from control, control +turmeric treated, diabetic and turmeric treated diabetic group (table 4 &5). The results were tabulated and compared by applying one way ANOVA, one tailed (t) test. Kruscal walis test and P value was calculated using Prism program 5. P value <0.001 was considered significant.

**Table 3. Showing the thickness of bladder wall at different sites in all four groups of mice.**

<table>
<thead>
<tr>
<th>Sites</th>
<th>Normal U.B. (A) Wall thickness mm</th>
<th>Normal +TT U.B. (B) Wall thickness</th>
<th>Diabetic U.B. (C) Wall thickness mm</th>
<th>Diabetic + TT U.B. (D) wall thickness mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>165</td>
<td>180</td>
<td>250</td>
<td>255</td>
</tr>
<tr>
<td>2</td>
<td>210</td>
<td>160</td>
<td>365</td>
<td>202</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>185</td>
<td>385</td>
<td>190</td>
</tr>
<tr>
<td>4</td>
<td>222</td>
<td>260</td>
<td>430</td>
<td>250</td>
</tr>
</tbody>
</table>
Table 4: Showing mean thickness of wall of bladder in all groups of mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>Normal + TT (100mg/kg)</th>
<th>Diabetic</th>
<th>Diabetic + TT (100mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean in mm</td>
<td>230.0</td>
<td>255.7</td>
<td>353.5</td>
<td>245.8</td>
</tr>
<tr>
<td>SDM in mm</td>
<td>±57.07</td>
<td>±91.14</td>
<td>±71.38</td>
<td>±70.22</td>
</tr>
<tr>
<td>SEM in mm</td>
<td>±18.05</td>
<td>±28.82</td>
<td>±22.57</td>
<td>±22.21</td>
</tr>
<tr>
<td>25% Percentile-75% Percentile</td>
<td>170.3-286.8</td>
<td>183.8-319.0</td>
<td>272.5-407.5</td>
<td>189.5-379.3</td>
</tr>
<tr>
<td>10% Percentile-90% Percentile</td>
<td>151.5-301.0</td>
<td>162.0-418.0</td>
<td>245.5-439.0</td>
<td>182.6-379.0</td>
</tr>
<tr>
<td>Lower 95% CI of mean-Upper 95% CI of mean</td>
<td>189.2-270.8</td>
<td>190.5-320.9</td>
<td>302.4-404.6</td>
<td>195.6-296.0</td>
</tr>
</tbody>
</table>

Table 5: Showing the One sample t test.

<table>
<thead>
<tr>
<th>t, df</th>
<th>t=12.75 df=9</th>
<th>t=8.872 df=9</th>
<th>t=15.66 df=9</th>
<th>t=11.07 df=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value (two tailed)</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Significant (alpha=0.05)?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 6: Showing the Wilcoxon Signed Rank Test.

| Sum of signed ranks (W) | 55.00 | 55.00 | 55.00 | 55.00 |
| Sum of positive ranks   | 55.00 | 55.00 | 55.00 | 55.00 |
The measured thickness from ten sites of group three were found significantly increased. The observed hypertrophy of different layers of group three bladders was found higher in comparison to group one and two. Group four showed decrease of the thickness. Two tailed t-test analysis between and within the group comparison showed highly significant.

**DISCUSSION**

The results of the present study demonstrated that the urinary bladder structure in STZ-diabetic mice exhibited progressive structural changes. The light microscopic examination showed an apparent progressive increase in thickness of the urothelium and the muscle layer in six weeks diabetic mice. The morphometric and statistical result of the same revealed a significant increase in urothelial thickness as well as the muscle layer in six weeks diabetic mice when compared with the control group and turmeric treated diabetic group. It is suggested that diabetic associated fluid intake (polydypsia) and urine output (polyuria) could impose an extra demand on the bladder to void to excessive excreted urine resulting in overall hypertrophy of the bladder wall.[7]

Furthermore other authors found that experimentally induced diuresis in rat[8] and mice[9] caused bladder hypertrophy increased capacity and compliance that were similar to that observed in diabetic animals. They concluded that the similarities between the finding in diabetic and diuretic animals suggested that the bladder hypertrophy in diabetic mice might be a result of physical adaptation to increased urine production and that changes in the physical properties of bladder might be a significant factor in the development of bladder dysfunction in diabetes.

In the present study, progressive decrease in urothelial and smooth muscle thickness together with the decreased collagen content in turmeric treated mice was observed. This result indicates a decrease in structural changes in urinary bladder of turmeric treated diabetic mice with increase of duration of treatment. The degeneration of epithelial lining of bladder of diabetic mice might be secondary to the urinary bladder inflammation (cystitis) which is very common complication of diabetes mellitus.
In the turmeric treated mice, the inhibition and repairing of the degeneration of the epithelial lining of the bladder of diabetic mice might be the secondary effect of treatment of diabetes.

In the current study a decreasing trend in the weight of diabetic group (group-III) mice as well as turmeric treated group (Group-IV) initially; in diabetic mice given the turmeric treatment showed a decreasing rate of weight loss as well as it was less in group four (Graph-1) compared to other groups i.e. II & III.

Decrease in the level of fasting blood sugar as compared to diabetic group of animals is also observed (Graph-2). This might be due to anti diabetic effect of turmeric. Some authors reported that turmeric reduces blood sugar level in diabetic rats.[10]

The turmeric treated group (Group IV) shows that mean fasting blood sugar level decreases from 440.25±27.03 at the third week to363.25±17.57, when measured at sixth week, as depicted by Table No-2 of experimental study observation. Further it was also observed that rate of  weight loss was less in group four as compared to rate of  weight loss in diabetic group (Group-III). The turmeric treated group in morphometric study shows significant decrease in the mean thickness of bladder wall as compared to diabetic group i.e. from 353.5 to 245.8 mm as depicted in table-4 of experimental study.

The above changes in the turmeric treated diabetic animal would might be due to number of properties of active principle curcumin present in the turmeric. Recently it has been reported that curcumin (300 mg/kg) could enhance the effect of Vitamin-C in protecting endothelial cells though an anti-oxidant effect.[11]

Curcumin a powerful antioxidant is a component of turmeric found in Curcuma longa plant and has been used for centuries in treating inflammatory ailments and conditions.[12]

Curcumin is a potent scavenger of reactive oxygen and nitrogen species such as hydroxyl radicals and nitrogen dioxide radicals.[13]

It has also shown that short-term treatment of diabetic rats with curcumin prevents diabetes induced decreased anti-oxidants enzyme levels and kidney dysfunction.[14]
CONCLUSION

The present experimental study provide base to the concept of Prameha, which occur in Basti (urinary bladder) according to Ayurvedic classic. In this study, it was observed that there are specific inflammatory changes grossly as well histological in the region of Basti (urinary bladder) of diabetic mice. The comparative health of mice was significantly affected when they are exposed to diabetic factor (Streptozotacin). The weight & sugar level of diabetic mice have shown significant change when treated with turmeric extract as compared to control group. The results of the present study demonstrated that the urinary bladder structure in STZ-diabetic mice exhibited progressive structural changes. The light microscopic examination showed an apparent progressive increase in thickness of the urothelium and the muscle layer in six weeks diabetic mice. The morphometric and statistical result of the same revealed a significant increase in urothelial thickness as well as the muscle layer in six weeks diabetic mice when compared with the control group and turmeric treated diabetic group.

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3. हरिद्रा प्रमेह हराणाम। (A.Sa$q .Su30/22).
4. तत्तंकुदेतमालकर्णेन हरिद्रा महुरसंपुर्णा पायवेत। (SS.Ci.11/8).
5. मालेम प्रमुखं बल्लौं सुवं सम्यक्य प्रविष्टं। विकार किविकाराय प्रतिलोमं भवस्ती हि।।
मुखद्दरच्या प्रभावाद्वेयकोदारातथेऽत्र। मुखद्दराये कहियि बस्याचों भवस्ती हि।। लुठानिः।
6. हरिद्रा कुदुक्को तितका सृष्टिरूपस्य कथितपतुरू। कंत्यतंदुःतमेहायकाशेषापाण्डुर्गणपति। (Bha.Pra.6/198)


