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QUANTITATIVE EVALUATION OF PLASMA PROTEINS, LIPID PROFILE, LIVER AND KIDNEY FUNCTION PARAMETERS AFTER CHRONIC ADMINISTRATION OF "PRADARANTAK LOUHA" TO MALE SPRAGUE –DAWLEY RATS

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ABSTRACT

Background: Pradarantak Louha (PDL), a herbomineral Ayurvedic medicine has been used as a traditional medicine in the treatment of leucorrhoea for many years. **Objectives:** To evaluate the effect of Pradarantak Louha on major body organs**.** We assessed the possibility of side-effects after long term administration of **Pradarantak** Louha. **Materials and Methods:** To evaluate the effect of PDL, it was administered to the rats at a dose of 400 mg/kg for 54 days. **Result:** PDL does not change the plasma proteins (Total protein, albumin, and globulin) significantly. The change of bilirubin content was also not significant. In case of kidney function parameters, statistical significant increase was noted in both the creatinine content (p value: 0.015) and urea content (p value: 0.012). To assess the effect of PDL on cardiovascular health, lipid profile of rats were assayed and no significant changes were found. **Conclusion:** The outcome of this study implies that PDL is safe for our body but care should be taken when it is administered for long term and when it is accompanied by any kidney complications.

Keywords: Pradarantak Louha, Ayurvedic Medicine, Creatinine, Uric Acid, Plasma Protein.

INTRODUCTION

Traditional medicinal systems are the earliest enlightenment in health care system of mankind. In the very recent past, the use of traditional medicines is growing worldwide both in developed and developing countries.[1] Ayurveda, which means science of long life, is at least a 5,000-year-old system of traditional medicine (1500–1000 BC) designed to promote good health and longevity rather than to fight disease and was practiced by physicians and surgeons (called vaidya). Until 700 BC, this science was orally discussed between sages and physicians. Thereafter, two different textbooks were assembled: one by ''Charaka'' is called Charaka Samhita and the other by ''Sushruta'' is called SushrutaSamhita. Whereas Charaka Samhita deals

with the etiology, symptomatology, pathology, prognosis, and medical management of disease, SushrutaSamhita deals with various surgical instruments and procedures.[2] Pradarantak Louha (PDL) is included (pages 325-326) in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/ (Part-1) 116 dated 3-6-1991). It is a traditional Ayurvedic preparation widely used by the rural and ethnic people of Bangladesh to treat leucorrhea. It is a preparation of various metals, non-metals, animal constituents and medicinal herbs [Table 1, Table 2]. Safety of traditional medicine system is time tested and it is believed to be spiritual and known not to produce toxic effects. But no objective verifiable data exists to support many such claims. It is conceivable that a single herb extract or a pure active chemical constituent may cause some adverse effects under certain conditions and dose levels. For example, *SushrutaSamhita* describes the use of guggul (*Commiphoramukul*) for a wide variety of conditions, including rheumatism and obesity. But it has been shown to produce some anticoagulant effect under certain conditions. [3] In addition, there are lot of works and discussions going on globally about heavy metals and toxicity of heavy metal poisoning such as mercury, lead, arsenic etc. In 2003, a survey says that Ayurvedic theory attributes important therapeutic roles to mercury and lead and that perhaps 35-40% of medicines in the Ayurvedic formulary contain at least one metal. The intrigue phenomenon of its manufacturing converts these into complex mineral forms which are effective and nontoxic. However, improper processing/manufacturing of Ayurvedic medicines may result into severe toxicity.[4]Several studies done in other countries have had similar findings.[5,6] Another main component of Ayurvedic medicines is medicinal herbs as medicinal plants can be directly used as healing agent and their phytochemicals also serve as lead compound for developing potential drugs to cure various diseases in human. [7,8]Furthermore, there are several reports which state on the potential toxicity of the phyto products. Contamination of these products by pesticides, herbicides, naturally occurring toxins, microbes or adulteration by means of synthetic substitutes is a cause for concern. Toxicity manifestations include hepatotoxicity, nephrotoxicity, and neurotoxicity, hematological, mutagenic and cardiovascular toxicities. [9] Due to all the above concerns nowadays in the present era it has become very important to understand Ayurvedic medicines by carrying out certain safety studies. The outcome of those studies will be helpful for a clear judgment and revalidation of the safety and efficacy of Ayurvedic medicines in living organisms. Pharmacological evaluation of PDL would provide proper proof of safety of major organs like kidney, liver and heart. Since there is no strong clinical data about the safety of PDL for major body organs at the current situation mentioned above, it was decided to undertake a detailed evaluation about the effect of PDL on major body organs.

MATERIALS AND METHODS

Ayurvedic formulation: For the current study Pradarantak Lauha (PDL) was collected from Sree Kundeswari Aushadhalaya Ltd, Chittagong, Bangladesh.

Dose and Route of administration: In this study we administered the drug per oral route at a dose of 400 mg/kg of the body weight daily. Also, Ketamine were administered intra-peritoneally (500 mg/kg i.p.) for anesthesia purposes.

Experimental animals*:* For this research work, healthy albino rats (*Rattusnovergicus:* Sprague-Dawley strain,) eight-week old of male rats were used. These animals were weighed about 180±20 g. The rats were bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University under standard laboratory conditions (relative humidity 55–65%, room temperature 25.0 ± 2.0°C, and 12 h light-dark cycle). The animals were randomly assigned to control and treatment groups (10 rats per group) and housed in clear plastic cages containing wood shavings for bedding. At the end of the experimental period of 54 days and after overnight fasting, at 9:00a.m, the animals were sacrificed to get a blood sample. Before sacrifice ketamine (500mg/kg) were administered intraperitonially for anaesthetized the animals. Immediately, after sacrificing the animals' blood sample was collected from the post vena cava and then was transferred to the tubes having heparin without any delay.

Biochemical test: To collect the intended plasma and to remove red blood cells, the collected samples of blood were centrifuged at 4,000 g for 10 min using bench top centrifuge (MSE Minor, England). After separation, serum was collected using dry Pasteur pipette and stored in the refrigerator for analysis. The analysis of all the biochemical parameter was accomplished within 24 h of sample collection. After collection of plasma different methods were applied for bio-chemical tests. All the reagents and kits used for the biochemical tests are purchased as ready to use form and all test samples were prepared according to instruction guide of Human GmbH, Wiesbaden, Germany. The absorbances of all the test samples were determined using Humalyzer, Model No-3000 (Human GmbH, Wiesbaden, Germany).

Statistical analysis: The group data are expressed as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance. SPSS (Ver. 20) for Windows was applied for the analysis of data. Differences between groups were considered significant at $p< 0.05$, 0.01 and 0.001.

RESULT

After 54 days of PDL administration, biochemical parameters critical for normal and balanced physiological condition were considered. To assess the effect of PDL on blood proteins, change in total protein, albumin and globulin contents were measured but there was no statistically significant change [Table 3]. The change in bilirubin content was also measured and the decrease of bilirubin level after PDL administration was also not significant [Table 4]. To evaluate the effect of PDL on kidneys, measurement of creatinine, urea and uric acid level results in statistically significant increase in both creatinine (↑60.4167%; p: 0.015*) and urea (↑30.8694%; 0.012*). But the change in uric acid was not significant [Table 5]. Lipid profiles including triglycerides, total cholesterol, very low density lipoprotein, low density lipoprotein and high density lipoprotein was measured before and after PDL administration. But there was no statistically significant change [Table 6].

DISCUSSION

Effect on plasma protein contents: The level of total protein in the blood is normally a relatively stable value, reflecting a balance in loss of old protein molecules and production of new protein molecules. [10-13] After chronic administration of PDL preparation in the male rats the total protein content in the plasma was decreased (17.7334%. decr.) and it was not significantly different from its corresponding control value (p=0.119). The decrease of albumin and globulin content was 30.466 % and 13.608 % respectively. Both changes were not significantly different from their corresponding control values. In this study, there was no significant difference in plasma protein level between experimental group animals and control group animals shown in table 3 & figure 1.

Effect on liver: Blood bilirubin test measures the amount of bilirubin in the blood in order to evaluate liver function or to help diagnose anemia caused by the increased destruction of RBCs (hemolytic anemia). [14] After administration of PDL to male rats for 54 days, bilirubin level was decreased by 18.4441% in the plasma in comparison to their control group but it was not statistically significant shown in table 4 & figure 2. The plasma albumin content is another indicator of liver health as it is a protein made specifically by the liver. In this study, the decrease of albumin content was also not statistically significant. Study of bilirubin content and albumin content in blood reflects that PDL does not alter the normal physiologic condition of liver.

Effect on kidney: Creatinine is usually a good indicator of how well the kidneys are working.[16] The concentration of urea in the serum is useful in prediction of different types of health problems, like kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract. Low levels are also seen in trauma, surgery, opioids, malnutrition, and anabolic steroid use [19,22]**,** BUN (Blood urea nitrogen) is affected by tubular reabsorption of urea and several non renal factors like diet and urea cycle enzymes. Serum uric acid level is used to detect high levels of this compound in the blood in order to help diagnose gout and kidney failure. In this study PDL causes statistically highly significant (p=0.015) increase in the creatinine (60.417% incr.) content in plasma and an increase of urea level (30.8694% incr.) in the plasma was noted in comparison to their control group, the increase was statistically significant (p=0.012) shown in table 5 and figure 3. Also, it was observed that there was a negligible increase in the plasma uric acid content (1.806% incr.) in the PDL treated male rats, and this increase obviously was not significant (p=0.862). This study shows a statistically significant increase in the creatinine content and urea content in plasma in PDL treated male rats. So, it indicates that prolong administration of PDL may cause nephrotoxicity. These results suggest that administration of PDL should be carefully monitored to confirm proper functioning of kidneys.

Effect on lipid profile: Triglyceride and cholesterol is different from most tests in that it is not used to diagnose or monitor a disease. Both are used to estimate risk of developing a disease specifically heart disease. [23-26] In this study the change in triglyceride and total cholesterol level of PDL treated rat is not significant shown in table 6 & figure 4. Due to the very high tendency of LDL to block the artery and facilitate the heart diseases, of all the forms of

cholesterol in the blood, the LDL cholesterol is considered the most important form in determining risk of heart disease. In the present study, decrease was noted in the triglyceride level (11.7462 % decr.), and HDL (43.5008 % decr.) content in the plasma of the PDL treated male rats. In both cases, the decrease was not statistically significant; triglyceride (p=0.869), and HDL (p=0.621) whereas, there was increase in the total cholesterol (10.9886 % incr.), VLDL (44.5378 % incr.) and LDL (15.1560 % incr.). But none of those increases was statistically significant; total cholesterol (p=0.084), VLDL (p=0.209), LDL (p=0.201) (Table 6 and figure 4).

After chronic administration of Pradarantak Louha (PDL) for 54 days, the increase of LDL was not significant in PDL treated male rats and also HDL was decreased not significantly [Table 6]. These non significant changes predict that the cardiovascular health of PDL treated rats are good enough.[27] In addition, Increased levels of VLDL-cholesterol, have been found to be associated with increased risk of heart disease and stroke. PDL treated rats show no significant change in VLDL level. Accumulating all these above mentioned statistical changes in PDL treated male rats compared to control group indicates that cardiovascular health of PDL treated rats is sound and well. In the current study, we observed the effects of PDL on major body organs. It was found that PDL does not cause any significant change of the liver and cardiovascular system. But prolong exposure of PDL should be carefully monitored because in this study, PDL has increased both creatinine and urea statistically significantly. Care should also be taken when PDL is treated with impaired renal function. This kind of study should also be continued to establish clinical and pharmacological data of Ayurvedic medicines. In addition, based on this kind of study further more detailed study may be planned to observe the efficacy and safety of Ayurvedic medicines in the cellular and tissue level.

CONCLUSION

Being one of the most ancient healing systems, Ayurvedic medicine has been practiced for years. But still there is no sufficient reliable scientific data about the safety and efficacy of Ayurvedic medicines. In the present study, to assess safety of Pradarantak Louha and effect of this Ayurvedic medicine on major body organs, some important biochemical parameters were measured with and without PDL administration. Result of the present study showed that PDL is safe for almost all the major body organs but care should be taken while it is associated with nephropathy. To understand Ayurvedic safety principles and efficacy science more deeply, this type of study will be helpful.

Table1: List of plants and animal constituents used in the formulation of Pradarantak Louha.

Plants	Part Used	English/Common Name	Scientific Name	Family
Sunthi	Dry root	Dry ginger	*Zingiber officinale*	*Zingiberaceae*
Marica	Fruit	Pepper black	*Piper nigrum*	*Piperaceae*
Pippali	Fruit	Long papper, Pipli	*Piper longum*	*Piperaceae*
Haritaki	Fruits	Almond tree	*Terminalia chebula*	*Combretaceae*
Devadaru	Leaves, Heartwood,	Devadaru	*Cedrus deodara*	*Pinaceae*
Bibhitaka	Fruit	Belliric myrobalans	*Terminalia bellirica*	*Combretaceae*
Amalaki	Fruit	Amla	*Emblica officinalis*	*Phyllanthaceae*
Citra (citraka)	Root	Citra	*Plumbago zeylanica*	*Plumbaginaceae*
Vidanga	Fruit	False black pepper	*Embelia ribes*	*Primulaceae*
Vaca	Leaves, Rhizomes	Sweet flag, Calamus	*Acorus calamus*	*Araceae*
Havusa (hapusa)	Fruit	Juniper plant	*Juniperus communis*	*Cupressaceae*
Palaka (kustha)	Dried root	Saw-wort, Snow lotus	*Saussurea lappa*	*Asteraceae*
Patha	Root	Abuta	*Cissampelos pariera*	*Menispermaceae*
Ela	Seed	Cardamom	*Elettaria cardamomum*	*Zingiberaceae*
Sankha (bhasma)	Shell	Sea snails	*Turbinella pyrum*	*Turbinellidae*
Cavika (cavya)	Root, Fruit	Wild pepper	*Piper methysticum*	*Piperaceae*
Vrddhadaraka	Root	Elephant creeper,Guguli	*Argyreia Speciosa*	*Convolvulaceae*

Note: All ingredients are used as 1 part.

Table 2: List of minerals used in the formulation of Pradarantak Louha.

Minerals	English /Common Name	Scientific Name
Lauha (bhasma)	Iron calyx	*Calcined ferrum*
Tamra (bhasma)	Ash, Copper	Cuprum
Haritala (bhasma)		*Arsenic trisulphide*
Vanga (bhasma)	Tin calyx	*Calcined stannum*
Abhra (abhrakabhasma)	Powdered talc	Mica oxide
Vida lavana	Ammonium salt	Combination of Sodium chloride, Sodium sulphate, Alumina, Magnesia, Ferric oxide and Ferric sulphide
Sauvarcala	Black salt	Combination of Sodium chloride with some Sulphur content.
Audbhida lavana		Combination of Sodium chloride, Sulphide and Sodium bicarbonate
Samudra lavana	Sea salt	Sodium chloride
Saindhava lavana	Rock salt	Potassium chloride

Note: All ingredients are used as 1 part.

Table 3: Effect of PDL on Total Serum Protein, Albumin, Globulin and Albumin/Globulin contents (g/dl) in male rats.

Parameters	**Mean ±SEM**		**%Changes**	***p* Value**
	Control	**Test**		
Total Protien	7.2744±0.69553	5.9844±0.28955	↓17.7334%	0.119
Albumin	5.1736±0.72772	3.5974±0.20049	↓30.46582%	0.084
Globulin	4.5796±0.98631	3.9564±0.75582	↓13.6084%	0.623

Table 4: Effect of PDL on Bilirubin content (mg/dl) in male rats.

Parameters	**Mean ±SEM**		**% Changes**	***P* Value**
	Control	**Test**		
Bilirubin	0.2391±0.05808	0.1950±0.05777	↓18.4441%	0.117*

Table 5: Effect of PDL on Creatinine, Urea, Urea/Creatinine and Uric Acid contents (mg/dl) in male rats.

Parameters	**Mean ±SEM**		**% Changes**	***P* Value**
	Control	**Test**		
Creatinine	1.6000±0.09686	2.5667±0.32222	↑60.4167%	0.015**
Urea	22.4199±3.59719	29.3408±2.33467	↑30.8694%	0.012*
Uric acid	3.1078±0.18156	3.1639±0.25222	↑1.80645%	0.862

Table 6: Effect of PDL on Triglycerides, Total cholesterol, VLDL, LDL, HDL, TCHO/HDL and LDL/HDL contents (mg/dl) in male rats

Parameters	**Mean ±SEM**		**% Changes**	***p* Value**
	Control	**Test**		
Triglycerides (TG)	52.3684±5.23961	46.2171±4.46509	↓11.7462%	0.869
Total Cholesterol (TCHO)	60.6582±1.53422	67.3237±1.85046	↑10.9886%	0.084
VLDL	4.4737±1.04792	6.4662±1.07258	↑44.5378%	0.209
LDL	10.8135±3.45427	12.4524±2.46251	↑15.1560%	0.201
HDL	48.9642±1.11841	27.6644±0.54007	↓43.5008%	0.621

Note: *p<0.05, **p<0.01, ***p<0.001

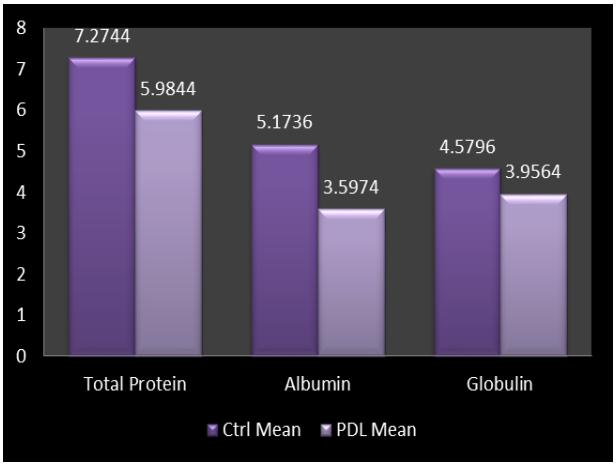

Figure 1: Graphical presentation of total protein profile test

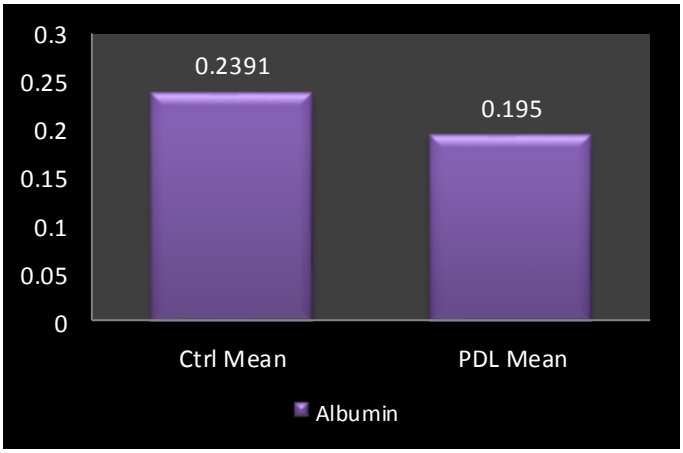

Figure 2: Graphical presentation of liver function test

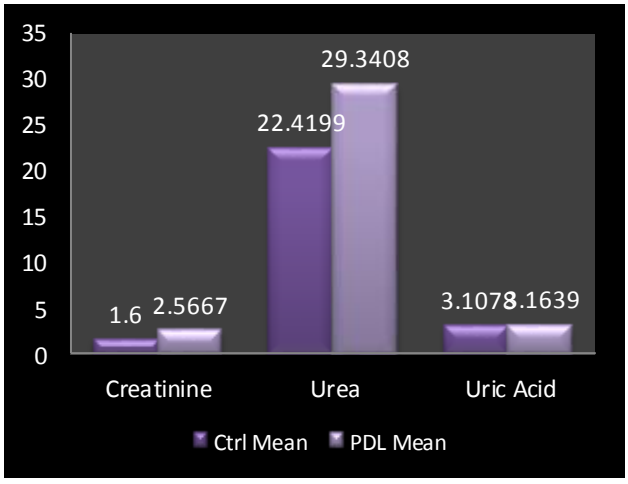

Figure 3: Graphical presentation of kidney function test

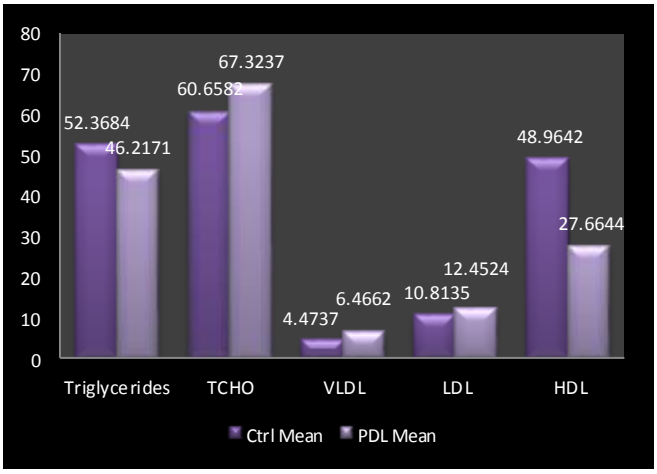

Figure 4: Graphical presentation of lipid profile test

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