ORIGINAL ARTICLE

Mercury Kushta Induced Biochemical Changes In Liver and Kidney of Wister Rats

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ABSTRACT

Background: Different treatment modalities including herbal medicines containing herbs, salts and metals are being practiced in South Asia for different diseases. These medicines may have serious side effects on liver and kidneys.

Objectives: To see the biochemical effects of Kushta which contains mercury, on liver and kidneys of rats.

Setting: This experimental study was conducted for a period of 6 months in the Department of Pathology, University of Health Sciences Lahore from 02-06-2019 to 19-12-2019.

Material and Methods: It was an animal experimental study in which a total of 42 *Wistar rats* were included and divided into five exposed and one control groups. Biochemical changes were observed in liver and kidneys of rats by using indigenous as well as patentmercury preparations.

Results: It was observed that serum ALT was raised uniformly in all exposed groups at the end of 4-weeks and then the levels were even more markedly raised at the end of 8-weeks weeks. Serum creatinine was found to be significantly raised after 4-weeks and the steady rise was then observed from 4 to 8 weeks of exposure to Mercury kushta.

Conclusions: Indigenous herbomineral preparation (kushta) of mercury produces deleterious effects on liver and kidney of ratsat cellular level thus it is both nephrotoxic and hepatotoxic.

Keywords: Mercury Kushta, Liver, ALT, Kidney, Creatinine

INTRODUCTION

Heavy metals are a group of elements that naturally occurs in our earth crust and bio- accumulate in environment, soil, plant and in human body[1]. Human body tissue requires some metallic elements for proper cellular function like copper, zinc and selenium in very low concentration otherwise they cause cellular damage in high concentrations. Heavy metals like, mercury, cadmium, arsenic, chromium and lead are toxic and harmful in low concentration providing no beneficial effects to humans[2].

Metals and their compounds have a long history of pharmacological importance. Metals and minerals are being practiced in South East Asian indigenous systems of medicines (i.e. Ayurveda, Unani-Tibb and Siddha) since over 2000 years. Metals and their products are meant for both external and internal uses and were prescribed for treating obstinate and untreatable diseases as well as in common ailments[3].

For centuries mercury has been used commercially and for medicinal purpose. In the past mercury was used as common constituent of many medicines but now that drugs are obsolete because of toxicity induced by mercury. Still it is used in thermometers and blood pressure cuffs in hospitals. Commercially used in batteries fluorescent bulbs and switches. Metallic mercury is used in electrodes in electrolytic production of sodium hydroxide and chlorine from saline[4].

While one of the dosage forms of traditional medicine practiced by the traditional healers, the "Hakeems", is herbo-mineral/metallic preparations popularly known as "KUSHTAS" commonly used in South East Asia. These herbo-metallic preparations are called Bhasma in Ayurvedic and Kushta in Unani system. The word kushta comes from Persian word "KUSHTAN" means "to kill" or "conquered"[5-6]. Kushta is prepared by constant heating of minerals/metals (calcination) with different herbal extracts, various other plants and fruit juices and then vigorous wet grinding. The preparation (kushta) thus contains oxidized metals such as arsenic, zinc, lead, and mercury, copper and tin which are known to be toxic on ingestion by clinical experts and toxicologists[6].

According to traditional medical literature these metallic preparations are subjected to physiochemical processing attributed to detoxification, purification and restoration of therapeutic properties. The philosophy of Unani medicine states that during the process of these preparations metal is killed and soul of herbal juices are incorporated in body of metal. Still such claims remain to be scientifically validated. There is possible risk of heavy metal poisoning with the use of these kushtas as they contain very toxic metals in high concentrations[3].

This study is concerned about the biochemical effects of kushta containing mercury on liver and kidney. Mercury kushta is commonly known as "kushta Para".

MATERIAL AND METHODS

This experimental study was conducted for a period of 6 months in the Department of Pathology, University of Health Sciences Lahore from 02-06-2019 to 19-12-2019.

A total of 42 Wistar rats of 6 – 8 weeks of age having weight of 200 – 250 grams were taken from University of Veterinary and Animal Sciences, Lahore. They were randomly divided into 6 groups each containing 7 rats. The groupswere labeled as Control Group – I and experimental

groups of II, III, IV, V, VI, VII. These groups were given mercury kushta for a period of 8 weeks. Schedule of feed was given as Table-I:

Table-I: BSA: Bovine Serum Albumin i.p: Intra-peritoneal i.v: Intra-venous

Group	Feed
I	Flour diet in pallet forms
II	Diet + 0.15 mg Hg kushta on alternate days
III	Diet + 0.3 mg Hg kushta on alternate days
IV	Diet +BSA i.v once at the start + 0.3 mg Hg kushta/day
V	Diet +.0.5mg of inj. Mercuric chloride 3times/wk i.p
VI	Diet + 250mg BSA/kg body wt i.v once at the start + 0.5mg of inj.mercuric chloride 3times/wk i.p

This was given on alternative days for 8 weeks. In group IV and VI, Injection BSA (Bovine Serum Albumin) was given at the start of the experiment and metallic preparations were given after 2-3 hrs. Inj BSA increases capillary permeability. Dose is calculated as 250mg/kg body weight[7].

Biochemical parameters Liver Function Tests (serum ALT) and renal function tests (serum creatinine and urinary spot proteins) wereassessed before starting the experiment as well as in the mid and end of the experiment. Serum ALT and creatinine was estimated using Randox Kits and Medi-test Combi 8, urinary strips were used to assess proteinuria. The results were entered in the relevant proforma

RESULTS

We described the observations and their interpretations together however a particular sequence was followed i.e. in the very beginning serological and biochemical parameters of hepatic and renal toxicity (serum alanine aminotransferase, serum creatinine and proteinuria) at start, 4 weeks and 8 weeks.

Serum ALT Levels: Normally, the ALT levels range from 17.5 – 30.2 U/L among healthy adult Wister rats[8]. Mean ± standard deviation of baseline serum alanine aminotransferase of the all the groups was calculated at the start, at 4 weeks and at 8 weeks (Table-II). It is evident, that serum alanine aminotransferase levels remains essentially the same among control animals from the start till the end of experiments. Secondly, it is also clear that all exposed animal groups have shown step-wise rise in their serum ALT levels from the start, through four weeks till the end of experiments. (Fig. I)

Serum Creatinine Levels: Normally, serum creatinine levels range from – 0.8 U/L among healthy adult Wister rats according to most of the published literature [8].Mean ± standard deviation of baseline serum creatinine of the all the groups was calculated at the start, at 4 weeks and at 8 weeks (Table-I). It is evident that serum creatinine levels remains essentially the same among control animals from the start till the end of experiments. Secondly, it is also clear that all exposed animal groups shown a step-wise rise in their serum creatinine levels from the start, through four weeks till theend of experiments. (Fig. II)

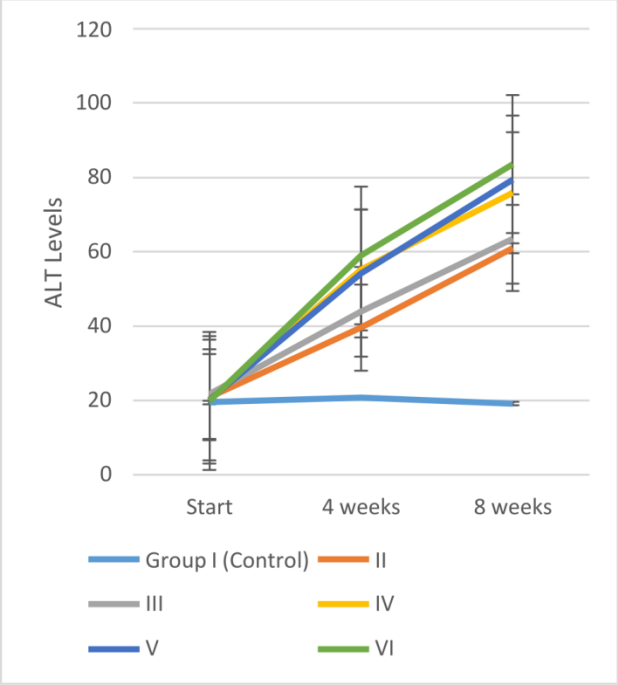

Fig. I: Serum ALT levels among study groups at various stages of experiments

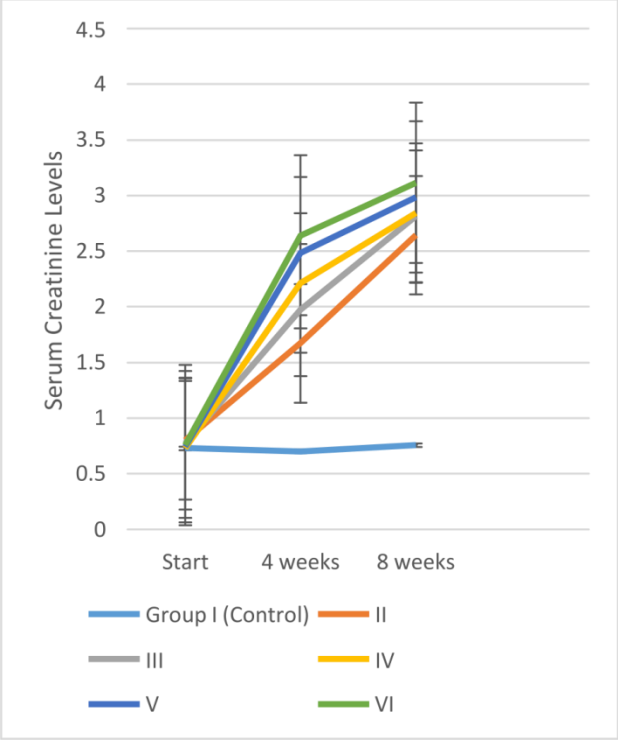

Fig. II: Serum Creatinine Levels among study groups at various stages of experiments

Table II: Levels of ALT, Serum Creatinine and Urinary Proteins among different groups.

Alanine Aminotransferase (ALT) Levels							
Time	Parameter	I	II	III	IV	V	VI
Start	Mean + St.Deviation	19.43± 2.440	20.86± 2.193	21.71± 2.563	20.14± 1.952	20.14± 2.545	19.86± 1.952
	Range	15-22	18-24	18-25	18-23	17-25	1823
At 4 weeks	Mean + St.Deviation	20.71± 1.496	39.57± 6.852	43.86± 5.757	55.14± 9.668	54.14± 7.946	59.00± 7.853
	Range	19-23	30-48	35-50	40-70	42-65	48-70
At 8 weeks	Mean + St. Deviation	19.14± 0.900	61.00± 5.228	63.43± 4.577	75.86± 5.080	79.43± 3.457	83.57± 5.563
	Range	18-20	55-70	58-70	69-82	75-85	75-90
Serum Creatinine Levels							
Time	Parameter	I	II	III	IV	V	VI
Start	Mean + St.Deviation	0.729± 0.1604	0.800± 0.816	0.771± 0.0756	0.729± 0.0488	0.743± 0.0976	0.757± 0.0535
	Range	0.4-0.9	0.7-0.9	0.7-0.9	0.7-0.8	0.6-0.9	0.7-0.8
At 4 weeks	Mean + St.Deviation	0.700± 0.081	1.671± 0.287	1.971± 0.205	2.214± 0.267	2.485± 0.195	2.642± 0.237
	Range	0.60-0.80	1.20-2.0	1.80-2.30	1.90-2.60	2.20-2.80	2.20-2.90
At 8 weeks	Mean + St.Deviation	0.757± 0.053	2.643± 0.310	2.814± 0.106	2.843± 0.113	2.986± 0.134	3.114± 0.186
	Range	0.7-0.8	2.0-2.9	2.7-3.0	2.7-3.0	2.8-3.2	2.9-3.4
Urinary Protein Levels							
Time	Parameter	I	II	III	IV	V	VI
At 4 weeks	Mean + St.Deviation	0	104.29 ±49.618	200.00 ±81.650	285.71 ±106.904	242.86 ±53.452	357.14 ±97.590
	Range	0	30-200	100-300	200-500	200-300	300-500
At 8 weeks	Mean + St.Deviation	0	285.71 ±106.904	357.14 ±97.590	414.29 ±106.904	414.29 ±106.904	500.00 ±0
	Range	0	200-500	300-500	300-500	300-500	500-500

Proteinuria: No proteinuria was observed among animals of the all the groups at the start of experiments. Mean ± standard deviation of urinary proteins of the all the groups was calculated at the 4 weeks and at 8 weeks (Table-I). It is evident that none of the control animals developed proteinuria tillthe end of 4 weeks of experiments. Secondly, it is also clear that all exposed animal groups shown a step-wise rise in their levels ofproteinuria from the start, through four weeks till the end of eight weeks of experimentation. (Fig.III)

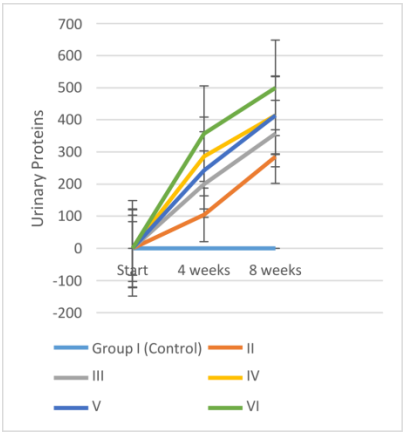

Fig. III: Urinary Proteins among study groups at various stages of experiments

DISCUSSION

It is well known and established fact that heavy metals like Mercury provoke deleterious effects on biochemical profile of liver and kidney in experimental animals. The present research investigated the derangements in biochemical parameters of liver i.e. serum ALT and that of kidney i.e. serum creatinine and spot urinary proteins for the estimation of micro-albuminuria in Wister rats exposed to indigenous herbo-mineral *unani* preparation of Mercury known asMercury *kushta* and compared these biochemical alterations with those produced by Mercuric Chloride.

In the present study, it was observed that serum ALT was raised uniformly in all exposed groups at the end of 4-weeks and then the levels were even more markedly raised at the end of 8-weeks. It is also important that the groups who were given bovine serum albumin (Group IV and VI) were more affected (mean = 79 and 84 U/L respectively) as compared to (mean = 61, 64 and 76 U/L) the rest of the exposed groups (II, III and V respectively). Thus, it may also be appreciated that the deleterious effects of Mercury intake were worse on the BSA-treated sick rats than healthy exposed rats. This elevation of serum ALT in the rats exposed to Mercury kushta is quite similar to the findings by Tanaka *et al.,* Nwokocha *et al.,* Kumar *et al.,* and El-Dermadash . [9-12] Liver is the site for biotransformation by which the toxic element or compound (Mercury *kushta*) has beentransformed in less harmful form to reduce toxicity. However, this will damage the liver cells

and produce hepatotoxicity. ALT is an enzyme that helps metabolize protein. When the liver (hepatocyte) is damaged, alanine aminotransferase enzyme is released from hepatocyte in the bloodstream and thus it acts as a marker of hepatotoxicity.

In the current study, two biochemical parameters of renal function were investigated i.e. serum creatinine and spot urinary proteins in rats exposed to Mercury kushta as compared to the control group. Serum creatinine was found to be significantly raised after 4-weeks and the steady rise was then observed from 4 to 8 weeks of exposure to Mercury kushta. Levels of serum creatinine remained within normal range in control group rats. The groups which were given injections of bovine serum albumin had more pronounced rise in their serum creatinine levels as compared to those groups who were not given the injection bovine serum albumin. Oriquat and coworkers also found that exposure to Mercuric chloride was associated with significantly raised serum creatinine after 3-weeks and 6-weeks of experiments [13]. Joshua *et al.,* and Toshiko *et al.,* also described similar findings in rats exposed to Mercury as compared to controls [9,14].

Spot urinary proteins were also found to be significantly raised after the exposure of Mercury kushta at the end of 4-weeks and then even more pronounced levels were found at the end of 8-weeks. Oda and El-Ashmawy found that exposure to Mercury compound was associated with significant reduction in serum total proteins especially serum albumin. Although serum albumin in reduced due to liver damage as well, but it also correlates with the development of albuminuria as a result of Mercury induced renal damage [15]. This observation is also supported by Zalups and Chaumont *et al.,* who reviewed the available literature on Mercury induced nephrotoxicity at cellular, subcellular and molecular levels [16- 17].

CONCULSIONS

The current study aimed at describing the biochemical alterations in liver and kidney of Wistar rats after ingestion of an indigenous herbal preparation known as Mercury kushta. It was found that indigenous herbo-mineral preparation (kushta) of mercury produces deleterious effects on liver and kidney of rats thus it is both nephrotoxic and hepatotoxic. Mercury kushta produces more pronounced effects when given to the bovine serum albumin(BSA) treated rats. As BSA causes serum sickness syndrome that increases capillary permeability and thus kushta causes more damage. That shows concomitant administration of injection BSA with mercury kushta increasesthe organ damage.

Thus, it is recommended that Mercury *kushta* maynot be used in any disease and health condition to avoid the hepatic and renal derangements.

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