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ORIGINAL ARTICLE

Mercury Kushta Induced Histopathological Changes in Liver of Wister Rats

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ABSTRACT

Background: Heavy metals are the natural constituents of the earth's crust but the indiscriminate human activities have drastically effected their biochemical balance and geochemical cycles. Heavy metals and their compounds have pharmacological importance. These are being used in south Asian countries as component of different medicines. These medicines may have serious side effects on liver.

Objectives: To see the histological changes of Kushta which contains mercury, on liver of wister rats.

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. Morphological changes were observed in liver of rats by using indigenous as well as patent mercury preparations.

Results: Morphological changes in liver of exposed rats included hepatocyte swelling, hepatocyte necrosis, hepatocyte apoptosis, disarray of hepatic architecture, development of portal tract inflammation, central vein congestion, sinusoidal congestion and dilatation, development of fatty change and damage to hepatic vascular and liver capsule were seen at the end of 8 weeks.

Conclusions: Indigenous herbo-mineral preparation (Kushta) of mercury produces deleterious morphological effects on liver of wister rats.

Keywords: Mercury Kushta, Liver, Histopathology

INTRODUCTION

Heavy metals are the natural constituents of the earth's crust but the indiscriminate human activities have drastically effected their biochemical balance and geochemical cycles. This results in the accumulation of metals and secondary metabolites in plants, soil or human body. Human body needs few metallic elements for proper cellular functioning. The advances of toxicology has improved our knowledge about human exposure to toxic elements. Prolonged exposure to heavy metals such as mercury cadmium, copper, lead, nickel, and zinc can cause serious health effects in humans such as developmental retardation, several types of cancer, kidney damage, endocrine disruption, immunological, neurological effects and other disorders. [1-4]

Heavy metals and their compounds have pharmacological importance. These are being used in south Asian countries as component of different medicines in three principal systems i.e. Ayurveda, Siddha and Unani-Tibb. These systems utilize drugs of natural origin constituting plants, animals, and mineral preparations.[2] They are now a days even used in the allopathic medications for different diseases, for example, platinum and arsenic (As) in cancers, silver and mercury in microbial infections, gold (Au) in chronic rheumatoid arthritis and lithium (Li) in manic depression. [5]

In South Asian countries, traditional healers the "Hakeems", practice herbo-mineral/metallic preparations in their medicine. These preparations are known as "KUSHTAS". These herbo-metallic preparations are called Kushta in Unani system and Bhasma in Ayurvedic. This word Kushta comes from Persian word "KUSHTAN" means "conquered" or "to kill". [6-7]. *Kushta*(*s*) are being used for centuries but still the present knowledge about kushta is based on old information and very few scientific reports are accessible. Kushta (s) are finely powdered preparations prepared by the constant heating of metals/minerals with different plants, herbal extracts and fruit juices and then grinded vigorously. Most of the kushta forms prepared only specify the nature of element but not the substance, chemical nature and intermediate products of the end product. [7].

In the environment methyl mercury is the frequently found compound and it is toxic in any form [8]. .Mercury kushta is commonly known as "kushta Para". This kushta is prescribed by the traditional healers for paralysis, asthma, cough, impotence and as a depurative. This study is concerned about the histopathological effects of kushta containing mercury on liver of wister rats.

MATERIAL AND METHODS

This experimental study was conducted for a period of 6 months in the Department of Pathology, University of Health Sciences, and Lahore.

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 groups were labeled as Control Group – I and experimental groups of II, III, IV, V, VI, VII. These groups were given

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mercury kushta for a period of 8 weeks. Schedule of feed was given as Table-I.

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 was calculated as 250mg/kg body weight. [9]

Table-I: BSA: Bovine Serum Albumin

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

i.p: Intra-peritoneal
i.v: Intra-venous

After completion of the experiment the rats were given ether anesthesia and their liver was taken out by dissection under aseptic measures and washed with normal saline to clear blood contaminates and fixed in formalin for 72 hrs. 3 to 5 mm sections were taken from the organ, and each sections was passed separately in a single tissue cassette after labeling its identification. These cassettes were processed as per standard protocol. Slides were prepared and stained for routine histopathology with haematoxylin and eosin (H & E). Reticulin stain was performed on the liver tissue sections only. Slides were microscopically examined to establish the diagnosis. Findings were written in the relevant proformas.

RESULTS

No discernable abnormalities were seen on gross examination. Following histopathological findings were noted microscopically in each section of liver (Table II).

Table-II: Histopathological findings noted in liver sections of each wistar rat.

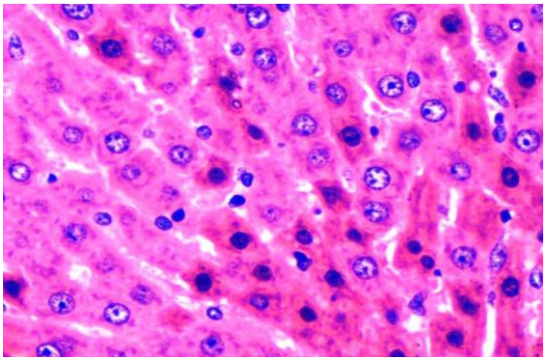
Liver Capsule
Hepatocyte swelling
Portal tract inflammation
Central Vein congestion
Sinusoidal congestion
Sinusoidal dilatation
Vascular damage
Disarray of liver architecture
Hepatocyte necrosis
Hepatocyte apoptosis
Fatty change

Observations were made and entered in a table (Table III). From these observations, it was deduced that 8-week exposure to the given dosage of Mercury *kushta* and injection mercuric chloride was significantly associated (p = 0.0001) with the abnormalities in liver capsule, disarray of liver architecture, hepatocyte swelling, hepatocyte necrosis, portal tract inflammation, fatty change, central vein congestion, hepatic sinusoidal congestion, sinusoidal dilatation, hepatic vascular damage in Wistar rats.

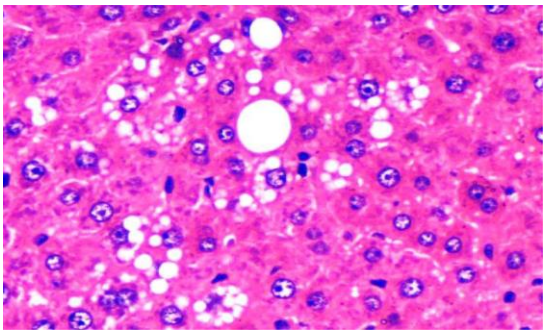
After 8 weeks exposure of the given dosage of mercury kushta and injectable preparation of mercuric chloride was significantly associated (p = 0.002) with the development of hepatocyte apoptosis. The morphological parameters of liver was almost entirely deranged at the end of experimental period in all study groups independent of dosage of Mercury *kushta*.

DISCUSSION

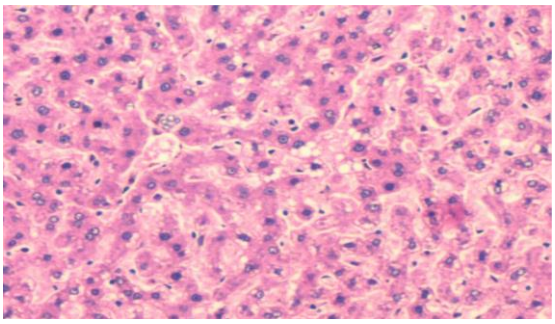
It is well known and established fact that heavy metals like Mercury provoke deleterious effects on biochemical and histomorphological profile of liver in experimental animals.



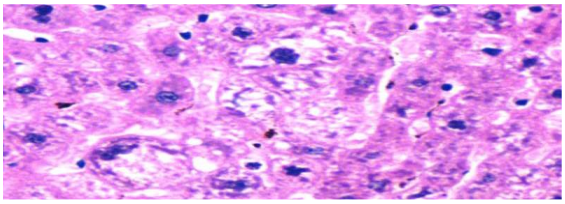
A: Apoptotic cells and some areas of necrosis in liver of group IV.



B: Fatty change in liver of Group IV rat



C: Sinusoidal dilatation and fatty change (Group-V)



D: Showing hepatocyte swelling (Group-V)

Table-III: Histopathological changes in liver of each group of wister rats. (Number of rats is given in each column)

Histopathological Features		Groups							
		I	II	III	IV	V	VI	Sub total	Total
Liver Capsule	Normal	7	7	7	7	2	4	34	42
	Abnormal	0	0	0	0	5	3	8	
Hepatocyte swelling	Absent	7	0	3	0	0	0	10	42
	Present	0	7	4	7	7	7	32	
Portal tract inflammation	Absent	7	6	6	0	0	0	19	42
	Present	0	1	1	7	7	7	23	
Central Vein congestion	Absent	7	0	2	0	0	0	9	42
	Present	0	7	5	7	7	7	33	
Sinusoidal congestion	Absent	7	6	4	1	1	0	19	42
	Present	0	1	3	6	6	7	23	
Sinusoidal dilatation	Absent	7	7	6	3	0	0	23	42
	Present	0	0	1	4	7	7	19	
Vascular damage	Absent	7	4	3	0	0	0	14	42
	Present	0	3	4	7	7	7	28	
Disarray of liver architecture	None	7	0	2	0	0	0	9	42
	Mild	0	4	5	4	0	0	13	
	Moderate	0	3	0	3	1	0	7	
	Severe	0	0	0	0	6	7	13	
Hepatocyte necrosis	None	7	1	0	0	0	0	8	42
	Mild	0	5	6	0	0	0	11	
	Moderate	0	7	1	7	2	3	20	
	Severe	0	0	0	0	5	4	9	
Hepatocyte apoptosis	None	7	1	0	0	0	0	8	42
	Mild	0	6	7	0	6	7	26	
	Moderate	0	0	0	4	1	0	5	
	Severe	0	0	0	3	0	0	3	
Fatty change	Absent	7	2	3	0	0	0	12	42
	Focal	0	4	2	0	0	0	6	
	Multifocal	0	1	2	7	6	4	20	
	Diffuse	0	0	0	0	1	3	4	

The present research investigated the derangements in histological morphology of liver in Wister rats exposed to indigenous herbo-mineral *unani* preparation of Mercury known as Mercury *kushta* and compared these alterations with those produced by Mercuric Chloride.

In the present study, hepatocyte swelling, hepatocyte necrosis, hepatocyte apoptosis, disarray of hepatic architecture, development of portal tract inflammation, central vein congestion, sinusoidal congestion and dilatation, development of fatty change and damage to hepatic vascular and liver capsule were commonly observed in a majority of rats in all study groups. Although certain study groups (which were pre-treated with bovine serum albumin) had difference in the frequency and severity of some of these histological alterations yet all exposed definitely showed hepatotoxic effects of Mercury. Deore and Wagh studied the hepatic changes induced by Mercury compound and found that vacuolation in cytoplasm, degeneration of nuclei, vacuolisation in stroma, cloudy swellings, pycnotic nuclei, necrosis, rupture of blood sinusoids, disarray of hepatic cords, loss of shape of hepatocytes were significantly associated with exposure to metal [10]. Moreover, they also found that severity of damage was found to be dose dependent and dependent on the time of exposure. Oda and El-Ashmawy found that exposure to Mercury compound was associated with moderate hepatocyte vacuolisation particularly in the periportal zones, dilatation of hepatic sinusoids with atrophy and disarray of hepatic cords as well as focal areas of hepatic necrosis in other experimental animal (Hamsters) [11]. Sheikh *et al.,* (2013) observed severe degree of fatty changes, vacuolisation, karyorrhexis in hepatocytes, sinusoidal dilatation and mononuclear cell infiltration in liver of *Wister* rats [12]. Another group of investigators, Sheikh *et al.,* (2011) reported that grossly liver turned pale and round border at the end of 4-weeks exposure to Mercury compound in Wister rats [13]. Microscopically, liver revealed moderate to severe degrees of fatty changes (vacuolisation) in hepatocytes, sinusoidal dilatation, necrosis with congestion and haemorrhage. Severe congestion, hemorrhage along with moderate infiltration of mononuclear cells around central vein was also noticed. Karyorrhexis and scatted red blood cells in hepatic cords were also present in exposed groups.

Jankeer and El-Nouri showed that lead (Pb) being another heavy metal induced almost similar histological changes in the liver of Albino mice [14]. They observed hepatocyte vacuolation, fatty change, necrosis in some hepatocytes, congestion within central veins, haemorrhage between hepatic cords, architectural disarray of the cords and infiltration of inflammatory cells which were mononuclear i.e. lymphocytes. Durgut *et al.,* investigated histological changes in various organs of the body in rabbits and found similar changes in liver in response to ingestion of heavy metals [15]. Thus, it is deduced that Mercury *kushta* induced hepatic morphological alterations quite similar to other compounds of Mercury used by previous investigators.

CONCULSIONS

The current study aimed at describing the histological alterations in liver 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 of rats. Morphological changes in liver of exposed rats included hepatocyte swelling, hepatocyte necrosis, hepatocyte apoptosis, disarray of hepatic architecture, development of portal tract inflammation, central vein congestion, sinusoidal congestion and dilatation, development of fatty change and damage to hepatic vascular and liver capsule. 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 permeabily and thus kushta causes more damage. That shows concomitant administration of injection BSA with mercury kushta increases the organ damage.

Thus, it is recommended that Mercury *kushta* may not be used in any disease and health condition to avoid the derangements in hepatic architecture.

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