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Original Research Article

Herbal Kushta of Cuprous oxide as a Nephrotoxic Agent - An Experimental Study

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Abstract

The aim of this research is to evaluate the effects of cuprous oxide preparations of Kushta on renal function tests. This study was conducted at Pathology Department of University of Health Sciences Lahore and it was an experimental study. Thirty albino rats were selected and divided into three groups. The control group A was fed on standard pellet rodent diet and tap water. The experimental group B was fed single dose of cuprous oxide preparation of Kushta (0.15mg/kg of body weight) for 24 weeks while experimental group C was fed double dose of cuprous oxide preparation of Kushta (0.5mg/kg of body weight) for 24 weeks. Renal function tests were calculated once at the start of experiment and then after every 6 weeks up to 24 weeks. Results showed that renal function tests in terms of serum creatinine level and proteinuria were deranged evident on biochemical analysis. When the Control group A on normal diet was compared with the experimental group B and C, statistically significant difference ($p<0.05$) was noticed in terms of renal function tests. It is therefore concluded that cuprous oxide preparations of Kushta deranged renal function tests in terms of serum creatinine levels and proteinuria.

Keywords: Kushta, heavy metals, cuprous oxide, renal toxicity

INTRODUCTION

Cuprous oxide is a trace element vital for all human beings (deMayo and Taylor, 1980). It is an essential requirement in the synthesis of chlorophyll and haemoglobin (Birge and Black, 1997). Cu metal is found naturally in the Earth's crust. Its natural air and water discharges are therefore considerable (Barceloux, 1999). Heavy metals are those metallic elements which are dangerous at higher concentration and have greater density than 5g/cm^3. Heavy metals are toxic in nature since they tend to bioaccumulate in the body. Some adverse effects of heavy metal products can also result from metal poisoning but is dose dependant. Usually people inhale or ingest small amounts of metals which gradually interfere with body functions and pathogens which may cause eventually death (Abu Bakar, 2008). In the Earth's crust, many metals are found as natural constituents but many human procedures have altered their geochemical cycles and biochemical balance. Some form of pharmacological activity for example results in the accretion of metals in certain parts of the plant in the form of secondary metabolites. Any contact beyond the upper tolerable range, with these heavy metals such as cadmium, cuprous oxide, lead, nickel and zinc can cause lethal effects to the humans. Heavy metals interrupt the metabolic processes in two ways. Once they disturb the functions of essential organs such as the heart, brain, kidneys, bones, liver, etc., and secondly, they shift critical mineral nutrients from their position of origin, and thus impede their natural organic purpose. However, it is not possible to make the surroundings free from these

metals. There are several ways for heavy metals to be introduced in the body for example food consumption, beverages, skin exposure, inhaled air, soils, manufacturing activity and the utilization of fuel from the remnants. A contact to heavy metals such as cadmium, cuprous oxide, lead, nickel and zinc is reported to be dangerous for the human health (Reena et al., 2011). Ingredients of "Kushta" by quakes and so-called hakims for the treatment of chronic conditions can damage the kidneys and can cause renal failure. Heavy metals are used in the synthesis of Kushta by hakims. The metals used in the preparation of Kushta aren't useful for health or digestion. They accumulate within the kidneys, creating a continuous contamination that ends up in severe renal impairment. Cuprous oxide exists in the blood in two forms: one is bound to ceruloplasmin (85-95%), and the second is the 'free' rest, loosely bound to albumin and small molecules. Cuprous oxide is transported in the blood by a protein ceruloplasmin. After absorption, it is transported to the liver. After oral intake it accumulates within the liver initially when it reaches the lethal level, pathological reaction starts. Cuprous oxide is absorbed in the duodenum and upper parts of the small intestine. Then 95 percent Cu ions are bound to protein ceruloplasmin and rest is with albumin and amino acids. A small amount is excreted in the urine. The main part of the Cu from the body is excreted in bile. Traffic and a good use of cuprous oxide in the body are dependent on the proper functioning of many organs such as liver, gall bladder and kidneys. If any of these organs are not functioning properly, the body cannot use and excrete Cu properly. Cuprous oxide is a strong oxidant metal causing inflammation and free radical damage to tissues. To avoid these toxic effects, it should be linked to binding proteins, ceruloplasmin and metallo-thionein. With increase in retention of cuprous oxide, it produces a toxic effect (similar to other heavy metals) on the body and contributes several chronic diseases (Abu Bakar, 2008). As the kidneys are highly sophisticated transport organs, they excrete metabolic waste. Such a function is optimized by a special circulatory structure in the kidney, which has a remarkable ability to adjust the haemodynamic inputs in order to maintain renal circulation (Burkitt et al., 2000). The basic functional unit of kidney is the nephrons. About 1.2 million of nephrons are present in each kidney, urine is synthesized by them. Each nephron is composed of Bowman capsule that surrounds the glomerular capillary tuft, the proximal convoluted tubule, the loop of Henle and the distal convoluted tubule, which empties into the collecting ducts (McCane and Huethe, 1994). All heavy metals are deposited in kidney tissue. When liver is being saturated with cuprous oxide and all binding sites are occupied then cuprous oxide compounds will begin to accumulate in the kidney (Chugh et al., 1975).

Renal lesions after using cuprous oxide preparations of Kushta can happen by three possibilities. It could be acting as haptens, could intervene in the body's defense mechanism and act as direct foreign toxic substances like other heavy metals (Druet et al., 1982). The toxicity of these preparations of heavy metals depend on various factors such as the toxicity of metals, the duration of the exhibition, the total absorbed dose, age of the exposed person and the route of exposure (Fuentealba and Haywood, 1988). Kidneys act as filtration unit of the body so when these cuprous oxide products have been used by humans they are filtered and trapped in the renal tubules and glomeruli, causing damage to the kidneys. Sometimes, it could be irreversible damaged the kidney and can lead to death. Kidney damage in terms of glomerulonephritis would be occurred by the use of these toxic metallic Kushtas. Glomerulonephritis is usually associated with rapidly progressive glomerulonephritis syndrome, which can occur mostly in the form of inflammation of glomeruli, including post infectious glomerulonephritis, IgA, lupus, Vasculitis renal nephropathy, membranoproliferative glomerulonephritis, and anti-glomerular basement (GBM) antibodies which are mainly caused by the toxicity of heavy metals.

MATERIAL AND METHODS

It was an experimental intervention, randomized controlled study in adult rats. The study was conducted at University of Health Sciences, Lahore. The animals were kept in the animal house and experimental work was further processed in the pathology department of University of Health Sciences, Lahore. The total duration of this study was 24 weeks. Thirty male and female albino rats of Wister strain, 6-8 weeks of age; weighing 200-250 Gms were procured from University of Health Sciences Lahore. Albino rats were separated in different cages and maintained in the animal house of the University of Health Sciences Lahore under controlled environment (temperature 22-25 C, humidity 65%±5) and light and dark cycle of 12 hours each. Each rat was to receive the prescribed dose on alternate days. Cage cards were used to indicate the group of albino rats.

1) **Group A**: is the control group. It included 10 healthy rats receiving normal diet and tap water for 24 weeks
2) **Group B**: It included 10 rats; Cu preparations of Kushta 0.15 mg/kg body weight, mixed and homogenized with wheat flour, and dispensed as pellets ,were given orally to the rats of this group on alternate days for 24 weeks.
3) **Group C**: It included 10 rats and Cu preparations of Kushta 0.5 mg/kg body weight, mixed and homogenized with wheat flour, and dispensed as pellets, was given orally to the rats of this group on alternate days for 24 weeks.

Experimental schedule

All rats were weighed before the commencement of the experiment. No sex differences were made and all rats were kept in separate cages. They received nutritionally standard diet and water. After acclimatization, rats were divided into three groups as mentioned above. Each rat was marked for its identification by giving a number on its back. Before the start of experiment urinary proteins and serum creatinine level were measured in all rats.

Cuprous oxide preparations of Kushta

Cuprous oxide preparations of Kushta used in this study were analyzed chemically from Biochemistry Department of University of Health Sciences Lahore, for exact quantification of the heavy metal. According to the body weight (0.5mg/kg body weight) doses were calculated, mixed and homogenized with wheat flour and dispensed as pallets. This dose has been used in various studies (Lei et al., 2008; Bertinato and Zouzoulas, 2009). Oral LD_{50} of rats for Cuprous oxide Kushta has been reported as 2mg/kg body weight (Onwuka, 2005). Doses have been translated from human dose to rat dose by normalization of the body surface area (Animal dose mg/kg=Human Dose mg/kg multiplied by Animal Km/Human Km); where Km is 5.9 for rat and 37 for human (Freireich et al., 1966). The doses were adjusted daily according to the weight of each rat.

Methods for determination of serum creatinine and proteinuria

Serum creatinine was measured by using commercially available kits prepared by Randox (Ref: CR510, LOT: 216982). Urinary proteins were determined by strip method (Cavaggioni and Mucignat-Caretta, 2000).

Collection of blood sample: Method for obtaining blood sample and 24 hour urinary proteins

Blood samples from each group were collected by cardiac puncture (Cunliffe-Beamer, 1983). Albino rat was taken out by holding its tail and was anaesthetized by placing it in a plastic container having a tight lid covering and a cotton ball soaked in ether alcohol until the rat was completely anaesthetized. The rat was when removed from the container and 1 ml of blood was drawn in 3 ml disposable syringe by cardiac puncture. Blood was allowed to stand for one hour before centrifuging it at a speed of 3000r/pm for 10 minutes. Samples were collected in metabolic cages. During this collection of 24 hour urine samples the animals were allowed only water to drink.

RESULTS

Renal functions tests were performed once before starting the experiment as well as every six week thereafter for 24 weeks. (Table 1)

Table 1. Descriptive statistics for Creatinine level in all groups at different time intervals

Groups	*N*	*Mean*	*SE*
6 weeks of experiment			
Group-A	10	0.16	0.01
Group-B	10	0.48	0.03
Group-C	10	0.40	0.05
12weeks of experiment			
Group-A	10	0.16	0.01
Group-B	10	0.58	0.02
Group-C	10	0.54	0.02
24 weeks of experiment			
Group-A	10	0.16	0.01
Group-B	10	0.73	0.03
Group-C	10	0.73	0.11

Serum creatinine level

Serum creatinine level was assessed in all 3 groups of animals at the start of experiment and then after every 6 weeks. During this follow up time period it was observed that creatinine level in Group-A animals was 0.16±0.01 when assessed after 6, 12 and 24 weeks. While in Group-B & C creatinine level was quite high as compared to Group-A rats at follow up time intervals, i.e., at 6, 12 and 24 weeks respectively.

24 hour urinary proteins level

24 hour Urinary proteins level in Group-A rats was 0.00 during the course of follow up time period i.e., (6,12 and 24 weeks). But 24 hour urinary proteins level in Group-B& C rats show high level of urea protein level at 6, 12 and at 24 weeks (Table 2).

Table 2. Descriptive statistics for 24 hour urinary Proteins level in all groups at different time intervals

Groups	*N*	*Mean*	*SE*
6 weeks of experiment			
Group-A	10	< 1.0	0.2
Group-B	10	142.00	60.73
Group-C	10	139.00	61.41

Table 2. Continue

12weeks of experiment			
Group-A	10	< 1.0	0.2
Group-B	10	213.00	63.00
Group-C	10	173.00	54.93
24 weeks of experiment			
Group-A	10	< 1.0	0.2
Group-B	10	340.00	65.31
Group-C	10	380.00	61.10

DISCUSSION

The present study demonstrated that cuprous oxide in dose dependent manner is renal toxicant and it changes serum parameter levels related to renal function. Serum levels of creatinine increased as a result of cuprous oxide exposure in comparison to the control group.

These observed changes are in accordance with other authors' results (Onwuka, 2005; Wang et al., 2014).

The significant role of kidneys in both excretory and endocrine process cannot be denied. Kushta and metal containing products hinder the function of kidneys.

The scope of the present study is to highlight the toxic effects of cuprous oxide preparations of Kushta on kidneys of albino rats. In the present study rats, were treated with various doses of cuprous oxide preparations of Kushta orally and markedly raised serum creatinine level were observed during the study except in control group. Six rats from group C and 10 rats from group D showed up to 1.1mg/dl. Elevated creatinine level signifies impair kidney function or kidney disease and this relation has been very well established in various other experimental conditions (Wang et al., 2014; Babaknejad and N Moshtaghie and Shahanipour, 2015). Serum levels of creatinine increased as a result of cuprous oxide exposure in comparison to the control group. These changes are in accordance with other authors' results (Sinkovic et al., 2008; Beckett et al., 2009).

Similarly in the present study significant association has been found between various doses of cuprous oxide preparation of Kushta and proteinuria. One of the most toxic effects of cuprous oxide on kidney is proteinuria.

After six weeks four rats showed proteinuria of 30mg/dl and six rats showed proteinuria of 100mg/dl of group B while in group C, one rat showed 30mg/dl proteinuria and four rats showed 100mg/dl proteinuria and five rats showed 500mg/dl proteinuria. After twelve weeks nine rats from group B and three rats from group C showed 100 mg/dl proteinuria and one from group B and seven from group C showed 500mg/dl proteinuria. At the end of experiment eight rats from group B and all rats from group C showed 500 mg/dl proteinuria. This showed the clear association between doses of metallic Kushta and proteinuria which led to the irreversible kidney damage. These findings are consistent with the other studies describing indication of kidney damage is proteinuria (Beckett et al., 2009). One of the most toxic effects of cuprous oxide on kidney is proteinuria (Babaknejad and N Moshtaghie and Shahanipour, 2015).

The present study demonstrated that cuprous oxide in a dose dependent manner is renal toxicant and it changes serum parameter levels related to renal function.

CONCLUSION

In conclusion, present study clearly suggests that cuprous oxide in dose-dependent manner by changing serum function tests related to renal function can induce renal toxicity. It is therefore concluded that cuprous oxide preparations of Kushta deranged renal function tests in terms of serum creatinine levels and proteinuria.

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