ARNEBIA BENTHAMII AGGRAVATES POTASSIUM DICHROMATE INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT
Liver and kidneys are exposed to a lot of exogenous and endogenous oxidants. Potassium dichromate is a potent hepatotoxic and nephrotoxic agent. Reactive oxygen species (ROS) and consequent peroxidative damage caused by dichromate are considered to be the main mechanisms leading to hepatotoxicity and nephrotoxicity. The present investigation aims at assessing the effect of aqueous extract of Arnebia benthamii on dichromate induced hepatotoxicity and nephrotoxicity in a rat model. The effect was seen by comparing the serum hepatic and renal marker levels in treated and toxic model with control as an index for hepatotoxicity and nephrotoxicity. Hepatic markers, alkaline phosphatase, alanine and aspartate aminotransferases were found to be significantly increased in the serum of rats treated with dichromate (10 mg/kg b.w, i.p.), suggesting hepatic damage. Likewise, marked increase in kidney function markers i.e., BUN and creatinine were observed in dichromate administered rats. Pre-treatment with aqueous extract of Arnebia benthamii further increased the levels of serum markers for hepatotoxicity, providing an insight towards its effect as hepatotoxic. However, no significant change in kidney function markers was observed in treated group as compared to the toxic group. In conclusion, our study suggests that use of Arnebia benthamii may worsen hepatic health, thus caution need to be taken while using it as folklore medicine.

Keywords: Nephrotoxicity, Hepatotoxicity, Potassium dichromate, Arnebia benthamii.

INTRODUCTION
Herbal remedies are being used for the treatment of various diseases and dysfunctions since ancient times. Recent research focuses on the identification of the novel, natural and safe therapies using medicinal plants and several in-vitro and in-vivo pre-clinical studies have validated the therapeutical value of newly identified phytochemicals. The use of traditional herbal medicines is increasing day by day and is being appreciated with Western models of integrative health sciences. Herbal medicines may prove to be a successful therapy where conventional medicines fail, as herbal extracts function through multi-target mechanisms in contrast to the conventional medicines. However, these herbal extracts may sometimes cause adverse effects due the presence of certain toxic components in them. In a survey in Iceland regarding adverse effects of herbal medicines, Herbalife products were seen to be involved in the most cases of hepatotoxicity. For this reason, use of some of the herbal remedies has been restricted now. In the present study, we used potassium dichromate induced toxicity as a model to investigate the effect of Arnebia extract on liver and kidneys. Chromium imparts tissue damaging effect and is a well known potent hepatotoxic and nephrotoxic. Potassium dichromate finds extensive use in metallurgy, chrome plating, chemical industry, textile industry, wood preservation, photography...
and photoengraving, refractory and stainless steel industry, and cooling systems. Exposure to chromium is known to cause allergic dermatitis, carcinogenicity and acute renal failure in humans. Excessive generation of ROS has been speculated to be involved in cell injury induced by Chromium Cr(VI), which subsequently damages cellular proteins, lipids, and DNA leading to oxidative stress. As chromium reduction intermediates [Cr(V), Cr(IV), and Cr(III)] generated under physiological conditions involve massive ROS production, they may impart toxic effects to the cells. Arnebia species represent one of the essential herbal drugs of indigenous systems of medicine. One member of this species, *Arnebia benthamii*, locally known as “Keh zaban” belongs to family Boraginaceae. It is an erect, herbaceous perennial, commonly found in the alpine and subalpine Himalaya at an altitude of 3000–3900 m. Being a main component of the commercial drug “Gaozaban”, it imparts antibacterial, antifungal and anti-inflammatory properties to it. Besides, Arnebia also exhibits stimulant, tonic, diuretic, expectorant and anti-cancerous properties. Arnebia species have been reported to contain multifarious compounds such as naphthoquinones, benzoquinones, alkaloids, triterpenoids, steroids and flavonoids. Among these, some naphthoquinone derivatives have been observed to cause haemolysis, while others have been shown to cause necrosis of tubular epithelial cells. In addition to this, hydroxy and amino derivatives of naphthaquinones have been implicated in nephrotoxicity. It has been observed that methylation of the amino group of 2-amino-1,4-naphthoquinone increases the severity of both haemolysis and renal damage caused by naphthaquinones. Moreover, various species of Arnebia have been reported to possess pyrrolizidine alkaloids (PAs), which are potent hepatotoxins. On the whole, in more than 6,000 plants of Boraginaceae, Compositae, and Leguminosae families, over 350 Pyrrolizidine alkaloids have been recognized. PA-containing plants affect livestock, wildlife, and humans. In humans, poisoning occurs as a result of consumption of these plants for food or medicinal purposes. Furthermore, some scholars of the Indian system of medicines have observed harmful effects of this extract on spleen. But its effect on liver and kidneys is not so clear. So this study was designed to investigate the effect of aqueous extract of *Arnebia benthamii* on hepatotoxic and nephrotoxic parameters in potassium dichromate treated rats.

**MATERIALS AND METHODS**

**Animals**

Adult Wistar albino rats (250 g) of same sex were purchased from the IIIM Jammu. This study was approved by the Institutional Animal Ethics committee, University of Kashmir. The animals were kept under controlled temperature condition (25°C). Animals received pelleted rodent feed (commercial rodent chow) and water ad libitum.

**Reagents**

Analytical-grade Potassium dichromate and all other chemicals were purchased from Sigma-Aldrich (USA). The kits used in the experiments were purchased from RFCL Limited, Uttarakhand, India.

**Preparation of Arnebia benthamii extract**

100g of dried plant powder was added to glass flask containing one litre of water. This was followed by continuous stirring for four days accompanied with mild heating. The solution was then filtered to remove any un-dissolved plant material. The clear aqueous solution obtained after filtration, containing the dissolved plant components, was then subjected to freeze drying and stored at -80°C till further use. The amount of plant extract obtained on an average was 10g/l.

**Study design**

Rats were randomly separated into 4 groups (5 rats per group) namely Control group (C), Extract Control group (E), Toxic group (D) and Experimental group (D+E). Rats in Control group were not subjected to any treatment but were permitted water ad libitum. In toxic group, toxicity was induced by injecting potassium dichromate intra-peritoneally (i.p.) in rats (10 mg/kg body weight) for 5 days at 24 h interval. The cumulative dose of potassium dichromate received by each rat was thus 12.5 mg. In extract control group, rats were treated with Arnebia extract (300mg/500ml of drinking water) (This concentration was selected as per the consumption of Arnebia benthamii by the local population for therapeutic purposes). Rats in experimental group were given Arnebia extract at a concentration of 300mg/500 ml of drinking water per 24 hours per cage for a period of ten days prior to potassium dichromate injection at a concentration (10mg/kg b.w.) for 5 days. The blood was collected from the rats after...
24 hours of final dose of Potassium dichromate. Before collection of blood, rats were starved for six hours. Serum was isolated for the assessment of liver and kidney functions.

RESULTS
1) Effect of Arnebia extract on potassium-dichromate induced hepatotoxicity
Levels of serum hepatic markers viz., AST, ALT and ALP were determined. Pre-treatment with aqueous extract of Arnebia benthamii was seen to further increase the levels of these markers in experimental rat models as compared to toxic group treated with potassium dichromate.

a) Effect of Arnebia extract on potassium dichromate induced increase in AST (Aspartate aminotransferase) level in serum
AST is a hepatic marker. Its level gets elevated in serum due to the damage caused to liver cells. Serum AST activity in rats exposed to potassium dichromate was significantly higher than those in control unstressed rats (C). Pre-administered Arnebia benthamii at a dose of 300 mg/kg b.w., prior to potassium dichromate injection (i.p.) caused further increase in serum AST. However, extract control group of rats (E) did not showed any significant change in the level of AST.

b) Effect of Arnebia extract on potassium dichromate induced increase in ALT (Alanine aminotransferase) level in serum
ALT is a specific marker for hepatotoxicity, often used to indicate liver damage. Whenever liver is damaged, it releases ALT into the bloodstream raising its level in blood. ALT level increases by more than three times of upper limit of normal (ULN) during liver injury. In toxic group, ALT level was significantly increased as compared to that of control, whereas extract was seen to further increase this level in experimental group.

c) Effect of Arnebia extract on potassium dichromate treated rats on ALP (Alkaline phosphatase) level
ALP is a marker for hepatotoxicity. It shows increased concentration in serum at the time of hepatic damage. ALP level increases by more than twice ULN in liver injury. Serum ALP activity in rats exposed to potassium dichromate was significantly higher than those in control unstressed rats (C). Pre-administered Arnebia benthamii at a dose of 300 mg/kg BW prior to potassium dichromate injection (i.p.) augmented the increase in serum ALP due to potassium dichromate.

Fig 1a: Changes in AST level in extract treated rats compared to toxic and control. Effect of pre-administered Arnebia benthamii extract on changes in serum AST level; Control group (C), Extract control group (E), Toxic group treated with potassium dichromate (D) and Experimental group (D + E). Data are expressed as mean ± SEM of five rats in each group. p-value < 0.01.
2) **Effect of Arnebia extract on potassium-dichromate induced nephrotoxicity**

Levels of serum markers for nephrotoxicity (BUN and serum creatinine) were determined. Aqueous *Arnebia benthamii* extract was seen to further increase the levels of the serum creatinine and BUN in experimental rat models as compared to toxic group.

a) **Effect of Arnebia extract on potassium dichromate induced increase in serum BUN (Blood Urea Nitrogen) level**

BUN is a specific marker for nephrotoxicity. During renal damage, its level gets elevated in serum. Potassium dichromate increased the level of BUN in rats significantly as compared to control. However, extract pre-treatment did not cause any significant difference in serum BUN in experimental group as compared to dichromate treated group.

b) **Effect of Arnebia extract on potassium dichromate induced increase in Creatinine level in serum:**

Creatinine is filtered from the blood by the kidneys and excreted into the urine. With normal kidney function, the amount of creatinine in the blood remains relatively constant and normal. For this reason, an elevated blood creatinine level is a more sensitive indicator of impaired kidney function than the BUN. Potassium dichromate enhanced creatinine level in toxic group of rats significantly as compared to control. However, extract treatment did not cause any significant increase in the creatinine levels as well.
Fig 2a: Changes in serum BUN level; Control group (C), Extract control group (E), Toxic group treated with potassium dichromate (D) and Experimental group (D + E). Data are expressed as mean ± SEM of five rats in each group. P value<0.001.

Fig 2b: Changes in serum creatinine level; Control group (C), Extract control group (E), Toxic group treated with potassium dichromate (D) and Experimental group (D + E). Data are expressed as mean ± SEM of five rats in each group. P value < 0.01.

DISCUSSION
In the present study, we have preferred hepatic and renal markers because liver and kidney are major target organs of toxicity. Liver is vulnerable to toxicity by different chemicals as it is the primary organ of biotransformation of xenobiotic compounds. The various metabolizing enzymes present in liver transform most toxic substances to less toxic and more water-soluble form but sometimes can lead to their bioactivation. Hepatotoxicity due to Chromium occurs largely by accumulation of chromium in liver after exposures or ingestion of chromium. Intake of large quantities of Cr (VI) can lead to hepatocellular necrosis. In several in-vivo models, hepatotoxic effects of Chromium like large necrotic areas and destruction of the general architecture of the tissue as well as increased serum transaminases and lactate dehydrogenase have been reported. Moreover, Cr (VI) but not Cr (III) has been observed to impart a dose-dependent loss in cell viability in liver epithelial cell line. Likewise, kidneys are known to filter, detoxify, or bioactivate toxicants. Being highly vascularized and receiving about 25% of cardiac output, they are particularly susceptible to effects of stress and toxicity by metals like chromium. Furthermore, due to the existence of a cortico-medullary osmotic gradient, toxic agents are accumulated in papilla and medulla of kidneys. Moreover, as the renal tubules play a major role in the reabsorption of a number of endogenous and exogenous substances, both the tubular lumen and cells of kidneys are further exposed to high
concentrations of potentially toxic agents. Exposure to low doses of Cr (VI) usually results in transient renal effects but severe poisoning by Cr (VI) can lead to acute tubular necrosis and acute renal failure. In this study, both hepatic markers (alkaline phosphatase, alanine and aspartate aminotransferases) and kidney function markers (urea and creatinine) were found to be significantly increased in the serum of rats treated with potassium dichromate (10 mg/kg b.w, i.p.) as compared to the control, suggesting hepatic and renal stresses by potassium dichromate. Renal damage induced by K$_2$Cr$_2$O$_7$ has been previously associated with oxidative stress. Similarly, acute exposure to Cr (VI) has been reported to produce acute necrosis of renal tubules. It has also been reported that rats treated with a single subcutaneous injection of 15 mg/kg K$_2$Cr$_2$O$_7$ in a volume of 0.5 ml showed increased serum creatinine and BUN. Also, an oral dose of 4 mg/kg Cr (VI) has been observed to cause acute renal tubular necrosis before death from cardiovascular shock. Nephrotoxicity has been observed in rats treated with 2mg/kg Cr (VI). The exact mechanism of Cr (VI)-induced toxicity has not been elucidated yet. However, in-vitro studies have shown that the reduction of chromium inside the cells causes the generation of reactive oxygen species, which in turn cause damage to various tissues. Free radicals produce a number of toxic effects including DNA damage and lipid peroxidation, therefore, the toxic effects of Cr (VI) may be, at least in part, associated with the production of reactive species via Fenton or Haber-Weiss type reactions. The present study clearly shows that pre-treatment of experimental rat models with aqueous extract of Arnebia benthamii prior to potassium dichromate injection (i.p.) caused further increase in levels of hepatic markers, thereby providing an insight towards its effect as hepatotoxic.

As most plants of Boranginaceae, Compositae, and Leguminosae families including some species of Arnebia contain pyrrolizidine alkaloids, which are well known hepatotoxins, it is possible they may be present in Arnebia benthamii as well, which may be the cause of the effects observed. Significant damage caused to the liver cells in experimental rat model seems to be a combined effect of extract and dichromate, as the levels of serum hepatic markers are much elevated in experimental rat model as compared to toxic rat model. In conclusion, it seems that the extract enhances the extent of liver damage caused by the potassium dichromate, as no significant hepatotoxicity in extract control group was observed.

CONCLUSION
Although in folklore medicine Arnebia benthamii has been ascribed with beneficial medicinal properties, but the findings of our study suggest that it can cause significant hepatotoxicity. Its consumption should be completely avoided by the patients already suffering from any liver disorder.

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REFERENCES