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Is lead in Nagabhasma toxic to liver? – A histological evaluation

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SUMMARY

Ayurvedic medicines is known to use heavy metals in their preparation. Nagabhasma is one such form of a lead-based medicine. Even though lead is known to be toxic to several systems of the human body, according to Ayurveda, the metallic toxicity of the lead gets nullified and thereby imbibes medicinal property when it is prepared using many herbs and stringent traditional methods. Therefore, the present study is designed to evaluate the effect of such detoxified lead in various stages of authentically prepared Nagabhasma on the histopathology of liver in comparison with lead acetate and commercially available Nagabhasma-administered animals. Less than the human-equivalent doses of Nagabhasma at four intermittent stages of its preparation were administered orally for 30 days and 60 days (short term and long term exposure) to Wistar rats. In another set of experiment, test-material-administered animals were kept under observation for an additional period of two months to record the residual effect. Immediately after the administration and after the observation period, the animals were sacrificed to collect the liver for histopathological examination.

The histopathological results of the immediate and residual effects showed varying alterations in the microarchitecture of the liver as the stages of Nagabhasma preparation advanced. The final product (stage 4 bhasma), showed very less toxic effect in comparison with other stages. In conclusion, the results state that, by following the traditional procedures while preparing Nagabhasma, the metallic lead gets converted into non-toxic organometallic compound.

Key words: Ayurveda – Lead toxicity – Histology – Hepatocytes – Sinusoids

INTRODUCTION

Lead, a heavy metal, is known to be toxic to the entire organ system of our body, especially the liver. A large amount of ingested lead is usually stored in liver parenchyma (Mudipalli, 2007), and increased exposure to lead is known to elicit structural changes in the hepatocytes (Wachukwu et al., 2001; Onyeneke and Omokaro, 2016) and alter the liver function. Lead induced necrosis of the hepatocytes could be due to the depletion of glutathione resulting in oxidative stress (Jarrar and Taib, 2012). Earlier, it has been found that the subjects of the occupational exposure groups [lead battery workers] have remarkable rise in the hepatocellular serum enzyme [AST, ALT, and ALP] levels when compared to control (Allouche et al., 2011; Onyeneke and Omokaro, 2016). However, in contrary to this, in another study, despite the structural changes, no biochemical alterations were observed in the liver function tests which may be due to the adaptive response rather than the toxic effect (Abdel-Aal et al., 1989).

Lead is known to cause oxidative stress in certain organs like kidneys, liver and brain (Ercal et al., 2001; Patra et al., 2001). Despite the adverse effects, lead is used in the preparation of an Ayurvedic product, Nagabhasma. According to ayurveda, if lead is used as per the traditional way of preparation of bhasma, the lead undergoes a process of purification/detoxification known as

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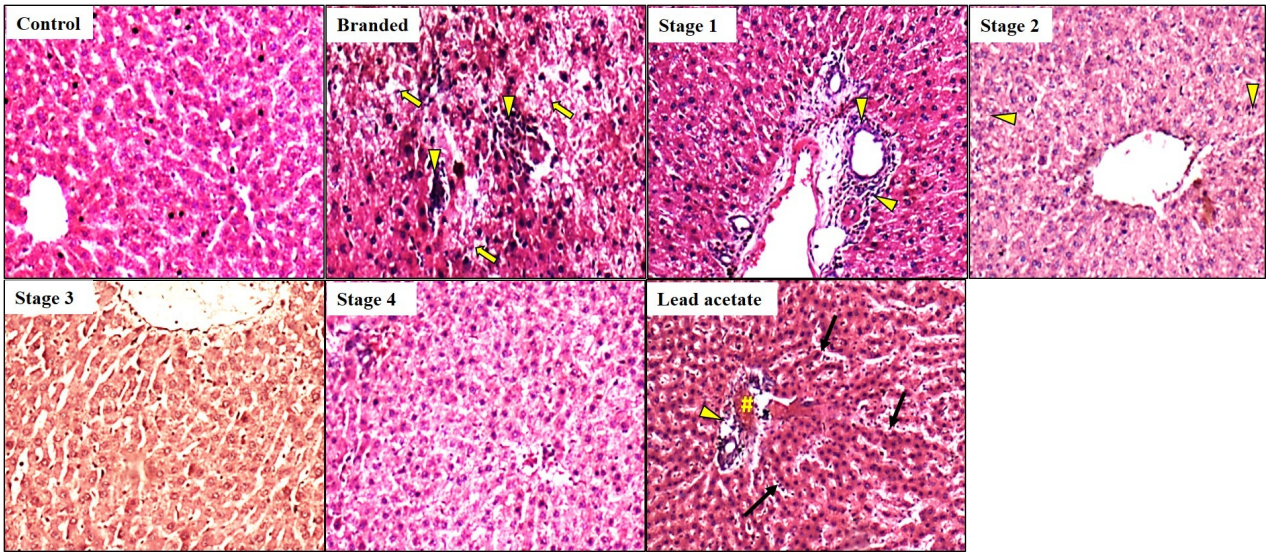

Fig 1. Histopathological changes in the liver immediately after 30 days of treatment. Arrow head – inflammatory infiltrates & portal triaditis, # - thrombus of blood vessels, yellow arrow – hydropic degeneration with cloudy swelling in cytoplasm, black arrow – Kupffer cell hyperplasia.

bhasmikarana that yields a non-toxic form of lead in Nagabhasma. However, there are reports from the Western countries to show that lead in Nagabhasma, imported from India, is toxic (Saper et al., 2004; Kanen and Perenboom, 2005; Dargan et al., 2008). A majority of the earlier studies have focused on the physicochemical properties of Nagabhasma (Nagarajan et al., 2012; Garg et al., 2012). However, the studies to show the histopathological changes due to administration of different stages of Nagabhasma to evaluate the detoxifying effect of lead is lacking. Therefore, this study is designed to understand the same effects in the liver.

MATERIALS AND METHODS

Nagabhasma preparation

Nagabhasma preparation was carried out authentically by the experts of the SDM College of Ayurveda, Udupi. The preparation was as per the steps described in Rasa Ratna Samucchaya. This elaborate procedure consists of 60 putas [steps] of purification. Out of these, bhasma at five major intermittent steps was procured, namely, Lead acetate (taken as a raw material for the preparation of Nagabhasma), Stage 1 (Gomutra Shodhana), Stage 2 (Samanya Shodhana), Stage 3 (Vishesha Shodhana) and Stage 4 (fully processed Nagabhasma).

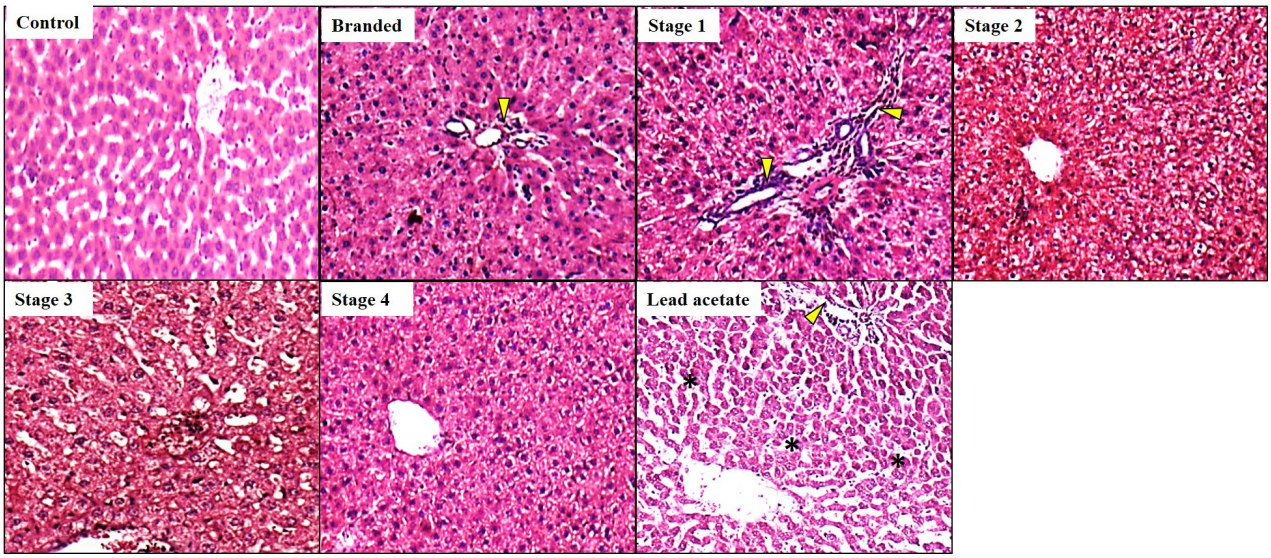

Fig 2. Histopathological changes in the liver of animals 2 months of observation after 30 days' treatment. Arrow head – inflammatory infiltrates & portal triaditis, # -thrombus of blood vessels, * - discontinuous cords of hepatocytes.

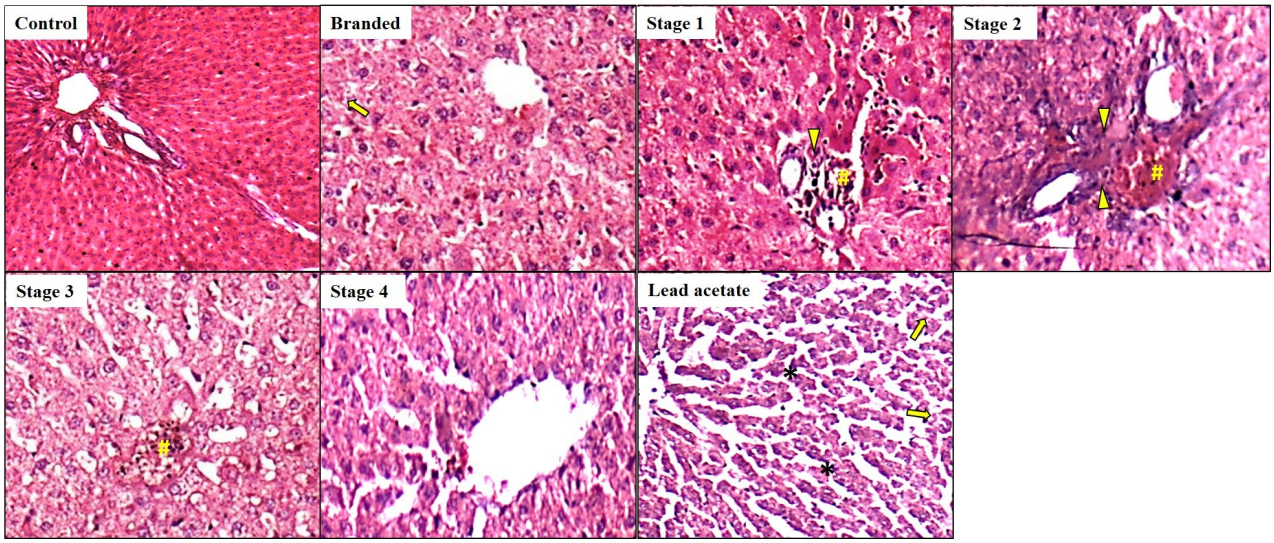

Fig 3. Histopathological changes in the liver of animals immediately after 60 days' of treatment. Arrow head – inflammatory infiltrates & portal triaditis, # - thrombus of blood vessels, yellow arrow – hydropic degeneration with cloudy swelling in cytoplasm, * - discontinuous cords of hepatocytes

Animals

After obtaining the Institutional ethical committee clearance (ref. no.IAEC/KMC/33/2012), Wistar rats of both sexes were utilized for the experiment. The animals were divided into seven groups of 6 animals each. The groups were as follows – 1. + Branded commercially available Nagabhasma , 2. + Stage 1 bhasma, 3. + Stage 2 bhasma, 4. + Stage 3 bhasma, 5. + Stage 4 (fully prepared Nagabhasma), 6. + Lead acetate used for preparation of Nagabhasma and 7. Untreated control group.

Dosage

For humans, the recommended dose of Nagabhasma is 125mg/50-60kgs/day. Accordingly, for the rats, the dose will be 0.25mg/100g/day. However, in the present study a much lower dose of 0.15mg/100g/day was used.

Study plan

The experiment was carried out to check the immediate and residual effect of intermittent stages of preparation of Nagabhasma by orally feeding the bhasma. Bhasma was administered for a period of 30 (short term treatment) and 60 days (long term treatment), and after the treatment period animals were sacrificed for further analysis of immediate effect. In another group, after administering the bhasma for 30 and 60 days, animals were left untreated for another two months, and then the residual effect of lead was studied.

Histopathology

After the treatment plan, the animals were sacrificed, and the liver was subjected to histopathological procedures to get paraffin sections. The sections were then stained with Eosin and Hematoxylin and analyzed with the help of a Pathologist.

RESULTS

Immediately after the 30 days of treatment with test materials, hepatocytes of the animals administered with stages 1, 2, 3 bhasma and lead acetate showed minor structural changes. There was inflammatory infiltrates [arrow head in figure 1] in between the hepatocytes and the sinusoids in the animals administered with branded, stage 2 bhasma and lead acetate. In addition to this, lymphocytic aggregation was also visible around the blood vessels in the portal triads [portal triaditis] [arrow head]. The branded bhasma-administered animals also showed sporadic spotty, well-defined foci of parenchymal necrosis [hydropic degeneration with cloudy swelling in the cytoplasm] [yellow arrow]. The lead-acetate-administered animals showed an increase in the sinusoidal Kupffer cells [Kupffer cells hyperplasia] [black arrow]. There was thrombus with luminal obstruction of the blood vessel [#] in the portal triad of animals administered with lead acetate when compared to the remaining groups. However, the animals administered with stage 4 Nagabhasma showed almost normal histological architecture which is comparable with that of the control group (Fig. 1).

In the liver of the animals two months after a 30 days' feeding period, discontinuous cords of hepatocytes were observed in the lead acetate group [*], whereas the other groups did not show any changes in the cords. There were fewer sinusoids in the animals administered with stage 2 bhasma. Inflammatory infiltrates were seen in branded, stage 1 and lead-acetate-administered animals. Portal triaditis [arrow head in Fig. 2] was also observed in the animals administered with

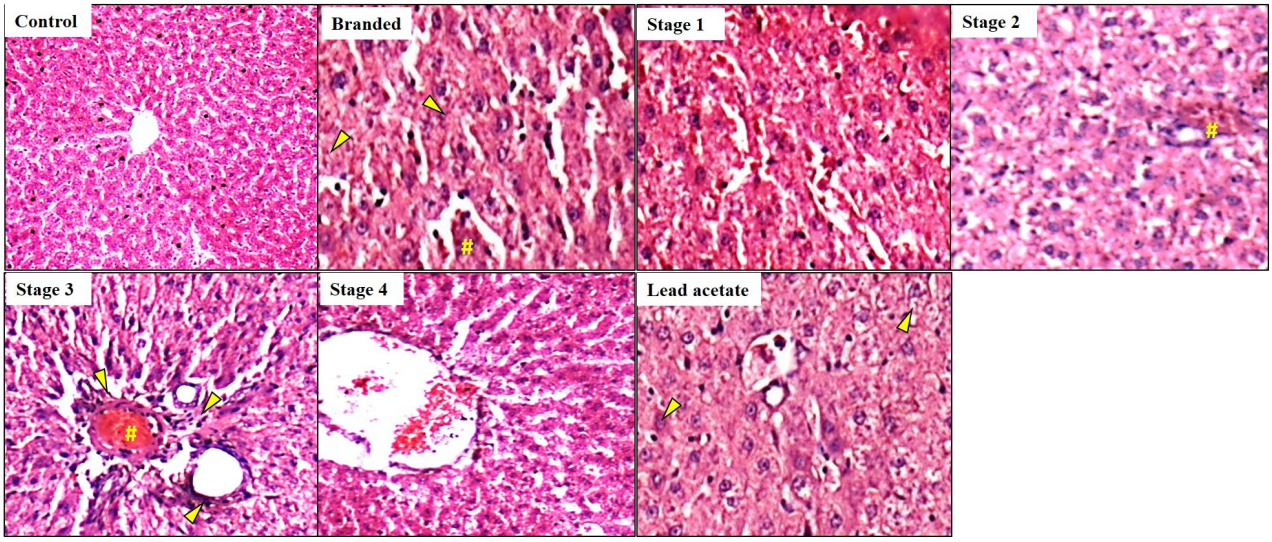

Fig 4. Histopathological changes in the liver of animals 2 months of observation after 60 days' treatment. Arrow head – inflammatory infiltrates & portal triaditis, # - thrombus of blood vessel.

branded, stage 1 bhasma and lead acetate. The animals of stage 4 Nagabhasma, showed nearly normal architecture of the liver when compared to rest of the bhasma treated group (Fig. 2).

Immediately after the 60 days' treatment period, hepatocytes of the stages 1, 2, 3 bhasma and lead acetate groups also showed minor structural changes. Discontinuous cords of hepatocytes [*] were observed in the animals of lead acetate group. Lymphocytic aggregation was also visible around the blood vessels in the portal triads [portal triaditis] in stages 1 and 2 bhasma groups [arrow head]. The branded bhasma and lead acetate groups showed sporadic spotty well-defined foci of parenchymal necrosis [hydropic degeneration with cloudy swelling in the cytoplasm] [yellow arrow]. Thrombus with luminal obstruction of the blood vessel [#] in the portal triad was observed in the animals of stages 1 and 2 bhasma. Congestion [#] of central vein was seen in stage 3 bhasma. Interestingly, the animals administered with stage 4 Nagabhasma exhibited normal histological architecture (Fig. 3).

In animals with long term treatment (60 days) and with two months of untreated period, portal triaditis was observed in the animals administered with stage 3 bhasma [arrow head]. In addition to these changes, the animals administered with stage 2 bhasma showed a decrease in the sinusoids. Luminal obstruction of blood vessels [star] was observed in the animals of stages 2 and 3 bhasma (Fig. 4).

DISCUSSION

'Bhasmikarana', a process in the Nagabhasma preparation with many different herbs, is responsible for the conversion of toxic metallic lead into a non-toxic form. This non-toxic form is considered to be beneficial to health when administered with a prescribed dosage. According to an earlier study on rats, a dosage of 6mg/100g/day was found to be non-toxic (Singh et al., 2010).

In the present study, despite giving less dosage, adverse histological changes were observed in the liver. It was observed that there was a gradual decreased histological adverse alterations as the animals were administered bhasma from stages 1 to 4 of purification. This shows that the lead metal in each of these stages gets converted into a less toxic form. Several studies have shown that lead-treated animals revealed numerous changes in the microscopic structure. Degenerative changes such as disorganized hepatic cords, dilatation of sinusoids, vascular congestion of the portal vein and central vein, periportal fibrosis were seen in the liver parenchyma (Haouas et al., 2014; Hegazy and Fouad, 2014). Inflammatory infiltrates around the portal triads [portal triaditis] and the periportal area was a common finding of the lead-treated animals (Verheij et al., 2009; Singh et al., 2010; Salinska et al., 2012; Haouas et al., 2014; Metwally et al., 2015). These inflammatory infiltrates may be due to the production of reactive oxygen species that may cause the imitation of an inflammatory response due to the interaction of lead with the proteins and enzymes of hepatic interstitial tissue (Johar et al., 2004). Vacuolations in the cytoplasm, proliferation of the hepatocytes and cellular necrosis were shown in the previous studies (Abdel-Aal et al., 1989; Sirimongkolvorakul et al., 2012; Haouas et al., 2014; Metwally et al., 2015; Omotoso et al., 2015). Necrosis in the hepatocytes might have a cause in the oxidative stress on these cells, due to a fall in the glutathione. Similar observations of the lead-acetate-administered animals (positive control group) were recorded in the present study.

Interestingly, in the present study, there was a gradual decrease in the adverse histological changes in the animals of stages 1 to 4 bhasma, suggestive of the progressive decrease of toxicity in the processed forms of lead metal.

The results of our earlier work on liver function tests suggest that there was a high level of liver enzymes, especially Alanine transaminase [ALT], Aspartate transaminase [AST] and Alkaline phosphatase [ALP] in the lead-acetate- and branded-bhasma treated-animals. It was also seen that there was a gradual decrease in the enzyme levels in the animals treated with stages 1 to 4 bhasma. Similar results were observed in both the immediate and residual effect (Quadros et al., 2017). Therefore, our present histopathological findings are in accordance with the liver function tests.

In the present study, the animals treated with stage 4 [fully processed] Nagabhasma did not show any significant histological changes. This indicates the non-toxic nature of metallic lead in stage 4 Nagabhasma, when compared to the rest of the treated groups. This clearly shows that by following the stringent traditional way of preparation of Nagabhasma, the metallic lead toxicity can be removed, thus rendering a non-toxic and health -beneficial organometallic compound. Further, evaluation of physical and physiological properties of the lead at various intermittent stages of preparation may help us to understand the lead toxicity on liver.

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