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HISTOPATHOLOGICAL ALTERATIONS IN KIDNEY DUE TO THE EFFECT OF VARIOUS STAGES OF *NAGABHASMA* (LEAD) PREPARATION

L. S. Quadros [1] and K. M. R. Bhat [* 2]

Department of Anatomy [1], Kasturba Medical College, Manipal University, Manipal, Karnataka, India.
Department of Anatomy [2], Ras Al Khaimah College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, UAE.

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Correspondence to Author:
K. M. R. Bhat

Professor and Chairperson – Anatomy, Dept. of Anatomy, Ras Al Khaimah College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, UAE - P. O. Box 11172.

E-mail: kumarbhat@rakmhsu.ac.ae

ABSTRACT: Background: *Nagabhasma* is a lead-based Ayurvedic medicine. Numerous reports indicate the toxic effects of lead used in this preparations on various systems of our body. But Ayurveda claims that the toxic effect of lead gets nullified thereby acquiring the medicinal property by following stringent and traditional method of preparation. **Objectives:** 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 kidney in comparison with lead acetate treated and commercially available *Nagabhasma* treated animals. **Materials and method:** Less than the human equivalent doses of *Nagabhasma* at four different stages of its preparation were fed orally for 30 days and 60 days (short term and long term) to Wistar rats. The animals were even kept under observation for a period of two months after the treatment period and then the residual effect was studied. After the treatment and observation period, the animals were sacrificed to collect the kidneys for histopathological examination. **Results:** The histopathological results of the immediate and residual effects showed massive alterations in the structural findings. **Conclusion:** The results supports that by following the traditional procedures while preparing the *Nagabhasma*, the metallic lead gets converted into non-toxic organometallic compound.

INTRODUCTION: Lead is a known metal to cause toxic effects on various systems of our body especially on the kidneys [1, 2]. It is an important metal causing oxidative damage in several tissues [3, 4]. Lead is known to cause oxidative stress in certain organs like kidneys, liver and brain [5, 6]. However, lead metal is used in the preparation of various drugs in traditional medicinal system such as *Ayurveda*.

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According to *Ayurveda*, lead metal is purified (detoxified and calcified) to form *bhasma*, an ash-based derivative called as *Nagabhasma*. However, reports suggest that the lead in *Nagabhasma* is found to be toxic [7, 8, 9]. Physicochemical properties of *Nagabhasma* have been studied by several authors previously [10, 11].

Since there is a paucity in the histopathological findings of the possible metallic toxicity of lead in various steps of preparation of *Nagabhasma*, this study intends to evaluate the same.

MATERIALS AND METHOD:

a. *Nagabhasma* preparation: *Nagabhasma* was prepared authentically by the experts of the SDM

College of Ayurveda, Udupi following the steps as mentioned in *Rasa Ratna Samucchaya*. The preparation includes 60 putas (steps) of purification. Out of these, *bhasma* at five major steps was procured. These are, 1. Lead acetate (taken as a raw material for the preparation of *Nagabhasma*) 2. Stage 1 (*Gomutra Shodhana*) 3. Stage 2 (*Samanya Shodhana*) 4. Stage 3 (*Vishesha Shodhana*) and 5. Stage 4 (fully processed *Nagabhasma*).

b. Animals: Wistar rats of both sexes were divided into seven groups each (n=6) 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. + Pure lead used for preparation of *Nagabhasma* and 7. Untreated control group. Institutional ethical committee clearance (ref. no. IAEC/KMC/33/2012) had been obtained for this experiment.

c. **Dosage:** For humans, the recommended dose of *Nagabhasma* is 125mg/50-60kgs/day. Accordingly, for the rats, 0.25mg/100g/day was used. However, in the present study much lower dose of 0.15mg/100g/day was administered to the animals.

d. Treatment plan: Experiment was carried out to check the immediate and residual effect of different stages of preparation of *Nagabhasma* by orally feeding for a period of 30 and 60 days (for immediate effect) and left untreated for a period of 2 months (for residual effect) After the treatment and observation periods, the animals were sacrificed to collect the kidneys for histopathological procedure.

e. Histopathology: The kidneys were subjected to histopathological procedures. The sections were stained with Eosin and Hematoxylin.

RESULTS:

a. Immediately after 30 days' treatment: Immediately after the 30 days' treatment with test materials the morphology of the proximal and distal convoluted tubules were significantly altered in animals treated with stage 3 and lead acetate. Renal tubular hyperplasia (characterized by increase in cellularity and cell size, cytoplasmic basophilia, nuclear enlargement and circumferential expansion) was observed in the animals treated with stage 3 of purification (#). Desquamation (denuded epithelium) of renal tubular epithelial cells and lysis of the tubules was observed in the animals treated with branded *bhasma* and stage 1 of purification (arrow). There was thickening and congestion of blood vessels in the animals treated with *bhasma* of stage 2 of purification ($). Pockets of inter-tubular haemorrhages was observed in the animals treated with lead acetate ($). Dilated tubules with atrophied lining epithelium was observed in the animals treated with lead acetate (@). Glomerular space was absent in the animals treated with stage 3 of purification (*). Inflammatory infiltrates (arrow head) was observed in the animals treated with stages 1, 2 and 3 of purification.

However, there was a gradual decrease in the amount of inflammatory infiltrates in animals fed with stages 1 to 3 of purification, suggestive of the lead amount being converted into a non-toxic form. Atrophy of the glomerular capillaries (glomerular atrophy) was clearly visible in the animals treated with lead acetate (*). When compared to all the treated groups and the control group of animals, the animals treated with stage 4 (fully processed) *Nagabhasma* did not show any histopathological changes **(Fig. 1)**.

b. Two months after 30 days' treatment: In comparison with the immediate effect of the 30 days' feeding period, the kidneys of the animals were examined 2 months after a 30 days' feeding period. Severe loss of morphology of the renal tubules was noticed in the animals treated with branded *bhasma*, stages 1, 2, 3 and lead acetate, indicating the cumulative residual effect of lead Renal tubular hyperplasia leading to obstruction of the lumen was observed in the animals treated with stages 2 and 3 of purification (#).

However, desquamation of epithelial cells was observed in the animals treated with branded *bhasma*, stage 1 and lead acetate (arrow). The blood vessels appeared to be normal but pockets of inter-tubular haemorrhages was observed in the animals treated with lead acetate ($). There was tubular dilatation and atrophy of the cells lining the tubules (@). Absence of glomerular space was seen in the animals treated with branded *bhasma*, stages 2 and 3 (star).

In addition to this, there was a decrease in the amount of inflammatory infiltrates in the animals treated with branded *bhasma*, stages 1, 2, and 3 (arrow head). Glomerular atrophy was also observed in the lead acetate treated group (*). As for the animals treated with stage 4 *Nagabhasma*, no abnormal histological changes were observed in the tubules and the glomerulus **(Fig. 1)**.

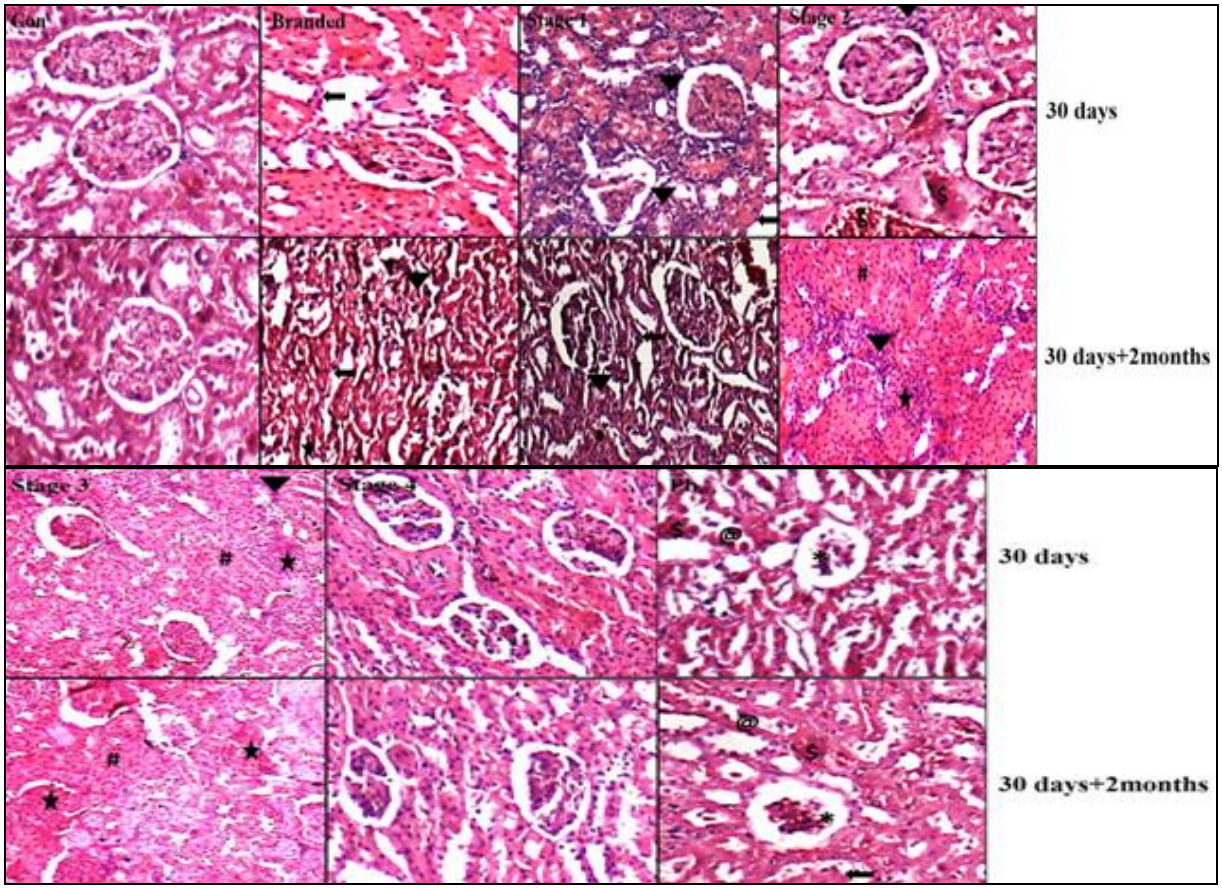

FIG. 1: HISTOPATHOLOGICAL CHANGES IN THE KIDNEY IMMEDIATELY AFTER 30 DAYS OF TREATMENT AND AFTER 2 MONTHS OF TREATMENT WITH TEST MATERIALS. # - RENAL TUBULAR HYPERPLASIA, ARROW – DESQUAMATED RENAL TUBULAR EPITHELIUM, $ - CONGETSED BLOOD VESSELS, @ - ATROPHIED RENAL TUBULAR EPITHELIUM, * - ABSENT GLOMERULAR SPACE, arrow head – INFLAMMATORY INFILTRATES

c. Immediately after 60 days' treatment: Immediately after the 60 days' treatment period, there was loss in the morphology of the proximal and distal convoluted tubules of the animals treated with lead acetate. As seen in 30 days' treatment, renal tubular hyperplasia was observed in the animals treated with stages 2 and 3 of purification (#), leading to obstruction of lumens of the tubules and gradually nephropathy. Due to the crude lead in test materials or crossing of lead through the basement membrane of the tubules, there was denudation of the tubular epithelial cells and the lysis of tubules was observed in the animals treated with lead acetate (arrow). Inter-tubular haemorrhage was seen in the animals treated with stage 1 ($) which may be suggestive of lack of blood supply for the proper functioning of the kidneys. Inflammatory infiltrates gradually reduced from stages 1 to 4, but were more pronounced in the animals treated with branded bhasma and stage 1 of purification. Absence of glomerular space was observed in the animals treated with stage 3 (star) and glomerular atrophy was observed in the animals treated with lead acetate (*). When compared to all the groups, the animals treated with stage 4 (fully processed) *Nagabhasma*, did not show much alterations. However, slight structural changes were observed in the tubular epithelial cells **(Fig. 2)**.

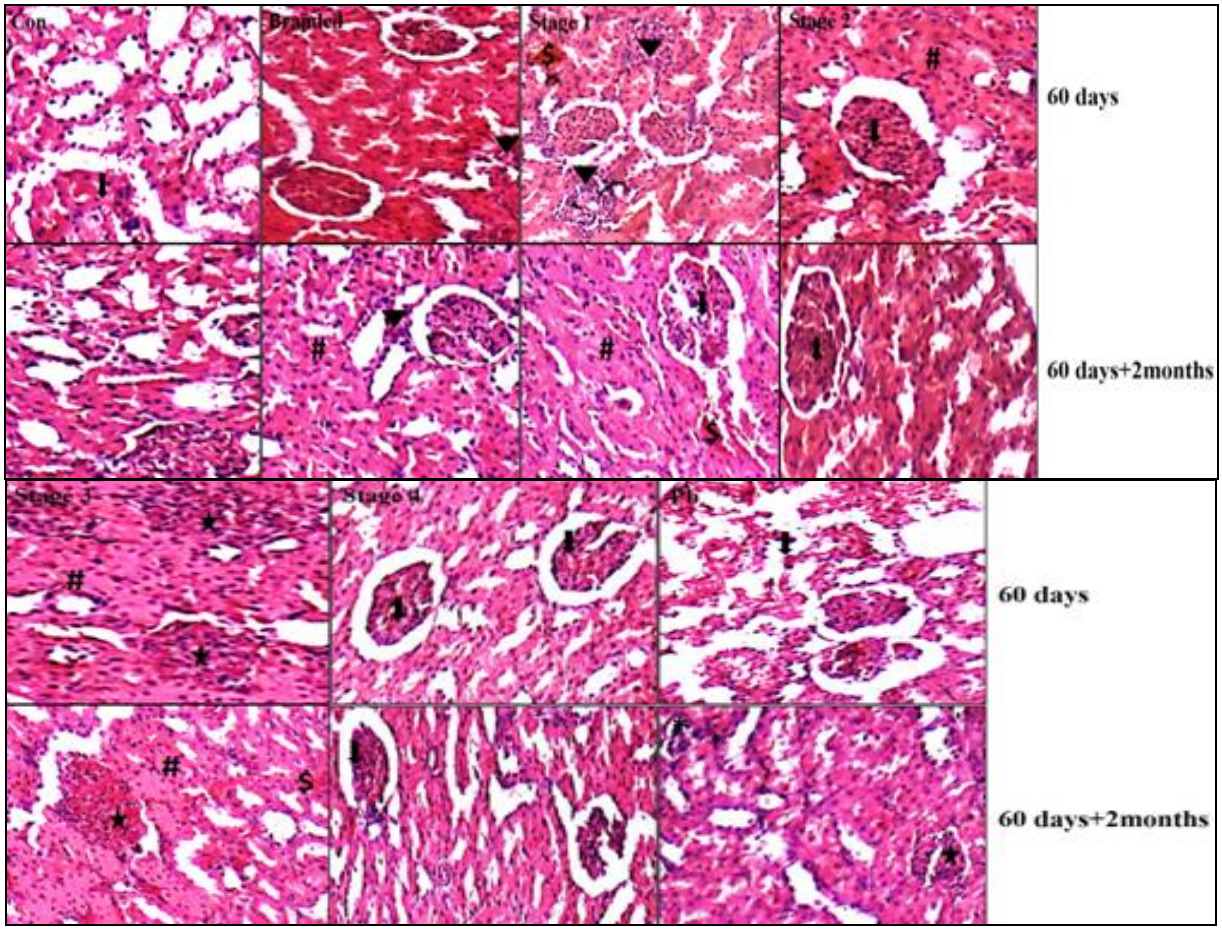

FIG. 2: HISTOPATHOLOGICAL CHANGES IN THE KIDNEY IMMEDIATELY AFTER 60 DAYS OF TREATMENT AND AFTER 2 MONTHS OF TREATMENT WITH TEST MATERIALS. # - RENAL TUBULAR HYPERPLASIA, arrow – DESQUAMATED RENAL TUBULAR EPITHELIUM, $ - CONGETSED BLOOD VESSELS, @ - ATROPHIED RENAL TUBULAR EPITHELIUM, * - ABSENT GLOMERULAR SPACE, ARROW HEAD – INFLAMMATORY INFILTRATES

d. Two months after 60 days' treatment: In comparison with the immediate effect of the 60 days' feeding period, the kidneys of the animals were examined 2 months after a 60 days' feeding period as shown in **Fig.2**. Severe loss in the morphology of the renal tubules was noticed in the animals treated with stages 2 and 3. Renal tubular hyperplasia leading to obstruction of the lumen was observed in the animals treated with branded *bhasma*, stages 1 and 3 of purification (#). The blood vessels appeared to be normal but pockets of inter-tubular haemorrhages were observed in the animals treated with stages 1 and 3 of purification ($). Absence of glomerular space was seen in the animals treated with stage 3 and lead acetate (star). In addition to this, there was a decrease in the amount of inflammatory infiltrates, but was specifically observed in the animals treated with branded *bhasma* (arrow head). Glomerular atrophy was also observed in the lead acetate treated group (*). However, no significant changes were observed in the the animals treated with stage 4 *Nagabhasma*, indicating the non-toxic nature of lead in it. The rest of the groups, including the control groups of animals, exhibited a normal glomerular structure.

DISCUSSION: *Nagabhasma* preparation follows a lengthy process which involves a process called *bhasmikarana* that converts the toxic lead metal into a health beneficial, non-toxic, organometallic compound that is acceptable by the body. Therefore, in this study we hypothesized that, the process of making this bhasma is very crucial in removing the metallic toxicity of the lead. A previous study conducted on animals, confirmed that at the dosage of 6mg/100g/day, no significant histological changes have been found in the organs including kidneys [12].

However, in the present study, keeping in mind the human equivalent dose, which is, 125mg/50-60 Kgs/day, the dosage for the animals would be 0.25mg/100g/day. However, in the present study, we have administered a dose of 0.15mg/100g/day, which is much less than the human equivalent dose.

Despite giving less dosage, histological changes were observed in kidneys. We have also observed that there was a decreased histological alterations as the animals were administered 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.

Renal tubular hyperplasia is the most common finding. This is suggestive of a chronic progressive nephropathy [13]. This could also lead to the obstruction of the lumens of the tubules **(Fig. 1** and **2)**. Several studies in the past have shown that the crude lead present in the test materials would cross the basement membrane of the tubules that may lead to desquamation of renal tubular epithelial cells and lysis of the tubules [14, 15, 16]. Similar finding was seen in the present study **(Fig. 1** and **2)**. Lead may also affect the blood vessels leading to the congestion in the lumen of the vessels and inter-tubular haemorrhages. This may interfere with the normal physiological function of the kidneys owing to the lack of blood supply [14]. Inflammatory infiltrates was a common finding in the lead treated animals [17]. The glomerular capillaries also undergoes atrophy which was seen in the present study. The results of our earlier work on renal function tests, suggest that there was a high level of blood creatinine and urea levels in the animals treated with lead acetate. Further, there was gradual decrease in the blood creatinine and urea levels in animals treated with stage 1 to 4 *bhasma*. However, no significant changes were observed in the animals treated with branded *bhasma* [18]. In both immediate and residual effect similar results were observed. Our present histopathological findings almost in line with the renal function tests.

CONCLUSION: In the present study, the histopathological observations did not show any significant changes in the glomerular and tubular structures in the animals treated with stage 4 (fully processed) *Nagabhasma* indicating the non-toxic nature of metallic lead in it 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 stages of preparation may help us to understand the lead toxicity on kidneys.

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CONFLICT OF INTEREST: The authors declare no known conflict of interest.

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