EFFECT OF YASADA BHASMA ON ORAL SODIUM PHOSPHATE INDUCED NEPHROCALCINOSIS IN RATS

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ABSTRACT
Nephrocalcinosis is a condition characterized by calcium salt deposits in the kidneys which may affect it's ability to function. Nephrocalcinosis is caused by a number of conditions including: the excess excretion of calcium by the kidney, renal tubular acidosis, medullary sponge kidney, hypercalcemia (high calcium levels in the blood), renal cortical necrosis, and tuberculosis. Nephrocalcinosis is relatively common in premature infants, partly from intrinsic kidney calcium losses and partly from enhanced calcium excretion when they are given diuretics. In the present study, nephrocalcinosis was induced in an animal model by administrating sodium phosphate (4100mg/kgbw) orally and the nephrotoxic rats were treated with Yasada Bhasma, a herbo mineral preparation at different doses. The levels of Alkaline Phosphatase, Acid Phosphatase and calcium were analysed in order to determine preventive effect of Yasada Bhasma. The kidney function was also assessed by determining the serum levels of urea, uric acid and Creatinine. The histopathology of kidney was also carried out to support the nephroprotective effect of Yasada Bhasma. Impaired kidney function was observed in rats with sodium phosphate induced nephrocalcinosis followed by acute renal failure. The levels of blood urea, serum creatinine and phosphorous were increased significantly in sodium phosphate induced nephrocalcinosis. The oral administration of Yasada bhasma produced the most significant reduction in the levels of blood urea, serum creatinine and phosphorous. Acute kidney injury is an important risk factor for urolithiasis and nephrocalcinosis in Oral Sodium Phosphate ingestion, and can coexist with hypercalciuria. A novel type of an ayurvedic drug (Yasada Bhasma) was produced which has high potentials for inhibition and prevention of nephrocalcinosis in Oral Sodium Phosphate treatment. Finally, studies in the mechanism of action of Yasada Bhasma and the product development as well as safety evaluation of the standard herbal extract are definitely required for future pharmacological applications of Yasada Bhasma as nephroprotective drug for Oral Sodium Phosphate induced nephritis.

KEYWORDS: Oral Sodium Phosphate, Yasada Bhasma, Nephrocalcinosis, Acute Kidney Injury, Urolithiasis and Hypercalciuria

INTRODUCTION
Phosphate nephropathy consists of damage to the kidneys caused by the formation of phosphate crystal within the renal tubules, damaging the nephron and can cause acute renal failure. It frequently occurs following the ingestion of oral sodium phosphate (OSP) solution for bowel cleansing prior to a colonoscopy1. Pathology findings are important in diagnosing phosphate nephropathy. There is a particular pattern of abundant deposit of calcium phosphate, principally in distal tubes and collecting ducts, important focal peritubular lymphocytic inflammation and minimal tubular damage, including occasional necrotic and sloughed epithelial cells, coarse vacuolation, loss of brush borders, nuclear enlargement, nucleolar prominence, and occasional binucleation 2,3. The risk of this complication is
increased with age, dehydration, or in the presence of hypertension or if the patient is taking an ACE inhibitor or angiotensin receptor blocker more recent attention has been directed to the possibility that in some patients the deposition of calcium – phosphate crystals after Oral Sodium Phosphate administration may result in AKI (Acute Kidney Injury). In 2003, a 71-year old case whose creatinine rose from 1.0 to 4.5 mg/dl in a 10-wk period after the use of Oral Sodium Phosphate solution administration. Metabolic disturbances such as hyperphosphatemia, hypocalcaemia, and axion-gap metabolic acidosis have also been reported in association with Oral Sodium Phosphate induced acute renal injury. Hyperphosphatemia after Oral Sodium Phosphate ingestion routinely occurs even in individuals with normal renal function, whose mean serum phosphate ranges form 3.7 to 7.3 mg/dl. Herein, we report a preclinical study of acute renal failure associated with oral sodium phosphate intake and nephrocalcinosis in the renal histopathology and compare this report with Oral Sodium Phosphate induced nephrotoxic rats treated with Yasada Bhasma.

MATERIALS AND METHODS

Drug material
The drug material used in this study is Yasada bhasma. It is a herbo mineral preparation obtained by calcining zinc metal with herbal drugs.

Preparation of drug material
The Yasada bhasma was prepared by treating zinc metal with sesame oil, butter milk, cow urine, fermented rice gruel, aqueous extract of Dolichos biflorus L, Achyranthes aspera L and fresh juice of Aloe vera Burm.f through the Ayurvedic methods via Shodhana (purification), Jarana (trituration) and Marana (calcination).

Pharmacological study
Animals
The study was performed using Wistar albino rats (60-90 days old) weighing 150-200g as animal model, which were maintained under standard conditions of temperature, humidity, diet and water ad libitum. The study was conducted after obtaining necessary animal ethical committee clearance.

Nephroprotective activity of Yasada Bhasma
The nephroprotective activity of Yasada Bhasma was studied by administrating sodium phosphate, which produced nephrocalcinosis, the nephrotoxic animals were treated with test drug. The animals were divided into five groups each group comprising of 6 rats (n=6).

Experimental Design

Group I: Normal rats, Group II: Nephrotoxic rats (administered with 4100mg/Kg.bw sodium phosphate), Group III: Sodium phosphate induced nephrotoxic rats received Yasada Bhasma (100mg/kgbw), Group IV: Sodium phosphate induced nephrotoxic rats received Yasada Bhasma (150mg/kgbw) and Group V: Sodium phosphate induced nephrotoxic rats received Yasada Bhasma (200mg/kgbw).

Assessment of renal function
At the end of experimental period, rats in all groups were starved for 12 hrs and sacrificed. Blood samples were collected by cervical decapitation and serum was separated after centrifugation at 5000 rpm for 10 minutes. The serum was used to estimate Enzymes, Plasma proteins, non-pertinacious nitrogen compounds and minerals.

Percentage change in the body weight was measured for each rat before treatment, during the middle of the treatment and at the end of the treatment. Serum creatinine and uric acid were estimated by Jaffé’s method and Kanai method respectively. Blood urea was estimated by urease enzymatic method. Serum and renal calcium was estimated by the method of clark and collip and serum inorganic phosphorous was estimated by the method of Fiske subbarow. Serum and renal activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) were measured colorimetrically following the procedure of king, in which the enzyme-substrate reaction takes place at pH 10 for ALP and 4.5 for ACP.
Histopathological studies
Two animals from each group were sacrificed on the day of blood withdrawal and kidneys were isolated. The kidney sections were stained with hematoxylin and eosin, and observed under microscope\textsuperscript{12}.

Statistical analysis
The data were given as the mean $\pm$ SE and comparison between groups was statistically evaluated by one-way analysis of variance (ANOVA). Statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSION
Sodium phosphate induced nephrocalcinosis
The results shown in Table 1 revealed that body weight was significantly increased in sodium phosphate induced nephrotoxic rats, when compared to control rats. The body weight of animals received sodium phosphate was increased by 86\% at the end of four weeks. However, Yasada bhasma treatment (100mg, 150mg and 200 mg/kgbw) in curative regimen, the extent of increase in body weight showed significant decline at both the higher doses. The increase in body weight in Oral Sodium Phosphate induced animals may be due to edema (accumulation of body fluids) which is accompanied with the decreased urine volume, and sodium phosphate induced acute tubular necrosis. Treatment with herbomineral preparation under study in Group 3, 4 & 5 rats caused improvement in the body weight comparable to group 2 nephrotoxic rats. The body weight gain in herbomineral preparation treated rats may be due to regeneration of glomerular membrane, tubular epithelium and interstitium through volatilization of calcium-phosphate crystals. This prevented accumulation of fluids and lead to normal functions of nephrons resulting in normal clearance of metabolic waste products from the body (Table 1).

Effect of Yasada Bhasma On Blood Urea Nitrogen, Creatinine and Phosphorous
Blood Urea Nitrogen (BUN) and serum creatinine are common parameters studied for the evaluation of renal function because Bun and creatinine are waste products which are excreted from the blood into urine through kidney. Impaired kidney function was observed in rats with sodium phosphate induced nephrocalcinosis followed by acute renal failure. The levels of blood urea, serum creatinine and phosphorous were increased significantly in sodium phosphate induced nephrotoxic rats. This was in agreement with earlier studies\textsuperscript{4} which reported that administration of sodium phosphate elevated the level of serum creatinine from 1.0 to 4.5 mg.

The oral administration of Yasada bhasma produced the most significant reduction in blood urea, serum creatinine and phosphorous (Table 2). Sodium phosphate treatment in Group 2 rats significantly increased the concentration of serum phosphorous by about 95\% when compared to control rats. Earlier workers\textsuperscript{13} reported that hyper phosphataemia occurs after oral sodium phosphate ingestion which resulted in a rise in the serum phosphate from 3.7 to 7.3 mg / dl. Acute renal failure (ARF) as a consequence of hyperphosphatemia is well described in the setting of tumor lysis, with documented Ca-Pi deposition in the tubules and tubular epithelium\textsuperscript{14}. The effect of herbomineral preparation was also observed in the level of phosphorous and the data indicated that the herbomineral preparation treated rats of Group 3, 4 and 5 showed reduction in the level of phosphorous and significant reduction was observed in group 5 rats treated with herbomineral preparation at a dose level of 200mg / kg bw (Table 2).

Effect of Yasada Bhasma On Uric Acid
Impaired Kidney function in rats treated with sodium phosphate was supported by increased levels of uric acid in serum of experimental animals. The Group 2 rats treated with sodium phosphate showed a significant elevation in the level of serum uric acid when compared to control rats. On treatment with herbomineral preparation, the levels of uric acid in serum of Group 3, 4 and 5 were found to be reduced but reduction was significant in Group 5 rats which was treated with herbomineral preparation at a dose level of 200mg/ kg bw. The increase in the level of uric acid in sodium phosphate rats may due to decreased glomerular filtration. The Kidneys of sodium phosphate administered rats were damaged through deposition of phosphate calcium crystal on glomerular membrane, and on podocytes. This resulted in decreased glomerular filtration. Hence, the concentration of uric acid was found to be higher in sodium phosphate induced nephrotoxic rats (Table 2).
Earlier studies reported that oral administration of sodium phosphate in rats produced acute kidney injury (AKI) which is characterized by degeneration of kidney cells. This caused transport of cellular contents including Alkaline phosphatase and Acid phosphatase from the damaged cells into circulation. Thus, the levels of Alkaline phosphatase and Acid phosphatase were found to be higher in serum of group 2 rats treated with sodium phosphate sodium phosphate induced acute kidney injury not only elevated the levels of serum Alkaline phosphatase and Acid phosphatase but also decreased the levels of the above enzymes in the tissue. This result coincides with previous study carried out by earlier workers who reported that renal activities of alkaline phosphatase and acid phosphatase were significantly reduced during acute kidney injury (AKI).

Effect of Yasada Bhasma On Calcium
Table 4 reveals the level of calcium in group II was found to be high in kidney tissue which was treated with sodium phosphate as compared to control rats. The increased concentration of calcium in kidney tissue of group 2 rats may be due to precipitation or crystals deposited in glomeruli, tubules and interstitium of kidney. These effects agree with earlier work carried out by earlier workers who reported that sodium phosphate induced nephrocalcinosis increased concentration of calcium as calcium phosphate crystals in the cortico medullary junction of the Kidney. The administration of herbomineral preparation normalized the calcium level in serum and tissue of sodium phosphate induced nephrotoxic rats and this may be due to supplementation of sufficient amount of magnesium which is the most important element in herbomineral preparation. The main components of calcified structures in the magnesium did not complex with these structures. Calcification could be prevented by an increased of dietary magnesium.

Effect of Yasada Bhasma On Kidney Tissue
The most important and sensitive criterion for the deleterious action of phosphate is the appearance of calcification in soft tissues especially in Kidney, stomach and aorta. Sodium phosphate induced nephropathy was characterized by calcification, degeneration and necrosis. In the present study it is observed that oral administration of sodium phosphate in rats produced degeneration of glomerular epithelium, dysfunctioning and calcium phosphate tubular deposits and interstitial fibrosis. This resulted in acute kidney injury (Fig 2 & 3). Following the treatment with Yasada bhasma (200mg/kg bw) congestion of glomeruli disappeared. Necrotic tubular cells also became normal (Fig 4). Administration of sodium phosphate of concentration of 4100mg /kg between for 3 days also produced mineral hyper phosphatemia which may be due to diminished proximal tubular reabsorption to transporters in the luminal membrane of renal tubules allow only reabsorption of filtered phosphate. In minimal hyper phosphatemia, the excess phosphate may complex with calcium in the serum. The ensuing fall in serum ionized calcium concentration provides the signal for increased release of parathyroid hormone which also contributes to the redemption in phosphate reabsorption. Earlier studies reported that oral administration of phosphate sodium produced acute Kidney injury (AKI) which is characterized by distal tubular injury and calcium phosphate deposits. Oral administration of sodium phosphate resulted in the deposition of tissue crystals. The proinflammatory action of innate immune system in the formation of intra luminal calcium phosphate crystals after Oral Sodium Phosphate leads to recognition by special epithelial Toll-like Receptors (TLRs) which resulted in the formation of chronic kidney damage (CKD). This statement coincides with previous report carried out by the earlier workers. Animal studies also suggest that hyperphosphaturia, independent of hypercalciuria, can be important in the pathogenesis of nephrocalcinosis and renal failure. HyP mice, a mouse model for XLH, develop nephrocalcinosis when treated with phosphate and Vitamin D. Additionally, Ritskes-Hoitinga et
N. Agnel Arul John et al. IRJP 2 (1) 2011 202-209

al observed nephrocalcinosis in rats fed a high-phosphate diet and demonstrated a protective effect of parathyroidectomy, male gender, and hypermagnesiuria. Thus present study confirmed that Yasada bhasma treatment reduced sodium phosphate induced renal damage significantly in the rats. Further present toxicological studies also revealed the non toxic nature of Yasada Bhasma.

CONCLUSION
Phosphate-based laxatives are widely used for bowel cleansing before colonoscopy and abdominal surgery. These agents can be associated with different patterns of acute renal failure, for which the pathogenesis and risk factors remain to be determined. Ayurvedic Traditional Medicine holds promise for its role in the prevention or crystallization after OSP intake through the use of Yasada Bhasma, a herbal mineral compound, though no evidence of dissolution properties exist.

REFERENCES


<table>
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<tr>
<th>GROUPS</th>
<th>INITIAL (g)</th>
<th>FINAL (g)</th>
</tr>
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<tbody>
<tr>
<td>GROUP I</td>
<td>128.1 ± 2.485 *</td>
<td>129.5 ± 1.224 *</td>
</tr>
<tr>
<td>GROUP II</td>
<td>121.66 ± 2.581 *, **</td>
<td>138 ± 2.449 *, **</td>
</tr>
<tr>
<td>GROUP III</td>
<td>119 ± 1.673</td>
<td>124.16 ± 1.169</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>130.33 ± 1.505</td>
<td>136 ± 1.549</td>
</tr>
<tr>
<td>GROUP V</td>
<td>122.5 ± 2.738 **</td>
<td>127.16 ± 2.483 **</td>
</tr>
</tbody>
</table>

* and ** - Significant at p<0.05 (n=6)

Table 1: Effect of Yasada Bhasma on Body Weight in Sodium Phosphate induced nephrotoxic rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CREATININE (mg/dl)</th>
<th>BLOOD UREA NITROGEN (mg/dl)</th>
<th>URIC ACID (mg/dl)</th>
<th>INORGANIC PHOSPHOROUS (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td>1.05 ± 0.0473 *</td>
<td>21.96 ± 0.5085 *</td>
<td>4.2 ± 0.0707*</td>
<td>3.21 ± 0.0735*</td>
</tr>
<tr>
<td>GROUP II</td>
<td>6.5 ± 0.0209*, **</td>
<td>78.26 ± 0.2338*, **</td>
<td>14.9 ± 0.0894*, **</td>
<td>9.82 ± 0.0816*, **</td>
</tr>
<tr>
<td>GROUP III</td>
<td>5.425 ± 0.0524</td>
<td>61.26 ± 0.2338</td>
<td>11.2 ± 0.0707</td>
<td>8.44 ± 0.0765</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>3.13 ± 0.0605</td>
<td>39.1 ± 0.1788</td>
<td>8.28 ± 0.0816</td>
<td>6.29 ± 0.0664</td>
</tr>
<tr>
<td>GROUP V</td>
<td>1.68 ± 0.0516 **</td>
<td>25.23 ± 0.2065 **</td>
<td>4.78 ± 0.0816 **</td>
<td>3.72 ± 0.0816 **</td>
</tr>
</tbody>
</table>

* and ** - Significant at p<0.05 (n=6)

Table 2: Effect of Yasada Bhasma on Serum Creatinine, Uric acid, inorganic phosphorous and Blood Urea Nitrogen in Sodium Phosphate induced nephrotoxic rats
### Table 3: Effect of Yasada Bhasma on Alkaline Phosphatase and Acid Phosphatase

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>ALKALINE PHOSPHATASE (IU/L)</th>
<th>ACID PHOSPHATASE (IU/L)</th>
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<tbody>
<tr>
<td></td>
<td>Tissue</td>
<td>Serum</td>
</tr>
<tr>
<td>GROUP I</td>
<td>151.08 ± 0.9174 *</td>
<td>80.7 ± 0.3898 *</td>
</tr>
<tr>
<td>GROUP II</td>
<td>61.16 ± 0.8164 *, **</td>
<td>201.33 ± 1.211 *, **</td>
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<tr>
<td>GROUP III</td>
<td>88.33 ± 0.8755</td>
<td>175 ± 0.7071</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>121.08 ± 0.8495</td>
<td>141.91 ± 0.801</td>
</tr>
<tr>
<td>GROUP V</td>
<td>146.66 ± 0.8164</td>
<td>88.58 ± 0.4915</td>
</tr>
<tr>
<td>GROUP VI</td>
<td>151.5 ± 1.000 **</td>
<td>83 ± 0.7071 **</td>
</tr>
</tbody>
</table>

* *, ** - Significant at p<0.05 (n=6)
* and * - compared between Normal and OSP induced nephrotoxic rats (p<0.05)
** and ** - compared between OSP induced nephrotoxic rats and YB (200mg/kgbw) treated rats (p<0.05)

### Table 4: Effect of Yasada Bhasma on serum and tissue calcium in Sodium Phosphate induced nephrotoxic rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>SERUM CALCIUM (mg/dl)</th>
<th>TISSUE CALCIUM (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td>7.17 ± 0.0605 *</td>
<td>1.158 ± 0.0584 *</td>
</tr>
<tr>
<td>GROUP II</td>
<td>3.33 ± 0.108 *, **</td>
<td>6.02 ± 0.0816 *, **</td>
</tr>
<tr>
<td>GROUP III</td>
<td>4.91 ± 0.1068</td>
<td>4.93 ± 0.108</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>5.92 ± 0.0516</td>
<td>3.13 ± 0.0983</td>
</tr>
<tr>
<td>GROUP V</td>
<td>7.05 ± 0.1095</td>
<td>0.933 ± 0.108</td>
</tr>
<tr>
<td>GROUP VI</td>
<td>7.09 ± 0.0735 **</td>
<td>0.646 ± 0.0516 **</td>
</tr>
</tbody>
</table>

* *, ** - Significant at p<0.05 (n=6)
* and * - compared between Normal and OSP induced nephrotoxic rats (p<0.05)
** and ** - compared between OSP induced nephrotoxic rats and YB treated rats (p<0.05)
Fig 1: Photomicrograph of normal rat showing normal architecture of kidney tissue X250

Fig 2: Photomicrograph of MSP treated rat kidney showing glomerular congestion X-250

Fig 3: Photomicrograph of MSP treated rat kidney showing acute tubular necrosis X-250

Fig 4: Photomicrograph of Yasada bhasma treated nephrotoxic rat kidney showing regeneration of glomeruli and tubular cells

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