Original Research Article **Novel drug delivery System**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

NEPHRO AND HEPATO PROTECTIVE POTENTIAL OF *BACOPA* PHOSPHOLIPID COMPLEX AGAINST ALUMINUM-INDUCED TOXICITY IN RATS

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ABSTRACT

Phytosomes are advanced herbal formulations, binding individual components of herbal extracts to phospholipids resulting in a dosage form that is better absorbed than the conventional herbal extracts. Aluminum is commonly used in daily life, but it can accumulate in mammalian tissues especially brain, bone, liver, kidney and it can be potentially toxic. To examine the protective effect of *Bacopa* phospholipid complex (BPC) against Aluminum maltolate (AlM) induced nephro and hepato toxicity. Serum aminotransferases, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, total protein, albumin and lipid profile levels were estimated. Further, tissue alkaline phosphatase (ALP), ALT, AST and architecture of kidney and liver were assessed. AlM administration showed significant increase (P≤0.01) of ALP, ALT, AST, urea, creatinine and decrease in total protein and albumin levels in serum, kidney and liver. Increased levels of total cholesterol, triglycerides and LDL and decreased HDL were also observed with AlM treatment. Degenerative changes observed in kidney's bowman's capsule, glomeruli and renal tubules with vacuolization. Dilatation and bleeding areas in liver, vacuolization and degenerative changes in central vein were observed. Whereas synchronous administration of *Bacopa monniera* (Bm) and BPC with AlM showed significant (P≤0.01) decrease ALP, ALTAST, urea, creatinine and increase in total protein and albumin levels in serum and in tissues were observed. Tissue damage induced by AlM was reduced in AlM+Bm and AlM+BPC groups. Compared to Bm, BPC showed better therapeutic activity. Bm and BPC reduces hepatic and renal damage induced by AlM. Novel formulation of Bm with phospholipids showed increased absorption and enhanced bioavailability of BPC compared to Bm alone.

KEYWORDS: ***AlM, Bacopa monniera, Bacopa phospholipid complex, Lipid profile, Hepatotoxicity and Nephrotoxicity***

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Received on: 31-07-2017
Revised and Accepted on: 10-11-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2018.9.1.p30-43

INTRODUCTION

Bioavailability is a major hurdle in the translation of preclinical potential of medicinal plant extracts into their therapeutic effects, especially active phyto constituents such as polyphenolics, triterpenoids and flavonoids cannot be absorbed due to their poor solubility ceases their ability to cross lipid rich bio membranes[1]. The provision of less expensive topical medicines is the obvious driving force to identify procedures to facilitate the commercialization of bioequivalent products, with respect to oral delivery[2]. In this case phospholipid-based delivery systems have been developed to improve the novel formulations with herbal constituents and dietary ingredients. Among different strategies, phytosome technology is emerging model for better therapeutic benefits, assured delivery to the target regions without compromising nutrient safety[3]. Phospholipids are small lipid molecules derived from glycerol bonded to two fatty acids, with the third hydroxyl, normally one of the two primary methylenes, bearing a phosphate group[4]. In this context, lecithin has been used as a matrix to improve the lipophilic property and targeted delivery of bioactive compounds[5]. Generally eggs are considered to be a rich source of lecithin[6]. Lecithin is a phospholipid mixture of phosphatides consisting mainly Phosphatidylcholines, Phosphatidyl ethanolamine, Phosphatidyl serine, Phosphatidyl inositol and also contain phosphorous and nitrogenous (e.g., choline) compounds[7]**.** *Bacopa monnieri* (Bm) (family Scrophulariaceae), is second in the list of most essential Indian medicinal plants with ample number of therapeutically important bacosides[8]. *Bm* extract has various medicinal properties such as it acts as anticonvulsive, memory enhancer, analgesic, cardiotonic, sedative, anti cancerous, anti-anxiety agent, anti ulcerogenic activity, it relieves and prevents stress. Further, it is neuroprotective, antioxidant, and hepatoprotective agent [9]. It is used in traditional Ayurveda since centuries "Medhya Rasayan" (nootropic), In Bm, Bacoside A is considered as a major active component known to have protective activities against morphine-induced, chemical-induced liver toxicity[10]**.** It is used to treat skin diseases, leprosy, epilepsy, eczema, asthma, hoarseness of the voice, and diseases of the nervous system[11]**.** The neuro protective and cognition enhancing properties of Bm extract are due to the mechanisms explained by several researchers such as chelation of metal ions, scavenging of free radicals, enhanced antioxidant defense system and hepatoprotective activities[12-14]. Bm has been reported as a neural nourisher with restoring depleted synaptic activity and leading to enhanced memory function. In previous scientific studies, it has been shown to exert a remarkable and unique effect on neurotransmitters[15]. Secondary metabolites of Bm such as bacoside A and B, bacogenin A1 and A2, bacopasaponin D, pseudojujubogenin, hersaponin, monnierin and brahmine are found be responsible for its therapeutic activities[16]. Effort has been made to prepare Bacopa phospholipids complex to increase oral bioavailability. Complexation between Bm and phospholipid can enhance the therapeutic efficacy of the plant molecule, improves stability, sustained delivery and protection from physical and chemical degradation in gut area. Thus novel phytosome delivery of Bm has significant scope for enhancing the activity and to overcome problems associated with conventional type of pytomedicine**.** Aluminum (Al) has been suggested as a potential neurotoxic metal implicated in the progression of a number of neurodegenerative diseases, including Alzheimer's disease (AD). Mechanisms of aluminum toxicity include inhibition of enzyme activity and protein synthesis, alterations in nucleic acid function and changes in cell membrane permeability[17]. Pulmonary and oral intakes are the two major routes that Al can enter the body. A small portion of Al is absorbed by the gastrointestinal tract however, oral intake causes greatest toxicological implications[18]. Approximately 20% of the daily intake of Al has been stated to come from cooking utensils, migration of Al into food mainly occurs via acidic attack on plain Al materials[19]. Al provoked heptotoxicity, nephrotoxicity and neurotoxicity. Furthermore, Al-induced hepatic dysfunction and DNA cross-linking in rat [20]. Kidneys are the first target for Al, in the process of excretion Al may accumulate in tissue and shows adverse effects on kidney[21].Al-mediated alterations in iron (Fe) homeostasis have been indicated as pivotal factors that render this metal toxic. Indeed, the interaction between Al and Fe contributes to the formation of reactive oxygen species (ROS). To support this, various models of Al toxicity and oxidative damage has been systematically observed in the brain and other organs of animals exposed to the metal [22,14,23, 24].Hence, in view of the above facts, the aim of the present study was to determine therapeutic potential of BPC against AlM induced hepato and renal toxicity in albino rats through evaluating, biochemical enzymes (ALT, AST and ALP), urea, creatinine, total cholesterol, triglycerides, LDL, HDL and histopathological observations of kidney and liver.

MATERIALS AND METHODS

Chemicals

Al $(NO_3)_3.9H_2O$, Maltol, p-nitrophenyl phosphate, Acetone, Autospan(Clinical reagents) kits and diethylether were purchased from the Sigma Chemical Company (USA). H_2O_2, BSA and other chemicals were obtained from Merck, Arkray Health care pvt., Ltd, Qualigen, Himedia and SD fine Chemical Company.

Aluminum maltolate (AlM) preparation

AlM was prepared from maltol (3-hydroxy-2methyl-4H pyran-4-one) following the method [25]. Maltol and $Al(NO_3)_3.9\ H_2o$ were mixed in 3:1 ratio. The pH was adjusted to 8.6 and heated for few minutes. Al complex is hydrolytically stable from pH 2.0 to 12.0. This complex enhances free Al existence by 60-70% at neutral pH compared to any other inorganic or organic Al complex. AlM was used in the present investigation to overcome the Al speciation chemistry which is a complex phenomenon.

Preparation of Bacopa monniera ethanolic extract

Bm plants were collected from Thummala Gunta fields, Tirupati, Andhra Pradesh, India. Plants were identified and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. (Voucher No.1213). The plants were dried

in shade and then powdered. The powdered plant material was taken in a conical flask and extracted with 90% ethanol in a mechanical shaker with temperature control (Room temperature) and constant stirring at 200 rpm. It was left for 24 h and solids were filtered using Whatman No.1 filter paper[26]. The procedure was repeated three times until complete extraction was done. The residue obtained after removing the solvent, dried in vacuum and macerated with acetone to give free flowing powder.

Preparation of Bacopa phospholipid complex (BPC)

The specific amount of Bm and egg lecithin were added to ethanol with continuous stirring and sonicated then ethyl ether was added, stirred vigorously for a few minute; allowed it to stand for 15 min with occasional stirring. The suspension was filtered through a filter paper moistened with alcohol and filtrate was collected. After cooling, residue was dissolved in ethyl ether. The ether solution was poured into acetone slowly and with gentle shaking and collected the residue. The dried precipitate is crushed in mortar and sieved. Powdered complex was placed in amber colored glass bottle stored at vacuum desiccator [3, 27].

Treatment

Albino rats of 12 months of age, weighing 250-300 g were used for this study (Institutional Ethical Committee Reference number 1677/PO/Re/S/2012/CPCSEA/IAEC-37dt.6/6/16). Animals, obtained from the Sri Venkateswara Traders, Bengaluru, were kept in polypropylene cages under hygienic conditions. They were fed with standard pellet diet (Hindustan Lever Limited, India). Rats were kept on a 12-h light:12-h dark cycle and checked for health status frequently

Experimental design

Animals were equally randomized into six groups

Group-I: Control: Rats in this group were administered with (0.9%) saline solution.
Group-II: Aluminum maltolate treated (AlM)
Group-III: *Bacopa monniera* extract administered (Bm)
Group-IV: *Bacopa* Phospholipid complex administered (BPC)
Group-V: Aluminum maltolate plus *Bacopa monniera* treated (AlM+Bm)
Group-VI: Aluminum maltolate plus *Bacopa* phospholipid complex administered (AlM+BPC)

Blood samples

At the end of the treatment blood samples were withdrawn from the medial canthus of the eye, blood samples were collected in clean test tubes and allowed to clot, then centrifuged for ten minutes at 3000 rpm. Serum was separated and stored in eppendorf tubes at – 20^{o}C to be used for biochemical analysis.

Biochemical assays

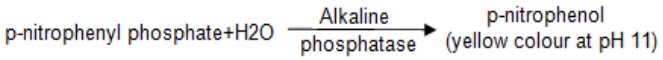
Serum aminotransferases ALT, AST and creatinine were performed according to the methods of [28,29] respectively, while serum urea was determined by enzymatic colorimetric method[30]. Serum total lipid, cholesterol, triglycerides, high-density lipoprotein(HDL), were determined using Autospan(Clinical reagents) Arkray Health care pvt., Ltd., India. VLDL was calculated using the formula TG/2.2 mmol/l. Low density lipoprotein (LDL) cholesterol was determined by differential subtraction of the sum of the cholesterol fractions from the total cholesterol

Preparation of Tissue Homogenates

After the experimental period, the rats were sacrificed and liver tissues were isolated and homogenates were prepared in buffer and centrifuged at 10,000 rpm for 10 minutes, supernatant was collected and used for further biochemical assays.

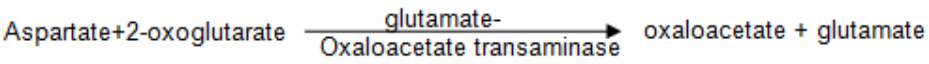
Determination of Alkaline Phosphatase (ALP)

Alkaline phosphatase was estimated by using the method[31]. For assaying phosphatases, p-nitrophenyl phosphate can be used as substrate which is hydrolysed to p-nitrophenol and Pi. P-nitrophenol is a colourless at acidic or neutral pH but alkaline pH of 11.The activity of ALP was expressed as µmoles of phenol liberated/mg protein/hr.



Determination of Aspartate Transaminase(AST)

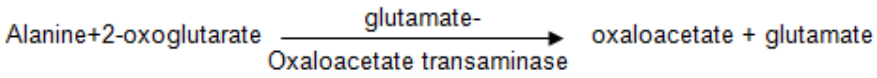
Activity of aspartate transaminase (AST) was estimated by using the method[32]. Aspartate is converted to oxaloacetate by ketoglutarate oxaloacetate transaminase. Oxaloacetate produced in this reaction is reduced by malate dehydrogenase which is accompanied by oxidation of NADH to NAD utilization of NADH in the second reaction and this will eventually be equivalent to the amount of aspartate in the reaction mixture.



The enzyme AST was expressed as µmoles of pyruvate formed/mg protein/hour.

Determination of Alanine Amino Transaminase (AAT)

Activated alanine amino transaminase (AAT) was estimated by using the method[32]. Alanine is converted to oxaloacetate by ketoglutarate- oxaloacetate transaminase. Oxaloacetate produced in this reaction is reduced by malate dehydrogenase which is accompanied by oxidation of NADH to NAD utilization of NADH in the second reaction .The enzyme AAT activity was expressed as µmoles of pyruvate formed/mg protein/hour.



Total proteins

The total protein content was estimated by the method of[33] 2% homogenates were prepared in 10 % TCA and centrifuged at 1000g for 15 minutes. The supernatant was discarded and the residue was dissolved in a known amount of 1N sodium hydroxide. From this 0.2 ml was taken and 4 ml of alkaline copper reagent and 0.4 ml of folin phenol reagent (1:1folin phenol and distilled water) were added. The contents were allowed to stand for 30 minutes at room temperature and the developed color was read at 600 nm in a spectrophotometer against a reagent blank. The amount of total proteins present in the sample was measured by using bovine albumin standard and the values were expressed as mg/g wet weight of the tissue.

Histopathology

Histopathological evaluation of liver and kidney tissues was performed in control and treated groups[34]. The tissues were processed and stained with hematoxylin-eosin and examined under light microscope.

RESULTS

Liver and kidney marker enzymes

AIM treatment showed significant increase in serum amino transferases (ALT & AST) compared to control group (Table 1). In kidney and liver Amino transferases and alkaline phosphatase (ALT, AST & ALP) levels were found to be elevated markedly in AIM group compared to control group. Bm and BPC administered groups showed normal levels of ALP, ALT and AST as in control (Fig 1 to 6). Where as in AIM+Bm and AIM+BPC groups, the enzymes (ALP, ALT and AST) were significantly decreased both in serum and tissues (Figure 1 to 6).

Kidney function markers

Serum urea and creatinine levels were significantly elevated in AIM treated group compared to control group. Whereas in Bm and BPC administered groups, these levels were almost similar to control group and co-administration of Bm and BPC with AIM resulted in significant reduction of serum urea and creatinine levels compared with AIM alone treated group (Table 1).

Lipid profile

There is a significant increase in total cholesterol, triglycerides, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels and decrease in high density lipoprotein (HDL) in AIM treated group compared to control group in serum (Table:1). Bm and BPC are maintained normal levels of total cholesterol, triglyceride, LDL, VLDL and HDL (Table 1) as in control. Synchronous administration of Bm and BPC with AIM reduced the levels of total cholesterol, triglyceride, LDL, VLDL and enhanced levels of HDL compared to ALM group (Table 1).

Total protein and albumin levels

The total protein and albumin levels are fell much lower in AIM treated group compared to control group in serum. Bm and BPC showed normal levels of protein and albumin as in control. Whereas co-administration of Bm and BPC with AIM significantly restored the content of protein and albumin compared to AIM alone treated group (Table 1).

Histopathological studies

Kidney architecture

Histological observation of control kidney exhibited well formed glomeruli and bowman's spaces in renal tubules with basal prominent nuclei (Figure 7). Kidney of AIM treated group showed degenerative changes in bowman's capsule, shrunken and broken glomeruli, obstructions in renal tubules and vacuolization (Figure 8) compared to control group. In Bm and BPC administered groups kidney's architecture was similar to control kidney (Figure 9&10). Whereas in AIM+Bm (Figure 11) and AIM+BPC (Figure 12) damage or degenerative changes induced by AIM were reduced compared to AIM treated kidney.

Liver architecture

Control liver exhibited central vein (CV) and hepatic cord arrangement around the CV with prominent nuclei, cell membrane and sinusoidal spaces (SS) (Figure 13). Dilatation and bleeding areas, loss of sinusoidal spaces and congestion in central vein were observed in the AIM treated liver, significant cytoplasmic loss was seen at the microscopic level (Figure 14). Whereas co-administration of both Bm and BPC with AIM, regeneration in CV, better formed SS were observed. These changes were more prominent in AIM+BPC (Figure 18) compared to AIM+ Bm group (Figure 17).

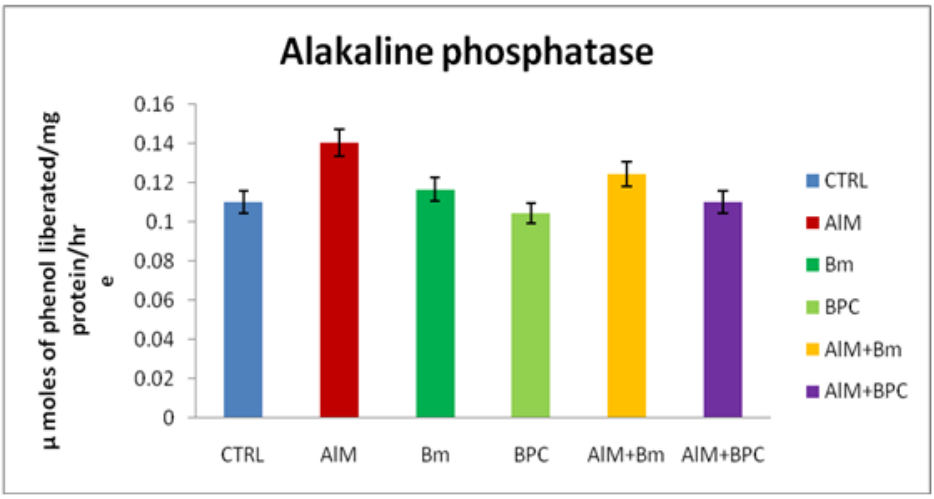
Table 1

Alteration in liver and kidney marker enzymes in control and treated groups.

Parameter	CTRL	AIM	Bm	BPC	AIM+Bm	AIM+ BPC
Albumin (g/dl)	4.12±0.42	2.82±0.22*	3.92±0.26	4.02±0.32	3.98±0.32*	4.01±0.13*
Total protein(g/dl)	5.6±0.3	4.2±0.7**	5.3±0.29	5.8±0.5	4.9±0.3$^{*@}$	5.1±0.2***
ALT (U/L)	32.43±4.35	90.14±4.7*	34.42±5.03	31.43±8.09	57.43±5.11*	56.23±9.12*
AST (U/L)	64.43±1.25	110.57±17.9*	62.32±10.8	60.93±11.4	75.99±15.8$^{\#}$	72.65±1 1.9**
Urea (mg/dl)	24.14±2.86	27.93±1.84***	24.24±1.9	24.24±4.1	25.77±0.44***	25.98±0.43$^{\#\#}$
Creatinine (mg/dl)	0.4±0.20	0.9±0.12*	0.4±0.09	0.3±0.4	0.6±0.140***	0.5±0.12$^{\#}$
Serum total cholesterol (mg/dl)	152±2.6	162±8.1*	155±8.3	158±3.8	157±0.3$^{*@}$	154±3.2$^{*@}$
Total triglycerides (mg/dl)	30.45±1.9	32.56±0.8$^{\#\#}$	30.25±1.9	30.76±2.8	31.40±0.5***	30.86±0.3*
HDL cholesterol (mg/dl)	78±11	60±2.1*	73±9.1	79±4.2	63±3.9$^{\$}$	72±2.4*
LDL cholesterol (mg/dl)	67.91±2.1	95.49±0.6*	76.4±2.8	73.8±1.8	87.8±27$^{\#\#\#}$	75.9±0.8*
VLDL cholesterol (mg/dl)	6.09±0.2	6.51±0.1**	6.09±0.3	6.23±0.4	6.28±0.1$^{\#}$	6.17±0.8$^{@}$

Values are expressed as mean ± SD for six animals (N=6)* = P≤0.0001, ** = P≤0.001, ***=P≤0.01, @= P≤0.02,# = P≤0.002, ## =P≤0.03, ### =P≤0.003, *@=P≤0.04 and $=P≤0.008. Statistical change with respect CTRL versus AIM, AIM versus AIM+Bm and AIM+ BPC(Student t-test).

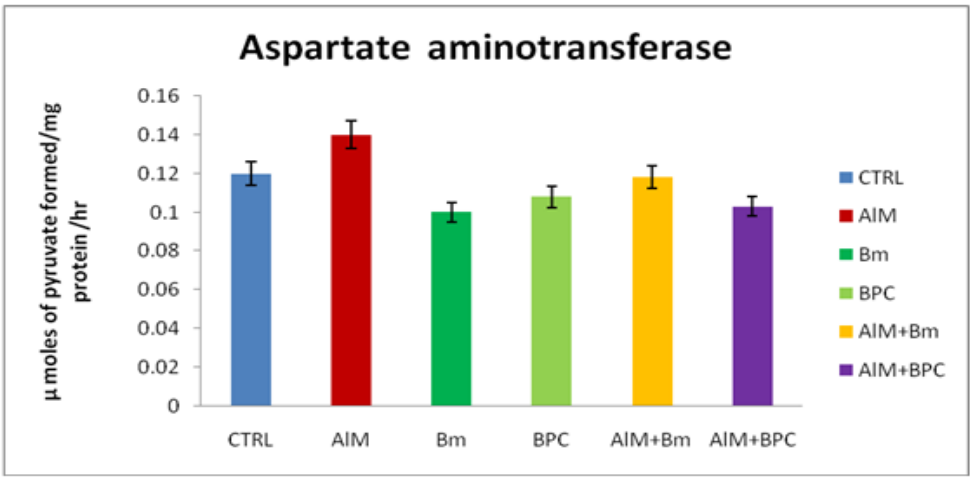
Kidney Markers



Results are expressed as mean ± SD (N=6) Statistically significant compared to treated animals with control (P≤0.01) (Student t-test).

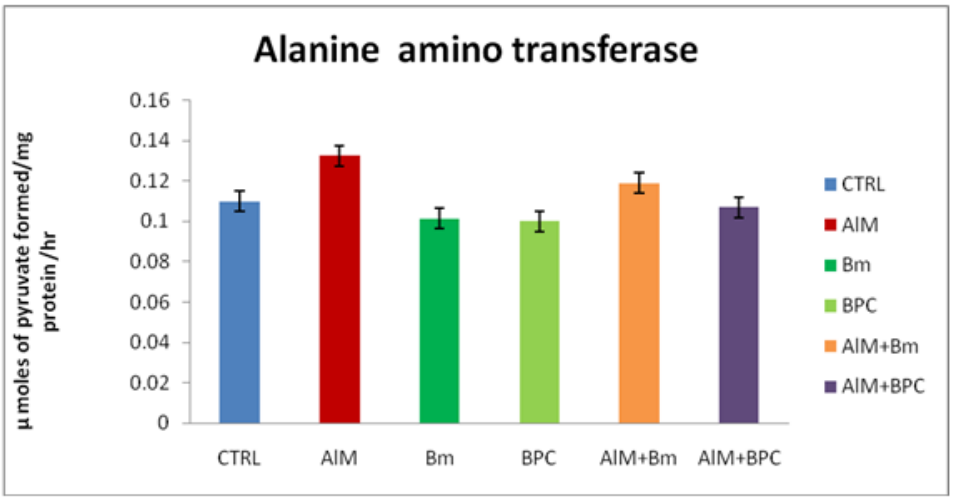
Figureure 1

Effect of Bm and BPC on Alkaline phosphatase (ALP) levels in kidney of AIM treated albino rats.



Results are expressed as mean ± SD (N=6) Statistically significant compared to treated animals with control (P≤0.01) (Student t-test)

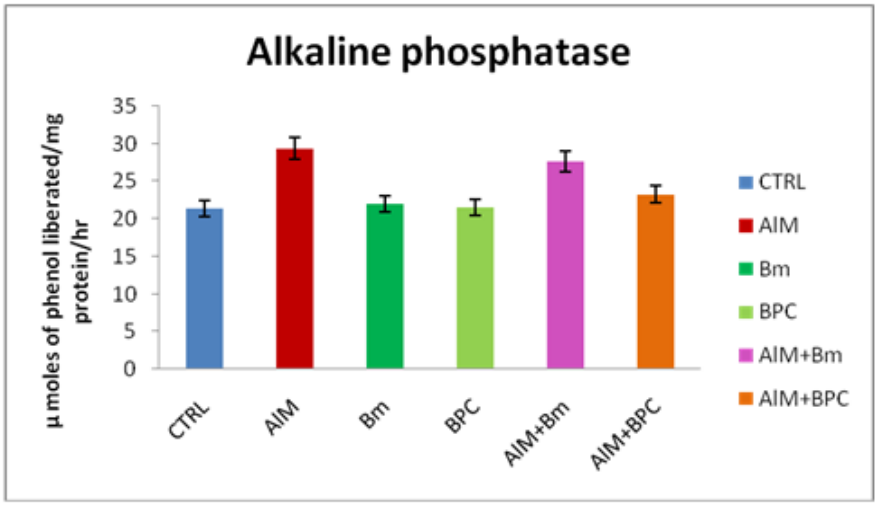
Figureure 2
Effect of Bm and BPC on Aspartate amino transferase (AST) levels in kidney of AIM treated rats.



Results are expressed as mean ± SD (N=6) Statistically significant compared to treated animals with control (P≤0.01) (Student t-test)

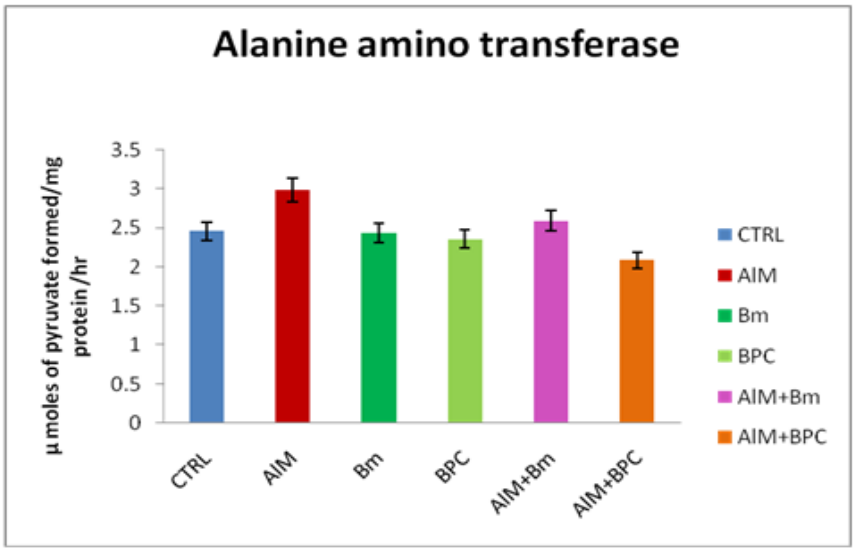
Figureure 3
Effect of Bm and BPC on Alanine amino transferase (ALT) levels in kidney of AIM treated rats.

Liver Markers



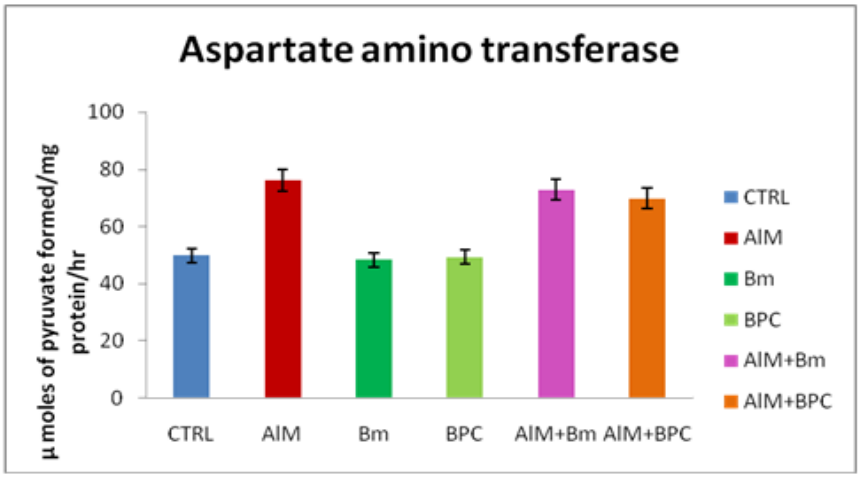
Results are expressed as mean ± SD (N=6) Statistically significant compared to treated animals with control (P≤0.01) (Student t-test)

Figureure 4
Effect of Bm and BPC on Alkaline phosphatase (ALP) Levels in liver of AIM treated albino rats.



Results are expressed as mean ± SD (N=6) Statistically significant compared to treated animals with control (P≤0.01) (Student t-test)

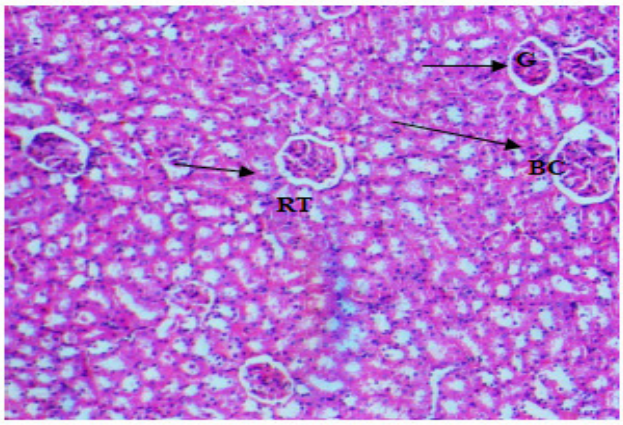
Figureure 5
Effect of Bm and BPC on Aspartate amino transferase (AST) levels in liver of AIM treated rats.



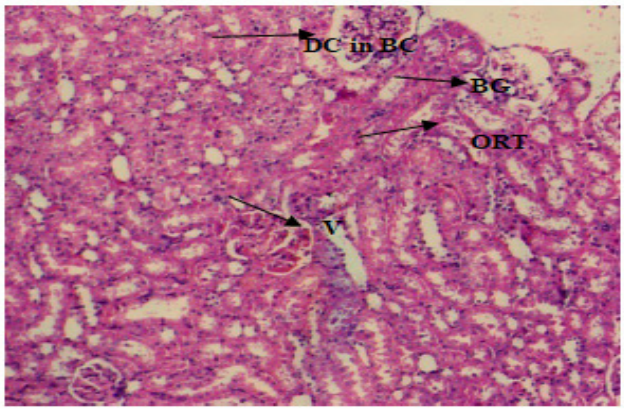
Results are expressed as mean ± SD (N=6) Statistically significant compared to treated animals with control (P≤0.001) (Student t-test)

Figureure 6
Effect of Bm and BPC on Alanine amino transferase (ALT) levels in liver of AIM treated rats.

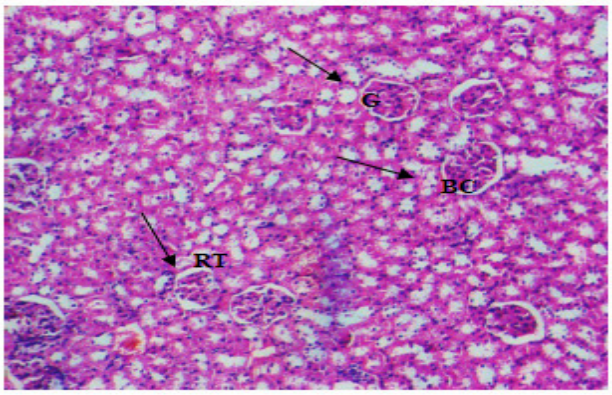
Histopathological studies of Kidney



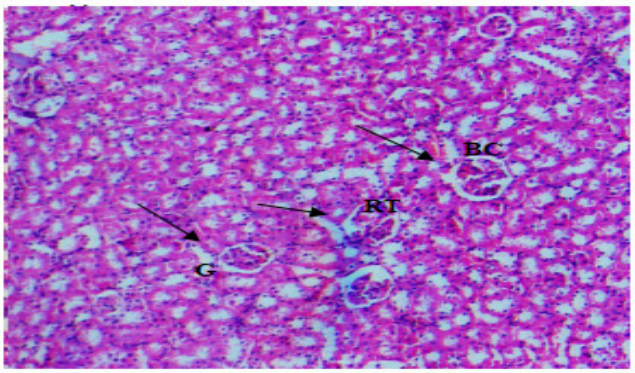
Figureure 7
Control group showing the normal architecture of the Kidney with normal Bowman's capsule (BC) with Glomeruli (G) and Renal tubules (RT) at magnification of 40X.



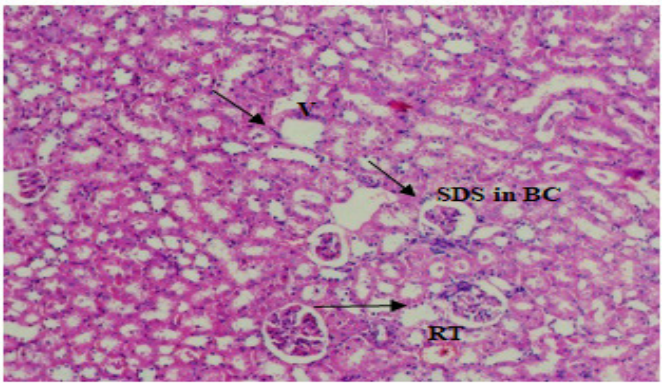
Figureure 8
AIM treated group showed degenerative changes (DC) in bowman's capsule (BC), shrunken and broken glomeruli (BG), obstructions in renal tubules (ORT) and vacuolization (V) at magnification of 40X.



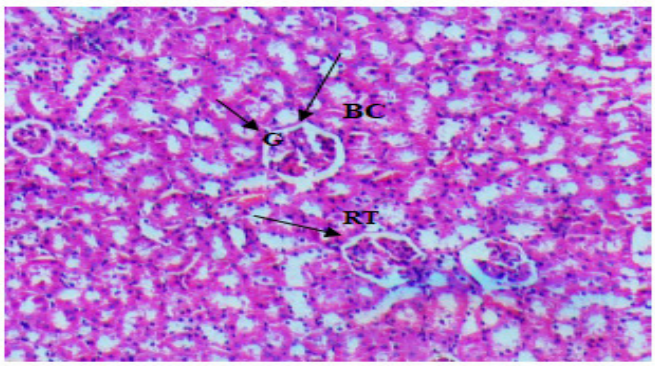
Figureure 9
Bm group showing the normal architecture of the Kidney with normal Bowman's capsule (BC) with Glomeruli (G) and Renal tubules (RT) at magnification of 40X.



Figureure 10
BPC group showing the normal architecture of Kidney with Bowman's capsule (BC),Glomeruli (G) and Renal tubules (RT) at magnification of 40X.

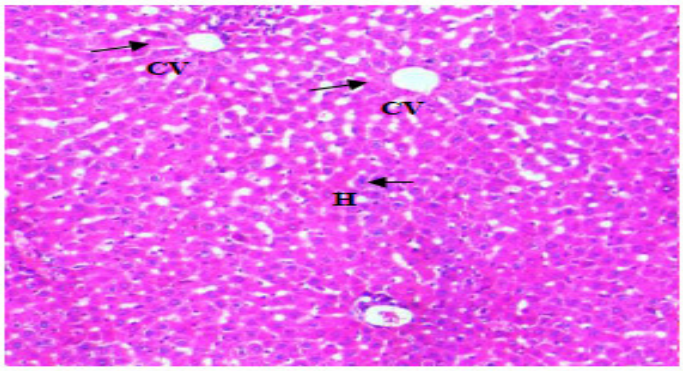


Figureure 11
AlM+Bm group showing the slight degenerative changes (SDC) of the Kidney within Bowman's capsule (BC) with Glomeruli (G) and Renal tubules (RT) with vacuolization (V) at magnification of 40X.

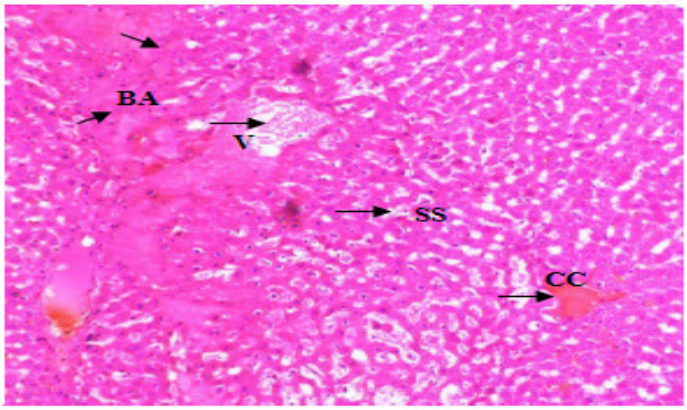


Figureure 12
AIM+BPC group showing the slight degenerative changes (SDC) of the Kidney within Bowman's capsule (BC) with Glomeruli (G) and Renal tubules (RT) with vacuolization (V) at 40X magnification.

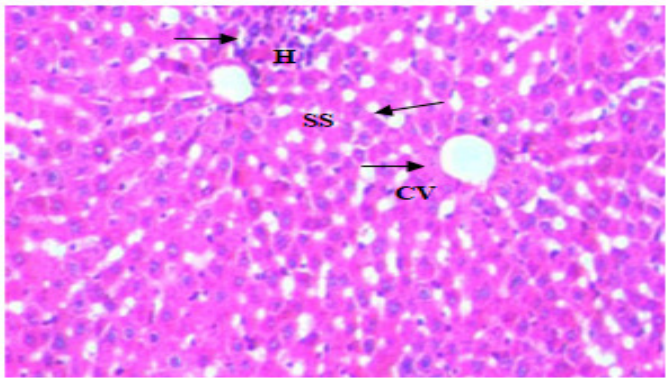
Histopathological studies of Liver



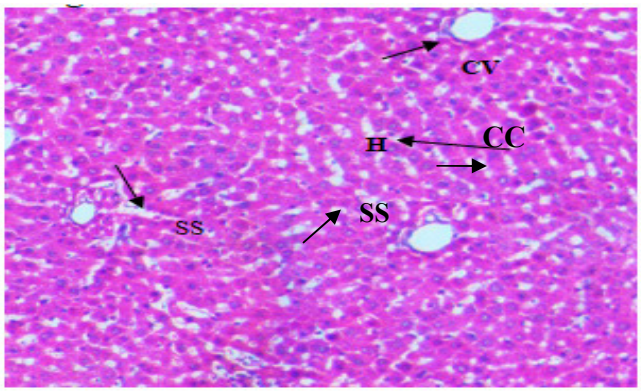
Figureure 13
Control group anatomic section of liver, the normal cyto-architecture central vein (CV) and active hepatocytes (H) with normal sinusoid space (SS) at 40X magnification.



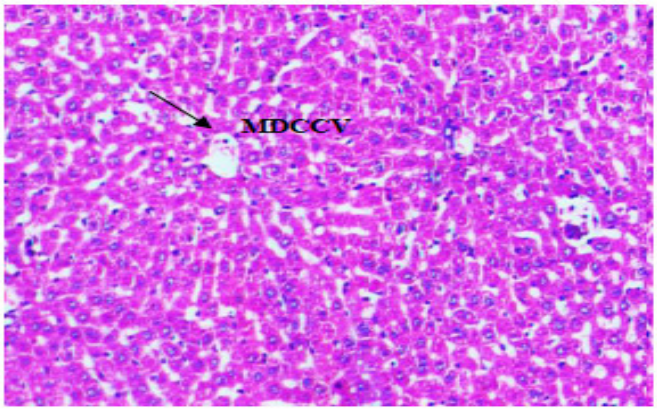
Figureure 14
Dilatation (D) and bleeding areas (BA), loss of sinusoidal spaces (LSS) and congestion in central vein(CC) and vacuolization (V) were observed in the AIM treated liver at 40X magnification.



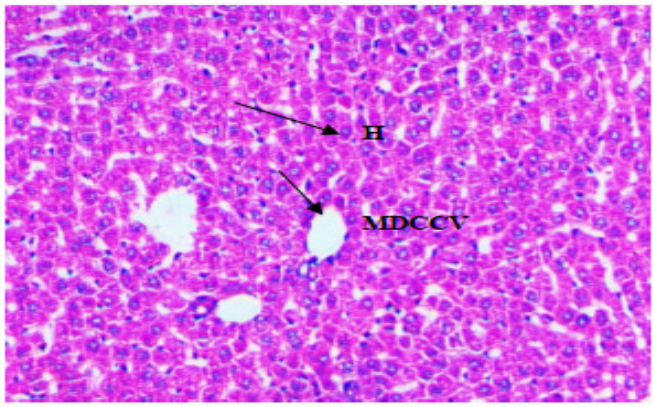
Figureure 15
Bm treated group showing the normal cyto-architecture showing central vein (CV) and active hepatocytes (H) with normal sinusoid space (SS) similar to control at 40X magnification.



Figureure 16
BPC treated group showing the normal cyto-architecture central vein (CV) and active hepatocytes (H) with normal sinusoid space (SS) similar to control at 40X magnification.

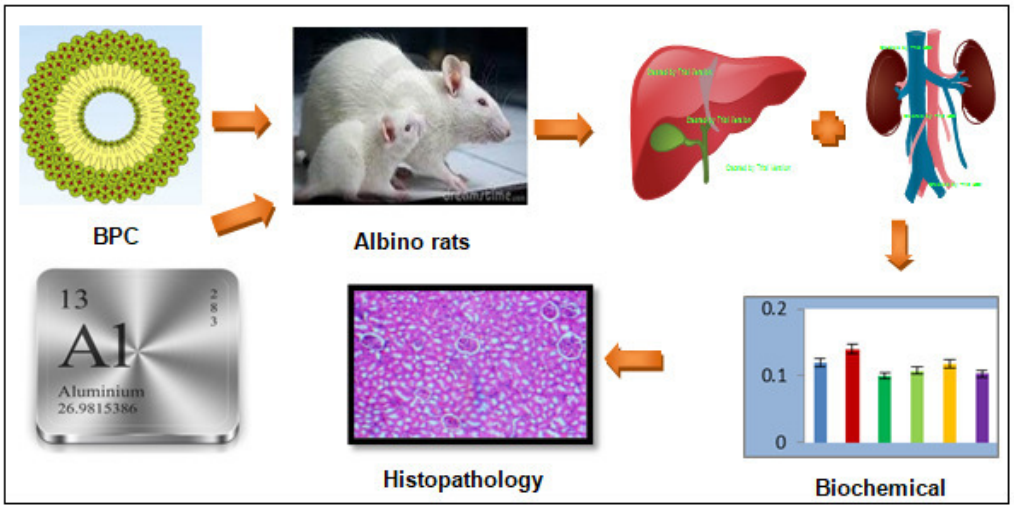


Figureure 17
AIM+Bm group anatomic section of liver tissue less necrotic changes with mild degeneration in central vein compare (MDCCV) and less vacuolization to AIM group at 40X magnification.



Figureure 18
AIM+BPC group anatomic section of liver showing less necrotic changes with mild degeneration in central vein compare(MDCCV), less vacuolization and active hepatocytes(H) to AIM group at 40X magnification.

Schematic diagram for "Bacopa phospholipid complex (BPC) against Aluminum maltolate (AIM) induced nephro and hepato toxicity"



Graphical presentation of work

DISCUSSION

The present study is designed to explore the protective role of *Bacopa* phospholipid complex against aluminum maltolate complex (AIM) induced hepato and renal toxicity in albino rats. Kidney plays a vital role in whole-body homeostasis in regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. Liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, metabolism and regulation of glycogen storage[20]. Generally liver and kidney function is assessed by the measurement of transaminases such as AST ALT and ALP. Previous study indicating that intracellular enzymes are released into the circulation due to any damage and necrosis of liver and kidney [35,39] In the current study, serum and tissues (kidney and liver) amino transferases and alkaline phosphatase (ALT, AST & ALP) levels were found to be elevated markedly in AIM group compared to control. Previous reports demonstrated that Al accumulation within the liver and kidney is associated with a number of biochemical changes, which include the release of enzyme markers of liver and kidney during injury which leads to alterations in the oxidant status [24,35-37]. The renal and hepatotoxicity induced by AIM was clearly observed by elevations in the ALP, AAT and AST enzyme levels. These enzymatic alterations may be due to defective excretion consequences upon the renal impairment of kidney by AIM. Our results are in consonance with previous reports[35,37-39,20]. The present study showed that AIM induced elevations in serum urea and creatinine levels. According to previous reports exposure of high concentrations of Al leads to its accumulation in the kidney which worsens the renal function by increased renal excreation of Al, thus making kidney vulnerable to Al-mediated toxicitys[23,34]. Increased serum urea and creatinine concentration can be a consequence of critical accumulation of Al in the kidneys, eventually resulting in renal failure[35,40]. Al accumulation was more in the liver than in the brain, muscle, heart or lung[23].Total cholesterol, triglycerides,

LDL were increased and HDL was decreased possibly due to activity of lipase in blood vessels, which breaks up triglycerides. In the present study High levels of cholesterol and low concentration of albumin were observed which might be due to hepatic dysfunction [35,38-39]. AIM treatment shows decreased levels of total protein and albumin indicating poor liver functions or impaired synthesis and alteration in protein metabolism[41,35]. This is an indicative of the onset of hepatocellular damage due to liver dysfunction and disturbance of the biosynthesis of these enzymes, with alteration in the permeability of liver membrane[41]. Histopathological observations of kidney showed degenerative changes in, bowman's capsule shrunken and broken glomeruli, obstructions in renal tubules and vacuolization in AlM treated group. Number of previous reports stated that degeneration in renal tubules, reduction in proximal tubule lumen, atrophy of glomeruli and necrotic changes in epithelial layer of proximal tubules' loss of sinusoidal spaces and vocalization [24,39,37,20]. Dilatation and bleeding areas, loss of sinusoidal spaces and congestion in central vein were observed in the AlM treated liver, significant cytoplasmic loss was seen at the microscopic level. Al induced liver showed cytoplasmic and nuclear degeneration and prominent vacuolization. Al overload in liver may lead to cholestasis disturbance of hepatic microsomal functions[24,36]**.** The hepato-toxicity was clearly observed in AlM with increase in the ALT, AST and ALP levels. Cellular leakage, loss of functional integrity of cell membrane and causes tissue dysfunction in liver [42]. Al+Bm and Al+BPC treated groups showed significant decrease in these biochemical enzymes (ALT, AST and ALP), urea, cretatinine, total cholesterol, triglycerides, LDL and HDL were increased. Bm and BPC which explores its protective modulatory effect on transaminases, total cholesterol, triglycerides, LDL, urea and cretatinine levels. Enhanced levels of total protein, albumin, regenerative changes of kidney's bowman's capsule with glomeruli and obstructions in renal tubules and recovery of central vein, sinusoidal spaces in hepatocytes of liver induced by AlM. Bm administration significantly decrese the elevation of serum transaminases ALT and AST[9].It suggested that Bm is able to stabilize the dysfunction in kidney and liver and indicate the improvement of functional status of the nephrocytes and hepatic cells[10].

CONCLUSIONS

Administration of BPC ameliorates the biochemical and histological alterations induced by AIM. It was found that oral administration of BPC showed better absorption and bioavailability than Bm alone administration. This might be due to novel formulation of Bm with phospholipids (BPC). Futher studies are needed to elucidate the mechanism of better therapeutic potential of BPCs.

ACKNOWLEDGMENTS

One of the authors, Ms T. Madhavi is highly grateful to University Grants Commission (UGC) for providing financial assistance in the form of –Rajiv Gandhi National Fellowship (RGNF) F1-17.1/2015-16/RGNF-2015-17-SC–AND-7719/ (SAIII/Website) to carry out this research work.

CONFLICT OF INTEREST

Conflict of interest declared none.

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