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THERAPEUTIC EFFECT OF *BELLAMYA BENGALENSIS* LAMARCK BY INHIBITION OF OXIDATIVE STRESS IN EXPERIMENTAL ARTHRITIC MODEL

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ABSTRACT: Objective: Oxidative stress and generation of free radicals leads to several chronic inflammatory diseases like arthritis, diabetes mellitus, cancer, *etc*. Arthritis is an immune-mediated systemic disorder known to impair the quality of life. In spite of development of drug discovery, therapeutic targeting of arthritis is still not satisfactory. *Bellamya bengalensis* has been long known for used in ailments like arthritis, asthma, conjunctivitis in traditional medicine. There are very few studies for clarification of scientific understanding of these therapies. In the present study, a purified fraction of *Bellamya bengalensis* mass (PBB) was assessed for oxidative stress in arthritis in Wistar rats. **Methods:** Arthritis was induced by 0.1 ml of Freund's complete adjuvant (FCA) in the left hind paw of Wistar rats. After that, PBB was administered orally at 100 mg/kg, 200 mg/kg and 400 mg/kg doses for consecutive 28 days. A thorough investigation of serum and tissue parameters, including autopsied histopathological analysis, related to systemic dysfunction due to oxidative stress during arthritis were evaluated at the end of the study. **Results:** FCA generates a cascade of free radicals, oxidative stress, and also causes alteration of serum parameters and various biomarkers indicating systemic damage. The purified extract of *Bellamya bengalensis* (PBB) exhibited dose-dependent significant anti-arthritic and antioxidant activities and restoration of biomarkers to near normal levels. **Conclusion:** This study demonstrated the therapeutic effect of *Bellamya bengalensis* Lamarck in FCA induced arthritic model. This might be mediated controlling the signaling pathways for the formation of reactive oxygen species followed by cellular damage.

INTRODUCTION: Arthritis is one of the most common compliance among the geriatric population.

In the United States, 49.6% of the geriatric population (aged 65 or older) are diagnosed with arthritis and are more common in female [1].

Arthritis is a chronic systemic immune-mediated inflammatory disorder [2]. Quality of life in arthritic patients deteriorates due to systemic abnormalities manifested in liver and kidney [3, 4]. Researchers have established a strong correlation between chronic inflammatory conditions and liver and

kidney damages [4, 5]. Chronic inflammatory disorders are associated with a high level of C-reactive proteins (CRP) in serum. A persistently elevated level of CRP is a marker for chronic kidney disease in patients with arthritis [4].

Identification of the pattern of systemic involvement of a disease at an early stage helps in providing better diagnosis and may help to cure the illness. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed drugs in inflammation and pain [6]. NSAIDs act by binding with cyclooxygenase (COX-1 and COX-2) in a non-selective way to prevent inflammation [7]. Some of the adverse effects that are associated with conventional use of NSAIDs are gastric perforations, ulcers, interstitial nephritis, *etc.* [6, 7]

The traditional and complementary medicine provides enough evidence of therapeutically important plant and animal products with ethnopharmacological value. *Bellamya bengalensis*, a mollusc inhabiting in fresh water is known to cure inflammatory diseases like arthritis, conjunctivitis, asthma in folklore medicine [8]. Earlier, it was reported that *Bellamya bengalensis* Lamarck effective on inflammatory arthritis in experimental animals, but its underlying mechanism still obscure [9]. Hence, the present study was designed to evaluate the role of mollusc on oxidative pathways in arthritis in detail to clarify its therapeutic mode of action.

MATERIALS AND METHODS:
Collection and Preparation of the Test Drug: Molluscwere collected from ponds near Kolkata and authenticated from Zoological Survey of India, Kolkata, West Bengal as *Bellamya bengalensis* Lamarck (Specimen No: 1242/Lot No-63). The mollusc was cleaned through demineralized water and acclimatized in laboratory conditions for 24 h.

The whole mass was dissected out carefully and was kept overnight in 1X phosphate buffer saline pH 7.4 at 4 °C. After that, it was ground and centrifuged at 5000 rpm for 10 min. The soluble fraction was collected after filtering through Whatman's filter paper (Ø125 mm) and lyophilized to make it powdered (PBB). The soluble fraction obtained (PBB) was further standardized using protein content and gel-electrophoresis techniques. Gel-electrophoretic separation revealed characteristic

bands of peptides (MW range 43 KDa, 27 KDa, 20 KDa, 18 KDa, and 15 KDa). It was stored at -20 °C until use.

Study Design and Group Division:
Animals: Healthy Wistar albino rats of either sex (150–200g) were used for the study. They were maintained according to the guidelines of Committee for Control and Supervision of Experiments on Animals (CPCSEA) [10]. The animal experiments have been conducted after clearance from the Institutional Animal Ethics Committee of R.G. Kar Medical College, Kolkata (Ethical Clearance no.: RKC/IAEC/13/18 dated 15.03.2016).

Induction of Adjuvant Induced Arthritis: Adjuvant-induced arthritis was induced by injecting 0.1 ml of Complete Freund's Adjuvant (Sigma, St. Louis, USA) in the subplantar region of the left hind paw of the animals.

Experimental Design and Group Division: After induction of arthritis, drugs were administered orally for 28 days. The doses of the test drug were selected on the basis of the pilot study. 36 rats were randomly selected and divided into 6 groups. Diclofenac Sodium was used as the standard drug. The treatment schedule is as follows:

Group I (Normal Control, n = 6): Normal rats treated with normal saline, 2 ml/kg.

Group II (Arthritic Control, n = 6): FCA induced rats treated with normal saline, 2 ml/kg.

Group III (Standard, n = 6): FCA induced rats treated with diclofenac sodium, 10 mg/kg.

Group IV (Test group 1, n = 6): FCA induced rats treated with the test drug PBB 100 mg/kg.

Group V (Test group 2, n = 6): FCA induced rats treated with the test drug PBB 200 mg/kg.

Group VI (Test group 3, n = 6): FCA induced rats treated with the test drug PBB 400 mg/kg.

Preparation of Serum and Tissue Homogenate: All rats were in 16 h fasting condition before euthanasia. They were euthanized with an overdose of thiopentone sodium, 2 h after given the last dose at day 28. Serum biochemical analysis was done by collecting blood from retro-orbital plexus of the rats. Liver and kidney tissue were segregated out,

perfused with normal saline and kept in at -20 °C before biochemical estimations. Small portions of tissues were stored in buffer formalin for histopathology.

Estimation of Biochemical parameters in Serum: Total protein, globulin, albumin, glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP), calcium, magnesium, sodium, potassium, chloride, urea, creatinine, uric acid were measured by using commercial kits (Autospan, India).

Measurement of Hepatosomatic Index and Renal Index: The liver and kidney tissues were weighed, and hepatosomatic index and renal index were calculated as follows[11, 12]

Hepatosomatic Index (HSI) = Liver weight × 100 / Bodyweight

Renal Index = Kidney weight (right and left) × 100 / Bodyweight

Antioxidant Enzymes and Oxidative Stress in Tissues: After dissecting the rat, the liver and kidney tissues were excised out and washed in normal saline. 10% w/v tissues were then homogenized in 50 mM phosphate buffer saline, pH 7.4. The homogenized mixture was then centrifuged for 10 min at 5000 rpm. The supernatant was collected and was used for estimation of antioxidant markers in tissue.

Protein Estimation: [13] The protein concentration was estimated using standard protocol following Lowry *et al*.

Nitric Oxide Assay (NO): [12] 500 µl of Griess reagent (1g/L sulfanilamide, 25g/L phosphoric acid, 0.1g/L N-1-naphthylethylenediamine) was mixed with 100µl of tissue homogenate and incubated at room temperature for 30 min. The absorbance was measured at 540 nm. The quantity of nitric oxide in the sample was estimated using standard curve plotted with sodium nitrite.

Lipid Peroxidation Assay (LPO): [14] 500 µl of 10% liver homogenate was added to 1 ml of TBARS (15% w/v Trichloroacetic acid and 0.375% Thiobarbituric acid was prepared in 0.25N Hydrochloric acid). The reaction mixture was heated in boiling water for 30 min and then cooled

in running tap water. The resultant mixture was then centrifuged for 15 min at 3000 rpm, and the optical density of the supernatant was estimated at 532 nm. Lipid peroxidation was evaluated with the help of standard curve plotted with Malonaldehyde (MDA).

Reduced Glutathione Assay (GSH): [15] Reduced glutathione reacts with the 5, 5dithiobis (2-nitrobenzoic acid) (DTNB) to produce a yellow compound. The GSH concentration in the sample is directly proportional to the reduced yellow chromogen. 0.1 ml of the tissue homogenate was added to 0.06 ml of 20 mM EDTA (Ethylenediaminetetraacetic acid) and incubated on ice for 10 min. 0.25 ml TCA (Trichloroacetic acid) and 500 µl of water was added to the above reaction mixture and incubated for 5 min at room temperature. 2 ml of 0.4M Tris buffer and 0.01 ml of 0.1M DTNB was added to 1 ml of the supernatant and incubated at room temperature for 3 min. The absorbance was read at 412 nm.

Superoxide Dismutase Assay (SOD): [16] 200 µl of tissue homogenate was added to 1.2 ml of 0.052M sodium pyrophosphate buffer, 100 µl of 186 µmol phenazine methosulphate, and 1 ml water. Addition of 0.2 ml of 780 µmol NADH (Nicotinamide adenine dinucleotide initiated the reaction and stopped exactly after 1 min by addition of glacial acetic acid. The purple chromogen formed was estimated by reading the absorbance at 560 nm.

Histopathology: For the preparation of histological slides of liver and kidney tissues, the tissues were washed with normal saline and fixed with 10% formalin solution. The tissues were then paraffinized, cut into thin sections of 3-5 µm thickness, deparaffinized and rehydrated following standard protocols [17]. The tissues were stained using hematoxylin and eosin (H&E) and were observed under a light microscope (Olympus, Japan).

Statistical Analysis: In the present study, data were expressed as mean ± SEM (Standard Error of Mean). The statistical analysis was done by the analysis of variance (ANOVA), followed by post-hoc Dunnett test. The statistical software SPSS version 20.0 was used for evaluation of the data. Differences were considered statistically significant at $p < 0.05$ and $p < 0.01$.

RESULTS: Serum biomarkers are important tools that help to monitor the disease progression. Present study **Table 1** revealed that the level of total protein, globulin increased and decrease in albumin: globulin ratio when induced with Freund's adjuvant (Group II) as compared to normal control (Group I). However, treatment with PBB at a dose of 200 mg/kg and 400 mg/kg significantly decreased globulin concentration and increased albumin: globulin ratio when compared with Group II rats.

The FCA induced arthritic rats showed a marked increase in serum GOT, GPT, and ALP levels compared to normal control due to leakage of inflammatory mediators in serum. FCA induced rats showed alteration of hepatic markers in serum, which was significantly reduced (p<0.01) by PBB 200 mg/kg and PBB 400 mg/kg in a dose-dependent manner. The serum renal markers in **Table 1** also showed that the PBB extract significantly reduced the urea and uric acid concentration in the serum in 200 mg/kg and PBB 400 mg/kg, respectively.

Serum electrolytes play an important role in maintaining the physiological balance of the body. Sodium and potassium concentration in the serum was decreased by 38% and 45% respectively considerably when induced with FCA. Oral administration of PBB 200 mg/kg and PBB 400 mg/kg restored the homeostasis after 28 days of treatment. Loss of magnesium and calcium were also observed after induction of FCA by 62.81% and 70.01%. The levels of electrolytes were elevated significantly after 28 days (p<0.01).

TABLE 1: SERUM BIOCHEMICAL PARAMETERS OF DIFFERENT EXPERIMENTAL GROUPS (n=6)

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Serum protein markers						
Total protein (g/dl)	8.31 ± 0.44	16.61 ± 3.87	10.88 ± 2.55	7.35 ± 0.91*	7.75 ± 0.51*	8.42 ± 1.32*
Albumin (g/dl)	3.87 ± 0.32	1.12 ± 1.66	3.50 ± 0.49	3.07 ± 0.20*	4.30 ± 0.17*	4.54 ± 0.88*
Globulin (g/dl)	4.44 ± 0.75	12.13 ± 2.57	7.38 ± 3.02	4.28 ± 1.12**	3.45 ± 0.43**	3.88 ± 1.07**
Albumin: Globulin	1.10 ± 0.42	0.52 ± 0.09	0.69 ± 0.36	1.30 ± 0.17	1.34 ± 0.21	1.62 ± 0.15
Liver enzyme markers:						
SGOT (IU/L)	44.79 ± 3.78	154.90 ± 4.66	112.25 ± 4.22	101.47 ± 3.60*	79.90 ± 5.58**	57.35 ± 0.60**
SGPT (IU/L)	37.04 ± 2.45	144.4 ± 11.11	117.59 ± 6.48**	103.7 ± 10.68**	51.85 ± 10.43**	49.07 ± 5.63**
ALP (KA Units)	71.24 ± 8.31	158 ± 11.99	140.5 ± 10.45	118.7 ±12.78	75.97 ± 10.43**	71.24 ± 10.04**
Serum electrolyte markers						
Sodium (mEq/l)	116.4 ± 5.15	45 ± 2.93	58.21 ± 2.79	78.21 ± 3.86**	116.07 ± 3**	126.43 ± 4.89**
Potassium (mEq/l)	5.42 ± 0.51	2.48 ± 0.62	3.56 ± 0.81	5.33 ± 1.18	5.83 ± 1.12	6.21 ± 1.11*
Calcium (mEq/l)	13.83 ± 1.06	9.69 ± 0.64	9.63 ± 1.01	9.93 ± 1.61	11.40 ± 1.35	16.76 ± 2.79*
Magnesium (mEq/l)	2.47 ± 0.044	1.55 ± 0.10	1.14 ± 0.17	3.89 ± 2.42	4.25 ± 0.71	4.81 ± 1.13
Serum nephrological markers						
Urea (mg/dl)	21.86 ± 2.25	37.02 ± 1.22	30.18 ± 3.02	23.47 ± 1.52	18.48 ± 0.81	18.81 ± 0.53*
Creatinine (mg/dl)	0.90 ± 0.17	1.05 ± 0.17	0.93 ± 0.12	0.83 ± 0.31	0.83 ± 0.03	0.82 ± 0.16
Uric acid (mg/dl)	2.46 ± 0.43	5.06 ± 0.42	4 ± 0.36	3.79 ± 0.57	2.88 ± 0.37	2.03 ± 0.2

Statistical analysis was done by ANOVA followed by Dunnett Test, *p < 0.05, **p < 0.01 when compared to arthritic control. Values were expressed as data ± SEM for 6 rats.

Table 2 revealed the hepatosomatic and renal index obtained from the weight of the liver and kidney tissues of the rats. The hepatosomatic index of FCA induced rats with arthritis (Group II) was found to be increased by 42.1% and 20%, respectively, when compared to normal control (Group I).

Oral administration of PBB extract at 200 mg/kg and 400 mg/kg significantly reduced the value of the hepatosomatic index and renal index and was comparable to normal control (Group I) rats.

TABLE 2: HEPATOSOMATIC AND RENAL INDICES OF DIFFERENT EXPERIMENTAL GROUPS (n=6)

Group no.	Hepatosomatic Index	Renal Index
Group I	3.23 ± 0.19	0.0070 ± 0.0011
Group II	4.59 ± 0.19	0.0084 ± 0.0009
Group III	4.26 ± 0.53	0.0084 ± 0.0014
Group IV	4.31 ± 0.32	0.0060 ± 0.0007
Group V	3.39 ± 0.29*	0.0064 ± 0.0015
Group VI	3.03 ± 0.16*	0.0066 ± 0.0008*

Statistical analysis was done by ANOVA followed by Dunnett Test, *p<0.05, **p<0.01 when compared to arthritic control. Values were expressed as data ± SEM for 6 rats.

**TABLE 3: OXIDATIVE STRESS MARKERS IN LIVER AND KIDNEY TISSUES OF DIFFERENT EXPERIMENTAL GROUPS (n=6)**

Group No.	Liver				Kidney			
	GSH (µg/mg Protein)	LPO (µM/mg Protein)	NO (µM/mg Protein)	SOD (U/mg Protein)	GSH (µg/mg Protein)	LPO (µM/mg Protein)	NO (µM/mg Protein)	SOD (U/mg Protein)
Group I	1.46 ±0.26	0.027 ±0.008	0.056 ±0.008	0.080 ±0.008	1.84 ±0.41	0.005 ±0.004	0.0011 ±0.0003	0.090 ±0.012
Group II	0.07 ±0.17	0.053 ±0.012	0.178 ±0.011	0.019 ±0.033	0.07 ±0.12	0.038 ±0.006	0.0024 ±0.0003	0.008 ±0.024
Group III	0.81 ±0.20*	0.019 ±0.003	0.082 ±0.025**	0.037 ±0.004	0.64 ±0.05*	0.028 ±0.002	0.0014 ±0.0009	0.057 ±0.004
Group IV	1.08 ±0.11*	0.012 ±0.004*	0.061 ±0.023**	0.052 ±0.013	0.87 ±0.03*	0.014 ±0.0004*	0.0013 ±0.001	0.042 ±0.032
Group V	1.24 ±0.36**	0.011 ±0.003*	0.052 ±0.011**	0.055 ±0.026**	1.04 ±0.04**	0.013 ±0.002**	0.0011 ±0.0007*	0.073 ±0.032**
Group VI	1.27 ±0.08**	0.007 ±0.004*	0.035 ±0.014**	0.091 ±0.007**	1.18 ±0.08**	0.009 ±0.001**	0.001 ±0.0006*	0.086 ±0.032**

Statistical analysis was done by ANOVA followed by Dunnett Test, *$p<0.05$, **$p<0.01$ when compared to arthritic control. Values were expressed as data ± SEM for 6 rats.

Oxidative stress and generation of free radicals are one of the major reasons behind inflammation and tissue injury in FCA induced arthritis. The antioxidant markers like reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and Superoxide dismutase (SOD) contents were estimated in liver and kidney supernatant and were expressed by the enzyme activity per gm protein in tissue **Table 3**. Induction of adjuvant-induced arthritis by FCA reduced GSH and SOD levels in liver and kidney tissues. Administration of PBB extract for 28 days significantly elevated the levels of cellular tissue antioxidant at a dose of 200 mg/kg and 400 mg/kg. Lipid peroxidation is another important tissue antioxidant marker. The concentration of malonaldehyde is directly proportional to the peroxidation of tissue lipids. Malonaldehyde concentration was found to be raised in Group II rats as compared to normal control while a significant reduction in malonaldehyde concentration in PBB 200 mg/kg and PBB 400 mg/kg was observed (Group V and Group VI). Significant rise in the concentration of nitric oxide in Group V and Group VI rats compared to Group II rats indicated that the PBB extract is a strong antioxidant and can delay or suppress the tissue damage.

Histopathology of the Kidney and Liver Tissues:

GROUP I   GROUP II   GROUP III

GROUP IV   GROUP V   GROUP VI
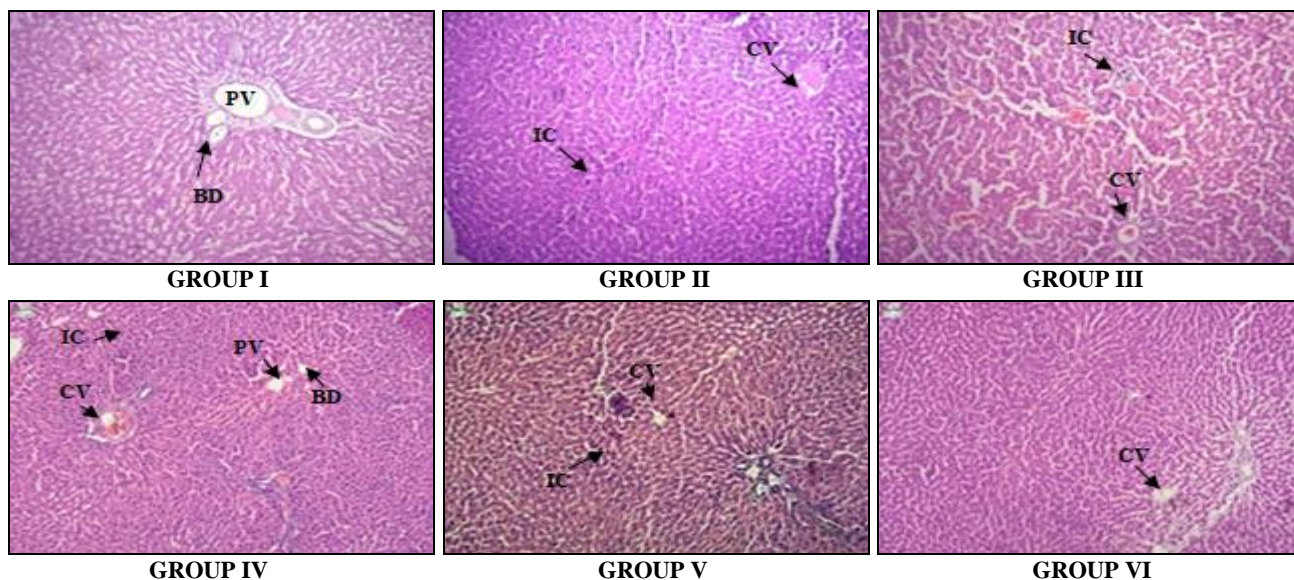
PHOTOGRAPH 1: HISTOLOGICAL SECTIONS OF LIVER TISSUES STAINED WITH HEMATOXYLIN AND EOSIN. [MAGNIFICATION 10X] PV = PORTAL VEIN, BD = BILE DUCT, IC = INFLAMMATORY CELLS

Photograph 1 revealed that the normal rats (Group I) showed normal characteristic features of liver, *i.e.*, hepatic lobules, portal veins, central vein, and sinusoids. Rats with adjuvant-induced arthritis (Group II) showed that the central vein was congested and infiltration of inflammatory cells. Group III, standard drug, Diclofenac sodium showed congestion of central veins and the presence of inflammatory cells. In Group IV (PBB 100 mg/kg) and Group V (PBB 200 mg/kg) revealed there was a decrease in inflammatory cells and reduction in congestion of central veins. Group VI (PBB 400 mg/kg) showed a marked reduction in congestion and the presence of very few inflammatory cells.
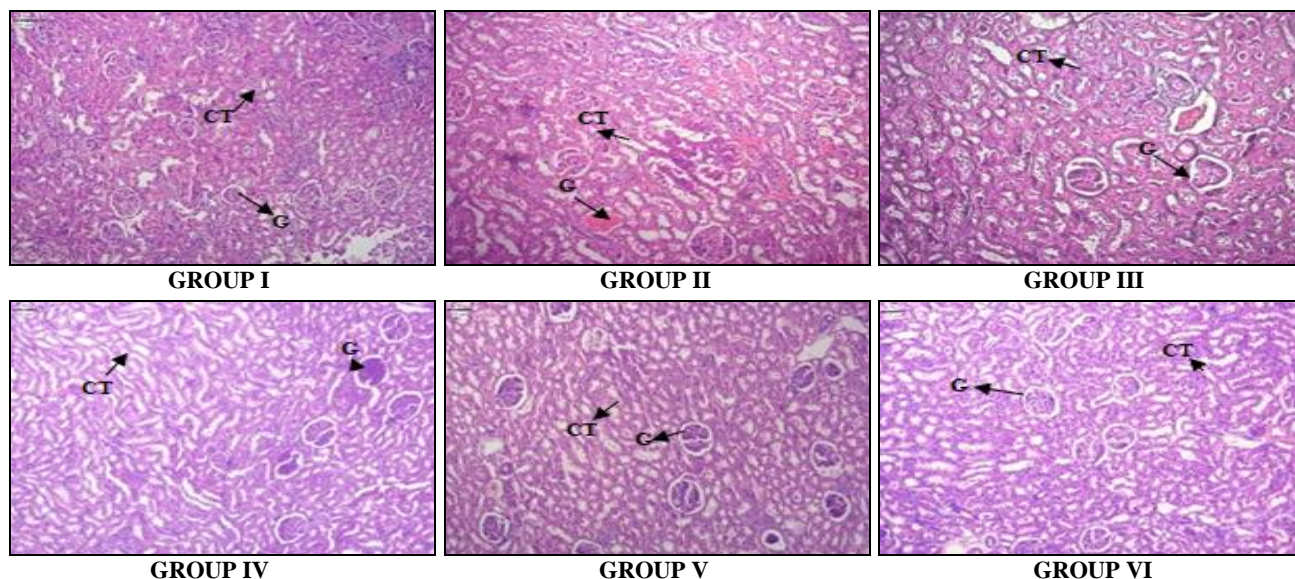
PHOTOGRAPH 2: HISTOLOGICAL SECTIONS OF KIDNEY TISSUES STAINED WITH HEMATOXYLIN AND EOSIN. [MAGNIFICATION 10X] G = GLOMERULUS, CT = CONVOLUTED TUBULES

The histological sections of normal kidney of normal control (Group I) showed preservation of normal corpuscular and tubular structure in renal cortex. It showed the presence of normal glomerular structure, Bowman's capsule, and convoluted tubules. Kidney sections of FCA induced control (Group II) revealed congestion in the glomerulus with degeneration of glomerular structure and infiltration of inflammatory cells and dilatation in Bowman's space. Treatment with Diclofenac sodium 10 mg/kg (Group III) and PBB 100 mg/kg (Group IV) showed a reduction in inflammatory cells and decreased in degeneration of glomerular structure. Treatment with PBB 200 mg/kg and PBB 400 mg/kg doses revealed a marked decrease in inflammatory cells and glomerulus was restored to near normal structure along with normal renal tubules **Photograph 2**.

DISCUSSION: Arthritis is a major systemic autoimmune disorder affecting 54.4 million adults only in USA [18]. The symptoms of this disease are associated with restricted musculoskeletal movement, pain, and alteration of biochemical parameters. Freund's complete adjuvant was first used by Pearson *et al.*, to induce polyarthritis in the hind paw of the rats [19]. Till date, this method is the most used and considered the best for induction of inflammation.

Assessment of serum biomarkers provides a sensitive tool to determine disease development and progression. The present study dealt with the effect of adjuvant-induced arthritis on biochemical parameters. An elevated level of globulin concentration is a marker of inflammation. Inflammatory mediators like histamine, bradykinin, and prostaglandins increase the permeability of vascular tissues to albumin, leading to reduction of albumin in serum. Treatment with PBB 200 mg/kg and PBB 400 mg/kg significantly increased the albumin level and decreased the globulin level in adjuvant-induced arthritic rats which may indicate that PBB might have inhibitory effects on inflammatory mediators.

Serum aminotransferases (SGOT and SGPT) and alkaline phosphate (ALP) are enzymatic liver

markers which increases in case of liver disease and also due to muscle injury during inflammation, a condition known as idiopathic inflammatory myopathy [20]. Bone erosion in arthritis is one of the reasons for the elevated level of ALP in serum [21]. Adjuvant-induced arthritis in Group II rats showed an increase in liver enzymes. However, treatment with PBB 200 mg/kg and PBB 400 mg/kg significantly decreased the concentration of liver enzymes (p<0.01). The study signifies that treatment with the extract has decreased bone erosion and have an organ protective role against adjuvant-induced arthritis. A study from Egypt has shown that SGOT and SGPT levels were elevated during inflammatory conditions and were significantly reduced by the earthworm extracts [22]. Another similar study showed that lipid extracts of freshwater mussels reduced the elevated serum liver biomarkers (SGOT, SGPT, and ALP) induced by Freund's adjuvant [23].

Increased level of creatinine, urea, and uric acid in adjuvant-induced rats is possibly due to the presence of amino acids arginine and citrulline that are synthesized in kidneys, increases the rate of hepatic ureagenesis [24]. PBB treatment at a dose of 400 mg/kg has significantly reduced the altered urea and uric acid levels. Studies have shown that uric acid concentration and creatinine level has a positive correlation with arthritis, which is by this study [25, 26].

Sodium (Na^+), Potassium (K^+), Chloride (Cl^-), Magnesium (Mg^{2+}) and Calcium (Ca^{2+}) are some of the electrolytes that play an important role as cofactors in biochemical reactions, maintaining electrical gradients and also takes part in intermediary metabolism [27]. Hyponatremia is often used as a marker of inflammation and is directly proportional to the increase in C-reactive protein (CRP), IL-6, and IL-1β [28]. Lower levels of potassium or hypokalemia are often associated with muscle cramps and arthritic disorder [29]. The present study revealed that the sodium levels and potassium levels were elevated in PBB 200 mg/kg and PBB 400 mg/kg significantly (p<0.01) when compared to FCA induced control. The degenerative disease of the bone leads to loss of magnesium and calcium levels in serum, which acts as a cofactor for enzyme activities for cellular metabolism [30, 31]. Oral administration of PBB

extract at 200 mg/kg and 400 mg/kg dose reduced the electrolyte deficiency in a dose-dependent manner.

Hepatosomatic index denotes the ratio of the liver weight to the body weight, and the renal index denotes the ratio of the kidney weight to the total body weight. PBB treated rats at 200 mg/kg, and 400 mg/kg lowered the hepatosomatic and renal index significantly (p<0.01) when compared to FCA induced arthritic control (Group II). The study indicates that the PBB extract reduced the inflammatory state in the body without imparting any toxicity to liver and kidney tissues. However, a study was reported that extracts of *M. edulis* did not show any significant changes from arthritic control induced by olive oil [32].

Oxidative stress refers to the excess concentration of reactive oxygen species (ROS) or oxidants within the body and is associated with a myriad of pathological conditions. ROS are byproducts of cellular metabolism, which includes superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-). These ROS have an inherent property to target different biological macromolecule and induce pathological state by damaging them [33]. Literature suggests oxidative damage and an elevated amount of ROS are inherently associated with inflammation [33]. The present study estimated the cellular antioxidant level in liver and kidney tissues of the rats. Low level of glutathione reductase (GSH) and superoxide dismutase (SOD) is detrimental to the cells [34]. Thus, diminution of NO and SOD in FCA induced arthritic rats reflects oxidative stress in the tissue. Treatment with PBB extract at a dose of 200mg/kg and 400mg/kg increased the GSH and SOD concentration to near normal value. The optimum concentration of nitric oxide (NO) and malonaldehyde (MDA) are required in the body to maintain normal physiological condition. Excess NO in cells binds with ROS and brings about protein damage, whereas a rise in MDA level in tissues reflects higher lipid peroxidation and cellular damage. FCA induced arthritic control revealed a higher amount of NO and MDA level in liver and kidney tissues. The decrease in lipid peroxidation activity and nitric oxide evident in PBB treated 200 mg/kg and 400 mg/kg rats, indicating lower damage to the cellular contents by

ROS. In other study, researchers have shown that mussel extracts are rich in antioxidants and act as a defense mechanism in FCA induced inflammatory rats [35].

Biochemical assessment of liver and kidney biomarkers in serum of FCA induced arthritic rats (Group II) indicated profound deviation from normal values. In the present study, histopathological findings additionally supported the results of biochemical findings. Histopathological analysis showed infiltration of inflammatory cells, necrotic hepatocytes in liver tissues and glomerular degeneration kidney tissue, respectively in FCA induced arthritic rats (Group II) and Diclofenac sodium treated rats (Group III). PBB treated rats at 200 mg/kg (Group V), and 400 mg/kg (Group VI) restored the normal architecture of the liver with a very lower number of inflammatory cells. The kidney tissues represented normal glomerular structure along with convoluted tubules in PBB 200 mg/kg and PBB 400 mg/kg group of rats. Therefore, from the above study, it can be stated that the therapeutic effect of the PBB extract might be mediated through its antioxidant property.

CONCLUSION: In the present study, *Bellamya bengalensis* Lamarck has shown to possess therapeutic action on liver and kidney tissues against Freund's adjuvant-induced arthritis. The extract further enhanced the defense mechanism by enhancing antioxidant signaling pathway and thereby reducing the inflammatory state of the rats.

CONFLICT OF INTEREST: The authors have declared that there is no conflict of interest.

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