6. DISCUSSION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of diseases, although relatively modest acquaintance about their mode of action is existing. There is an emergent interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Thus, in the present investigation, an attempt was made to evaluate the anti-inflammatory and diuretic potential of *P. daemia* in experimental animal models and to find probable mechanisms of these activities.

Carrageenan-induced paw edema in rats as an *in vivo* model of inflammation has been frequently used to assess the anti-edematous effect of natural products. Carrageenan-induced paw edema is a useful model in assessing the contribution of mediators involved in vascular changes associated with acute inflammation. Edema formation in the carrageenan-induced paw edema model is a biphasic response. In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin, and bradykinin affecting vascular permeability. The inflammatory edema reached its maximum level at the third hour and after that it started declining. In our study, test drug in all doses and indomethacin showed anti-inflammatory effects in carrageenan-induced rat paw edema. A previous study indicated that the 3rd hour of the edema induced by carrageenan, in which the edema effect reached its maximum (Kirkova *et al.*, 1992), is characterized by the presence of prostaglandins and other compounds of slow reaction (Spector and Willoughb, 1963). Ueno *et al.* (2000) found that the injection of carrageenan into the rat paw induced the liberation of bradykinin, and then further induced the biosynthesis of prostaglandin and other autacoids. However, in the carrageenan-induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism (Nantel *et al.*, 1999). In the present investigation, ethanolic extract (PDE) and all the fractions of *P. daemia* showed anti-edematous activity from first hour and maximum activity at 3 hr, suggesting that the anti-inflammatory activity may be due to inhibition of mediators of the inflammation such as histamine, serotonin, and bradykinin released during the first phase of inflammation and prostaglandins and bradykinin which released during the second phase of inflammation, with maximum activity in n-
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butanol fraction (PDB; 100 and 200 mg/kg) and water fraction (PDW; 100 and 200 mg/kg).

Further, present studies indicated that inflammatory effect induced by carrageenan is consorted with free radicals. The carrageenan-induced inflammatory response has been associated to neutrophil infiltration and the production of neutrophil-derived free radicals, for instance superoxide, hydroxyl radicals and hydrogen peroxide, as well as due to the release of other neutrophil-derived mediators (Dawson et al., 1991; Lu et al., 2009). Dudhgaonkar et al. (2006) reported that free radical, prostaglandin and NO are released by carrageenan for 1–6 hours of administration. A previous study has established that MDA production is due to free radical attack in the plasma membrane (Janero, 1990). Hence, carrageenan-induced inflammation results in the accumulation of MDA (Bilici et al., 2002). In different animal models of acute inflammation, carrageenan-induced inflammation has been employed to study the free radical generation in liver tissues after inflammatory states (Lu et al., 2009). Cuzzocrea et al. (1999) suggested that endogenous glutathione, an oxyradical scavenger, plays a key role against carrageenan-induced local inflammation. The enhancement of glutathione levels reduces MDA production. In the present study PDB and PDW were investigated for their effect on MDA levels in edematous paw at 3 hr after carrageenan injection. The liver tissues were analyzed for SOD and GPx activity. In the present study, PDB (100 and 200 mg/kg), PDW (100 and 200 mg/kg) and indomethacin showed significant rise in SOD and GPx levels. Further, there was a significant fall in MDA levels with PDB and PDW pre-treatment. These results suggest that free radical scavenging properties by increasing the activity of anti-oxidant enzymes SOD and GPx are responsible for anti-inflammatory activity of *P. daemia*.

Salvemini et al. (1996) have proposed that L-arginine-NO pathway plays an important role in the carrageenan-induced inflammatory response. The results of our study reassert that carrageenan administration leads in NO production. The expression of the inducible isoform of NO synthase has been purported as a vital mediator of inflammation (Cuzzocrea et al., 1997). In the present study, we found that the level of NO was lowered significantly by treatment with 100 and 200 mg/kg PDB and PDW. This indicates that the anti-inflammatory mechanism of *P. daemia* may be through the L-arginine-NO pathway.
Cytokines are critical to the pathogenesis of inflammatory disorders. The inhibition of their production and action can provide therapeutic benefits. Previous studies have shown significant correlations among cytokine production, COX-2 protein expression and PG synthesis in the paw in which edema was induced by intraplantar injection of carrageenan (Park et al., 2004; Lu et al., 2009). TNF-α is a major mediator in inflammatory responses, inducing innate immune responses by activating T cells and macrophages and stimulating the secretion of other inflammatory cytokines (Beutler and Cerami, 1989). The production of multiple proinflammatory cytokines like TNF-α in the edematous paw was also reduced by n-butanol and water fraction of *P. daemia*. These results indicate that PDB and PDW play a role in the anti-inflammatory activities in carrageenan-induced paw edema through the inhibition of TNF-α activity.

Inflammation induced by formaldehyde is biphasic. An early neurogenic component is mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandins and bradykinin are known to be involved (Wheeler Aceto and Cowan, 1991; Tjolsen et al., 1992; Chauhan et al., 1998). In our study, we found that PDE (50 mg/kg and 100 mg/kg, p.o) and all the fractions of *P. daemia* except PDP inhibited the edema produced by formaldehyde at 48 hrs, indicating the ability of *P. daemia* to inhibit chemical mediators of inflammation.

In addition, the effect of PDE, PDP, PDEA, PDB and PDW was evaluated against proliferative phase of inflammation using carrageenan-induced air-pouch model in which tissue degradation and fibrosis occurs. During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels occurs. These are the basic sources of forming a highly vascularized reddish mass, termed as granulation tissue (Swingle, 1974; Bhattacharya et al., 1992). PDE (50 and 100 mg/kg), PDEA (100 and 200 mg/kg), PDB (100 and 200 mg/kg) and PDW (100 and 200 mg/kg) when administered orally, significantly reduced the exudation and granuloma formation. PDP had no effect on exudation and granuloma formation.

It is reported that carrageenan induces inflammation by enhancing PGE₂ release and leukocytes migration. It also enhances the expression of COX-2 in epidermis, skeletal muscle and inflammatory cells in air-pouch models, suggesting
that prostaglandin E\textsubscript{2} production is linked through the expression of COX-2 (Sedgwick and Lees, 1986; Nantel et al., 1999). PDB and PDW showed reduction of the leukocyte count in the pouch exudates in our study. A reduction in neutrophil numbers within the pouch membrane was also observed, proving that the PDB and PDW inhibited neutrophil recruitment and/or migration into the pouch membrane and not just their exit into the pouch exudate. Furthermore, our study suggests that PDB and PBW were effective in inhibition of cell migration not only by decreasing the cell migration into pouch but also via inhibition of myeloperoxidase (MPO) enzyme. This enzyme is found in azurophilic granules of neutrophils and is released by activated leucocytes in the site of inflammation (Ko et al., 2005). This study also confirms that the pre-treatment of the animals with PDB and PDW at 100 and 200 mg/kg, p.o. doses, inhibits the production of pro-inflammatory cytokine i.e. TNF-\(\alpha\) that causes changes in the endothelium of capillaries by inducing activation and recruitment of leukocytes and exudation at the site of inflammation. This is caused by the increase in the expression of ICAM-1 (Tosi et al., 1992). TNF-\(\alpha\) also has synergistic effects with IL-4 and IFN-\(\gamma\) to increase VCAM-1 expression on endothelial cells (Thornhill et al., 1991). This finding revels that inhibition of cell migration in carrageenan-induced air-pouch model on administration of PDB and PDW may be due to a down regulation of cellular adhesion molecules expression (i.e. inhibition of TNF-\(\alpha\) at the site of inflammation) which is a key factor to recruit further leukocytes. In line with these comments, present investigation has also demonstrated that PDB and PDW also inhibited the expression of inducible NO\textsubscript{ synthase} (Strestikova et al., 2001), which explains the significant reduction of NO\(\times\) at the site of inflammation. NO plays an important role in the vascular and cellular components of inflammatory responses. NO is a potent vasodilator by virtue of its actions on vascular smooth muscle (Robbin et al., 2007). This data signifies that the inhibition of exudation by PDB and PDW in carrageenan-induced air-pouch model may be mediated by reduction in NO\(\times\) production by macrophage responsible for vascular leaking.

Acute reaction is unable to eradicate proinflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation. Chronic inflammation occurs by means of the development of proliferative cells (Arrigoni-Maratellie, 1988; Dunne, 1990). These cells can be either spread or in granuloma form. In our study, the effect of PDE, PDP, PDEA, PDB and
PDW was evaluated using cotton-pellet granuloma model, which is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The inflammation consists of 3 phases, which are 1) a transudative phase, defined as the increase in the wet weight of the pellet that occurs during the first 3 h, 2) an exudative phase, defined as plasma leaking from the bloodstream around the granuloma that occurs between 3 and 72 h after the implantation of pellet, and 3) a proliferative phase, measured as the increase in the dry weight of the granuloma that occurs between 3 and 6 days after the implantation (Swingle and Shideman, 1972). Anti-inflammatory drugs like NSAIDs decrease the size of granuloma which results from cellular reaction by suppressing granulocyte infiltration, forbidding generation of collagen fibers and inhibiting mucopolysaccharides (Della Loggia et al., 1968; Alcaraz and Jimenez, 1988). Although the anti-inflammatory drugs can inhibit both the transudative and proliferative phases, NSAIDs exert only slight inhibition, whereas steroidal anti-inflammatory agents strongly inhibit both phases (Swingle and Shideman, 1972). PDE, PDEA, PDB and PDW evidenced significant anti-inflammatory activity in cotton pellet induced granuloma and as a results were found to be effective in chronic inflammatory conditions, which showed efficacy in conquering the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation. On the other hand, the PDB and PDW effect on the dry weight of the cotton pellet was almost near to that of indomethacin. These data support the hypothesis of the greater effect of the fractions on the inflammation mediators in the immediate response of inflammation in rats. This effect may be due to the cellular migration to injured sites and accumulation of collagen. Maximum effect was observed with n-butanol fraction 200 mg/kg and water fraction 200 mg/kg.

An imbalance between free radical-generating and radical-scavenging systems results in oxidative stress (Sen, 1995) and it is documented that in chronic and sub-acute inflammation, ROS plays an important role in modulating the extent of inflammatory response and consequent tissue and cell injury (Robbin et al., 2007). MDA is a metabolic product of lipid peroxidation, the level of which is raised in oxidative stress. In present study, PDB and PDW at 100 and 200 mg/kg dose demonstrated significant reduction in MDA level in plasma, indicating reduction of oxidative stress by anti-lipoperoxidative activity, which might be the proposed
mechanism of anti-inflammatory action of PDB and PDW in the chronic inflammation model.

The present study was carried out to see the efficiency of *P. daemia* against arthritis. Arthritis is a chronic inflammatory disorder and the inflammation involves the release of mediators like cytokines (IL-IB and TNF-α), interferons and PGDF, which are responsible for the pain, destruction of bone and cartilage that can lead to severe disability. In the present study, rats were selected to induce arthritis since they explicate a chronic swelling in multiple joints with an influence of inflammatory cells and espoused by erosion of cartilage in joints and destruction of bones (Eric and Lawrence, 1996).

In AIA, inflammation is generally produced by accumulation of fluid surround joints. Inflammation is characterized by paw swelling, which is one of the physical markers of inflammation. Complete Freund’s adjuvant (CFA)-injected animals showed acute inflammation on the 3rd day after adjuvant injection, followed by subsequent chronic polyarthritis till the 21st day. Joint swelling in adjuvant induced arthritis occurs in two phase, the first phase being associated to non immune inflammation and is considered to be primary response, while the second phase begins after two weeks, which depends on the immune response, particularly a cell-mediated one and is referred to as secondary response (Vogel, 2002). We observed a significant reduction of swelling in adjuvant injected rat paws and thereby joints, starting from day 3 and throughout the experiment, indicating both the anti-inflammatory and the immune response attenuating properties of all the extract tested except PDP. Further, in present study we found that inhibition of primary lesions was maximum with PDB (200 mg/kg) followed by PDW (200 mg/kg).

Changes in the body weight of rats were also evaluated for assessing the course of the disease and the response to therapy of anti-inflammatory drugs. Reduction in body weight is normally observed in most of the autoimmune disorders, probably due to inflammatory cytokine, pain, loss of appetite, increased energy expenditure and enhanced protein catabolism (Argiles and Lopez-Soriano, 2002; Rall and Roubenoff, 2004; Shelton et al., 2005). As the incidence and severity of arthritis increase, the changes in the body weights of the rats also occur during the course of the experimental period (Winder et al., 1969). Previous findings suggest that absorption of $^{14}$C- glucose and $^{14}$C-leucine in rat’s intestine was reduced in the case of
Phytopharmacological action of *Pergularia daemia* with special reference to its actions and mechanism of action as diuretic and anti-inflammatory agent

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inflamed rats (Somasundaran et al., 1983) but on the treatment with anti-inflammatory drugs, the decrease in absorption is neutralized. It shows that the anti-inflammatory drugs correct the decreased/deranged absorption capacity of intestine during inflammation. In our study, the increased body weight during treatment with prednisolone, PDB and PDW may be due to the restoration of absorption capacity of intestine. From our results, it can be concluded that the n-butanol and water fractions of *P. daemia* possess potentially useful antiarthritic activity since they give a positive result in controlling inflammation in adjuvant-induced arthritis in rats.

CFA-induced polyarthritis is associated with an immune-mediated inflammatory reaction and the rat is unique in developing polyarthritis after CFA treatment (Cai et al., 2006). The initial reaction of edema and soft-tissue thickening at the depot site in this model is caused by the irritant effect of the adjuvant, whereas the late-phase arthritis and flare in the injected foot are presumed to be immunologic events (Ward and Cloud, 1965). The appearance of secondary lesions, i.e. non-injected paw swelling is a manifestation of cell-mediated immunity. The suppression of such secondary lesions by a drug shows its immunosuppressive activity (Singh et al., 2003; Bani et al., 2007). As the disease progresses, inflammation disseminates to other organ like heart, liver and lungs and is identified by extra-articular characters like formation of rheumatoid nodules on extensor surface of elbows, forearms, and hands. The nodules may also be developed in lungs. Connective tissue and blood vessels may also be damaged (Habermann and Cascino, 2006). Animals sensitized with CFA produce inflammation on hind paw and fore arm, show formation of nodules on ear and tail, swelling of connective tissue on nose and other organs are also affected. According to severity, score is given (Lin et al., 2007; Zhang et al., 2009; Vogel, 2002). An arthritic index for each animal is calculated as the sum of these scores. In our study, the average scores for each group of drug treated animals were compared with that of disease control animals. In disease control group, arthritic Index was significantly higher compared to normal control group while prednisolone, PDB and PDW treated groups showed significantly less score as compared to model control group. This indicates the protective effect and immunosuppressant properties of test drugs against adjuvant-induced arthritis.

Rheumatoid factor (RF) is an autoantibody directed against the Fc region of human IgG. Deposits of RF linked with IgG occur in various tissues like the
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synovium or joints, and interfere with the normal function of the joint and promote local inflammation, resulting in tissue damages and sometimes damage of blood vessels in the affected area (Goldbach et al., 2002). RA factor is observed positively in 80% of rheumatoid arthritis patients and is mainly increased in diffuse collagen disease (Kim, 1984). The RF test is used as a diagnostic marker for rheumatoid arthritis, since its presence is associated with an increased risk of developing RA in people with mild arthritic symptoms. Higher levels are detected in more severe forms of the disease, a condition that is a severe prognostic factor for patients (Jones et al., 1990; Nell et al., 2005). CFA-induced arthritis in rats is associated with an increase in the plasma levels of RF (Nielen et al., 2006). In the present investigation, significant rise in RF level was observed in disease control animals as compared to normal control, which was counteracted by prednisolone, PDB (100 and 200 mg/kg) and PDW (100 and 200 mg/kg). Since administration of PDB and PDW decreases serum agglutination in RA-tests, these may be effective in rheumatoid arthritis induced by self-immunological reaction.

The acute phase reactants are a class of serum protein, mainly glycoprotein whose concentration in the blood increases after various stimuli such as trauma or inflammation (Soothill et al., 1967). The magnitude of the acute phase protein response is roughly proportional to severity of the stimulus (Aronsen et al., 1972; Koj et al., 1974). Measurement of these proteins can therefore be used, like the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), which is largely a measure of fibrinogen (Hardwicke et al., 1952), to monitor the progress of an inflammatory disorder.

C-reactive protein is an acute phase protein present in normal serum that is produced by the liver during an inflammatory reaction. Since C-reactive protein levels in the blood rise more quickly after the inflammatory or infective process begins, due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages (Pepys MB et al., 2003) as well as adipocytes (Lau DC et al., 2005). CRP factor is a diagnostic index of bacterial infection, chronic rheumatoid arthritis, suppurative arthritis, gout, malignant tumor and rheumatoid fever (Kim, 1984). CRP binds to phosphocholine on microbes, which is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages, which express a receptor for CRP. A CRP factor is also believed to play another
important role in innate immunity, as an early defense system against infections. Present study revealed significant elevation in CRP levels in disease control animals as compared to normal control group while treatment with prednisolone, PDB (100 and 200 mg/kg) and PDW (100 and 200 mg/kg) significantly lowered CRP levels as compared to disease control group. This indicates that test drugs have protective effect in immunity and defense system.

Erythrocyte Sedimentation Rate (ESR) is an estimate of the suspension stability of RBCs in plasma. It is related to the number and size of the red cells and to the relative concentration of plasma proteins, especially fibrinogen and alpha and beta globulins. Increase in the rate is an indication of active but obscure disease processes. ESR is an indirect measurement of acute phase response for determining the disease activity in rheumatoid arthritis (Dervieux et al., 2003). Although CRP is a better marker for inflammation and though ESR is influenced by several factors such as the plasma concentration of fibrinogen, immunoglobulin, RF and Hb, the increased level of ESR in arthritic rats adds information reflecting the chronicity and severity of the disease better than CRP (Rajendran et al., 2008). Hence, a combination of the tests might be worthwhile. The basic principle of the ESR is that when anti-coagulated blood is placed in a vertical column, the RBCs normally settle quite slowly. This occurs for two main reasons: (1) RBCs repel each other due to the negative charges on their surfaces, or zeta potential, and (2) the large surface area to volume ratio of normal RBCs resists settling. When an inflammatory disease is there, high proportion of fibrinogens are present in blood. Fibrinogen’s positive charge decreases the RBCs’ zeta potential, leading to red blood cells to stick to each other and increased rate of rouleaux formation and hence increased ESR. An increase in the ESR is attributed to the accelerated formation of endogenous proteins such as fibrinogen and α/β globulin. The ESR count in the present study significantly increased in arthritic control group, whereas these counts were remarkably counteracted in the prednisolone, PDB and PDW treated groups and thus justifying their significant role in the arthritic conditions (William, 1996).

In autoimmune disease, there is increase in globulin concentration to generate more antibodies against autoantibody. In AIA, decreases in total serum protein and albumin levels have been reported (Lowe, 1964; Fahim et al., 1995). In AIA, changes in plasma protein levels with an increase in the globulin fraction and decrease in
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albumin fraction have been well documented (Cawthorne et al., 1976). These biochemical abnormalities result from a more basic liver malfunction (Lorber, 1975). General reduction of liver protein synthesis can be assessed by measuring albumin levels, because levels of this protein are lowered during inflammation and further, it has also been reported that albumin synthesis is reduced by IL-1 (Lewis et al., 1998). Also, the mediators released such as histamine, bradykinin and prostaglandin D during inflammation increase the permeability of vascular tissues to albumin leading to reduction in its serum levels (Lowe, 1964; Kohn and Barchet, 1976). In our investigation, we found that the decrease in AG ratio in disease control group was counteracting by the test drugs.

In arthritic condition, there is a mild to moderate rise in WBC count due to inflammatory response by released IL-1B. IL-1B increases the production of both granulocytes and macrophages colony stimulating factor (Eric and Lawrence, 1996; William, 1996). The present study shows that PDB and PDW treatments tend to normalize the WBC count and suppresses the migration of leucocytes into the inflamed area. In addition, other characteristic hematological alteration such as the decreased Hb count (Singh et al., 2003) was also restored by the n-butanol and water fraction of *P. daemia*. We propose that the reduction in the Hb count during arthritis results from reduction in erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells. Thus, increase in the Hb count brought about by test treatment further supports the anti-arthritic effect of PDB and PDW.

Arthritis is an inflammatory condition of the bone joints, associated with hyperalgesia and functional impairment. The hyperalgesia in arthritis is mediated by prostaglandins and other endogenous mediators (Schaible et al., 2006). In our investigation, the effect of PDB (100 mg/kg and 200 mg/kg) and PDW (100 mg/kg and 200 mg/kg) on arthritis-induced hyperalgesia was evaluated by visually attributed arthritic scores and the dorsal flexion pain test. Both the fraction and prednisolone effectively increased the pain threshold and reduced the flexion pain test score (Table 5.29). Further, the mobility and stance scores, which are commonly used to assess the functional impairment in arthritis, were determined. PDB and PDW treatment in both doses lowered the mobility score and improved the stance score, indicating a reduction in pain (Table 529). Though actual quantification of the mediators of pain
was not performed in this study, it is proposed that test drug significantly affects the levels/effects of such endogenous pain mediators (Kumar et al., 2006).

Earlier studies of diuretic agents have ascertained it is beneficial to pre-treat or “prime” the test animal with assorted fluids. The administration of saline has been found to be requisite to produce a graded response in the male rat with escalating dosage of aminophylline (McColl et al., 1956). As diuretics are utilized clinically in the management/treatment of edema, they would appear to be most imperative to prove efficiency in the presence of electrolyte and water. In the present investigation, we examined the diuretic potential of *P. daemia* in rats primed with saline. The results showed that all the extracts, except petroleum ether extract, increased urine output, which is expressed as the percentage of saline load excreted, as compared to control group (Table 5.31). The diuretic action of furosemide, PDE, PDP, PDEA, PDB and PDW was found to be 2.19, 2.04, 1.09, 1.66, 1.73 and 1.85 respectively when compared with 1 of control group (Table 5.31). Diuretic activity of alcoholic extract was found to be highest 0.93 followed by 0.79 of n-butanol (PDB) and 0.85 of water fraction (PDW). Further, we studied PDB and PDW for evaluation of probable mechanism as diuretic agent.

Diuretics have two separate connotations: increased urinary output *per se* and net loss of solute and water. These two processes are involved in the inhibition of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into the blood stream and as a consequence, promote the formation of urine (Krishna and Agrawal, 2006). The results of present study show that PDB and PDW at a dose of 200 and 400 mg/kg respectively administered orally produced an increase in urinary excretion and urinary sodium loss but no effect on urinary potassium as compared to control and standard drug treated groups.

Some herbal diuretics induce diuresis by stimulating the thirst center in the hypothalamus and thereby enhancing the fluid intake (Neuman, 2002). Such a mode of action is unlikely to be operative with PDB and PDW since the rats had no access to fluid intake during the 5 h experimental period. In addition, earlier studies revealed that diuretic drug causes increase in GFR due to either a direct effect on arterial pressure or glomerular blood flow (Bevevino et al., 1994) or by decreasing renal perfusion pressure (Bevenino and Mello Aires, 1994; Jouad et al., 2001) produces diuretic action, indicated by increase in GFR. Such a mode of action is unlikely to be
operative with PDB and PDW since administration of the PDB and PDW caused the
diuretic response, without affecting GFR.

The PDB and PDW-induced diuresis was significant with raised urinary Na+
levels and was not accompanied with a reduction in urinary K+ levels. In addition,
there was no alkalinization of urine and without alteration in Na+/K+ ratio i.e.
aldosterone secretion index. These data indicate that the drugs/fractions are not acting
as potassium sparing diuretics (Kreydiyyeh and Julnar, 2002; Rang et al., 2003;
Prakash et al., 2008). The PDB and PDW were also unlikely to be acting as thiazide
diuretics: they only increase urinary K+ level and alter the urinary Na+/K+ ratio (Rang
et al., 2003). But in this study, both urinary Na+ and K+ level were increased without
any alteration in Na+/K+ ratio. The ratio Cl−/Na+ + K+ (ion quotient) is calculated to
estimate carbonic anhydrase inhibition. Carbonic anhydrase inhibition can be
excluded at ratios between 1.0 and 0.8. With decreasing ratios, slight to strong
carbonic anhydrase inhibition can be assumed (Vogel, 2002). The present study
indicates PDB and PDW at dose 200 and 400 mg/kg had no significant effect on Cl−/
Na+ + K+ (index for carbonic anhydrase inhibitory activity), excluding carbonic
anhydrase inhibitory activity of both the fractions.

In this study, marked natriuresis (in terms of increased urinary Na+ level and
sodium saluretic index) was evident possibly because of the inhibition of Na+ reabsorption in the nephron (Ratnasooriya et al., 2009), thereby increasing the urinary
output. The diuresis induced by n-butanol and water fractions of P. daemia, was
similar to that of furosemide and was accompanied by marked increases in both
urinary Na+ and K+ levels. Further, the urine was slightly acidified (Table 5.30).
These characteristics strongly suggest these extracts are acting as loop diuretic. Loop
diuretics inhibit the Na+/K+/Cl− co-transporter system in the thick ascending loop of
nephron, thereby increasing natriuresis and kaleuresis (Rang et al., 2003; Kreydiyyeh
and Julnar, 2002) and also cause acidification of urine (Rang et al., 2003; Osorio,
1997).

Since the conductivity value is an indirect measure of the electrolyte
concentration in the urine, in the present study PDB and PDW at 400 mg/kg showed
increase in conductivity suggesting the diuretic effect of the PDB and PDW to be
saluretic rather than aquaretic, the latter being the typical effect of diuretics of plant
origin. The PDB and PDW did not affect plasma urea levels and hematocrit which
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indicates that the rapid physiological regulation of these important parameters was not altered after PDB and PDW treatment. Collectively, these observations indicate that the n-butanol and water fractions of *P. daemia* possesses oral diuretic activity, which is mediated via inhibition of the Na⁺/K⁺/Cl⁻ co-transporter system i.e. loop diuretic-like action.

AA metabolites, also called eicosanoids, are synthesized by two major classes of enzymes: cyclooxygenases (prostaglandins and thromboxanes) and lipoxygenases (leukotrienes and lipoxins). Eicosanoids bind to G protein-coupled receptors on many cell types and can mediate virtually every step of inflammation. They can be found in inflammatory exudates, and their synthesis is increased at sites of inflammation (Robbins et al., 2007). In the present study we evaluated the PDB and PDW against both enzymes using in vivo ulcerogenic activity for cyclooxygenase activity and estimation of lipoxygenase for activity of this enzyme.

Metabolism of AA by COX leads to the generation of PGs, causing vasodilation which increases the permeability of post capillary venules, thus potentiating edema formation. On the other hand, PGs serve a homeostatic function like cytoprotection in the gastrointestinal tract (Robbins et al., 2007). In our study, we found that PDW (100 and 200 mg/kg) showed gastrointestinal irritation with increase in ulcer incidence and increase in lesion area in rats which is typical of anti-inflammatory prostaglandin inhibitors such as the non-steroidal anti-inflammatory drugs NSAIDs (Rang et al., 2006). PDB exhibited negligible ulcerogenic activity with ulcer incidence of 1/5 (100 and 200 mg/kg), indicating inhibition of prostaglandins synthesis by PDB.

The LOX pathway utilizes AA to produce LTs, including the leukocyte chemoattractant LTB₄. Owing to the contribution of LTs to the pathogenesis of many inflammatory processes like increasing vascular permeability, chemotaxis and leukocyte adhesion. They also represent an important target for therapeutic regulation (Piper, 1984; Robbins et al., 2007). Lipoxygenase is known to catalyze the oxidation of unsaturated fatty acids containing 1-4 diene structures. The conversion of linoleic acid to 13-hydroperoxy linoleic acid is followed spectrophotometrically by the appearance of a conjugate diene at 234 nm on a UV/visible spectrophotometer. Interestingly, we found that PDB fraction showed a remarkable and significant inhibitory activity against lipoxygenase (IC₅₀ = 572.6 μg/ml), while IC₅₀ for PDW
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was found to be more than 1000 μg/ml. We thus propose that the anti-inflammatory effect by shown by through PDB might be due to inhibition of lipooxygenase enzyme.

Leukocyte aggregation at the site of inflammation is a fundamental event in the inflammatory process. Intraperitoneal injection of carrageenan leads to inflammation of the peritoneum consequential from macrophages in the carrageenan insulated tissue. It is followed by influx of neutrophils and mononuclear leukocytes, with increased levels of plasma/peritoneal fluid chemoattractants and with a sequential appearance of exudate proinflammatory cytokines. Interleukin-1, a pro-inflammatory cytokine, induces accumulation of polymorphonuclear cells by a variety of processes including adhesion and cell mobility (Meade et al., 1996). In our investigation, we compared the effect of the PDB and PDW at the doses of 100 and 200 mg/kg with indomethacin on the cell migration, in peritonitis model. Both the fraction of *P. daemia* inhibited the carrageenan induced leukocyte migration in peritonitis in mice. PDB and PDW significantly reduced the migration of neutrophils, indicating their anti-inflammatory action by inhibiting chemotaxis.

Since inflammation is characterized by increase in vascular permeability and exudation, we further studied capillary permeability using Evans Blue dye. The inflammatory response is also a physiological characteristic of vascularized tissues (Kang et al., 2008). Histamine (released from mast cells and basophils), serotonin, and to some extent bradykinin are responsible for increased capillary permeability. It is an important step as this enhances the migration of neutrophils and other immune cells, such as macrophages and leukocytes, to the inflamed area. Increased vascular permeability leads to exudation of the fluid, which is a consequence of increased vascular permeability and is considered to be a major feature of acute inflammation. Increased vascular permeability occurs as a result of contraction and separation of endothelial cells at their boundaries to expose the basement membrane, which is freely permeable to plasma proteins and fluid (Brown and Roberts, 2001). Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Acetic acid-induced capillary permeability increase in mice is a commonly used vascular permeability assay (Winter et al., 1962). In the present investigation, PDB and PDW produced a significant decrease in vascular permeability at doses of 100 and 200 mg/kg as indicated by decreased concentration of dye in peritoneal fluid, demonstrating that test drugs have the ability to inhibit the
permeability of small blood vessels in the process of acute inflammation. On the other hand, PDB and PDW in at both the dose level showed protection against clonidine-induced mast cell degranulation (Vyas and Vyas, 2009). Mast cells are found resident in tissues throughout the body, virtually in all vascularized types of tissues (Mekori and Metcalfe, 2000). Mast cells generate and store a large number of potent proinflammatory mediators that play a critical role in immune surveillance (Galli, 1993). Activated mast cell secrete a wide variety of preformed and neo-synthesized inflammatory mediators such as histamine, serotonin, leukotrienes, prostaglandins, heparin, tryptase and many cytokines like TNF-α which are observed in settings of acute and/or chronic inflammation e.g., vasodilatation, plasma extravasation and the recruitment and activation of granulocytes (Rattmann et al., 2008; Galli et al., 2005). Mast cell degranulation can be elicited by number substances, collectively known as the basic secretagogues of mast cells (Lagunoff et al., 1983). The most potent secretagogues include the synthetic compound 48/80 and clonidine (Vyas and Vyas, 2009), stimulates only certain subtypes of mast cells, such as rat peritoneal mast cells, to induce the release of inflammatory mediators via phospholipase D and heterotrimeric GTP-binding proteins (Vyas and Vyas, 2009). This effect of PDB and PDW on mast cell degranulation was suggested to be involved in its anti-edema effect by decreasing capillary permeability. Further our findings suggest that the anti-inflammatory effect of P. daemia in various models, like carrageenan induced paw oedema, carrageenan air pouch granuloma and adjuvant induced arthritis is due to its ability to decrease capillary permeability which results in decrease in fluid exudation.

It is possible that PDB and PDW inhibits the release of mediators like histamine (mast cell protective effect), 5-HT, prostaglandins, bradykinin, leukotrienes and PAF which play a significant role in increasing vascular permeability (Spector and Willoughby, 1963; Wilhelm, 1971; Humphery et al., 1984; Joris et al., 1987).

The vitality of cells depends on the integrity of their membranes (Ferrali et al., 1992). Exposure of red blood cell to ruinous substances such as hypotonic medium and phenylhydrazine results in lysis of its membrane accompanied by haemolysis and oxidation of hemoglobin (Augusto et al., 1982; Ferrali et al., 1992). The hemolytic effect of hypotonic solution is associated to excessive accumulation of fluid inside the cell, consequential in the rupturing of its membrane. Additionally, such injury to RBC membrane will make the cell more prone to secondary damage through free radical -
Phytopharmacological action of *Pergularia daemia* with special reference to its actions and mechanism of action as diuretic and anti-inflammatory agent

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induced lipid peroxidation (Augusto et al., 1982; Ferrali et al., 1992). This notion is in agreement with previous findings that the breakdown of bio-membranes leads to the formation of free radicals which in turn enhance cellular damage (Halliwell et al., 1988; Maxwell, 1995). The progression of bone destruction seen in rheumatoid patients for example, has been shown to be due to increased free radical activity (Cotran et al., 1999; Pattison et al., 2004). It is consequently anticipated that compounds with membrane-stabilizing properties should provide substantial protection to cell membrane against injurious substances (Liu et al., 1992; Maxwell, 1995; Perez et al., 1996; Shinde et al., 1999).

The early phase of inflammatory reactions, specifically the prevention of the release of phospholipase that triggers the formation of inflammatory mediators (Aitadafoun et al., 1996), is prevented by compounds with membrane-stabilizing properties. In our study, n-butanol and water fraction of *P. daemia* did not exhibit significant membrane stabilizing property. Thus, it could be inferred that the anti-inflammatory activity observed in present study, may be related to the inhibition of the late phase of inflammatory events, that is, the release of chemical mediators.

Neutrophils have been implicated in the pathogenesis of many inflammatory lung diseases (Ariga et al., 2004). The recruitment of leukocytes to sites of injury and infection is a multistep process involving attachment of circulating leukocytes (neutrophils) to endothelial cells and their migration through the endothelium. The first event is the induction of adhesion molecules on endothelial cells. TNF and IL-1 act on the endothelial cells of post capillary venules adjacent to the infection and induce the expression of several adhesion molecules, mainly VCAM-1 and ICAM-1. The next step in the process is migration of the leukocytes through the endothelium, called transmigration or diapedesis, providing opsonins (which assist in phagocytosis) and causing dilution of toxins (Robbins et al., 2007). Neutrophil adhesion to post-capillary venules is a critical first step in the inflammatory process. This has led to the development of the concept that inhibition of neutrophil-endothelial cell adhesion may represent a novel therapeutic strategy for the prevention of leukocyte-dependent injury in airway inflammation and asthma (Korthuis et al., 1994). Decrease in neutrophil adhesion to nylon fibers by drug indicates decrease in migration of neutrophils to the site of inflammation (Shinde et al., 1999). In our study, we observed significant reduction in adhesion of neutrophils to nylon fibers with PDB.
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(200 mg/kg) and PDW (200 mg/kg), indicating that test drugs possess anti-inflammatory action; they reduce the number of neutrophils at the site of inflammation thus decreasing their phagocytic action and the release of various enzymes and mediators that make inflammation worse.

Reactive Oxygen species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, arthritis and also the aging process (Guyton et al., 1997, Halliwell and Gutteridge, 1999). Currently, there is significant evidence that certain types of inflammatory tissue injury are mediated by ROS (Conner and Grisham, 1996). Oxidants such as superoxide and hydroxyl radicals, hydrogen peroxide and hypochlorous acid are formed at sites of inflammation, and appear to contribute to the tissue damage in some acute and chronic inflammatory diseases. Studies have implicated the oxygen free radicals in the process of inflammation and phyto-constituents may block the cascade process of arachidonic acid metabolism, and may serve as a scavenger of reactive free radicals which are produced during arachidonic acid metabolism (Sreejayan and Rao, 1996; Trouillas et al., 2003) and It has been suggested that many anti-inflammatory drugs might exert some of their effects by scavenging oxidants, and decreasing formation of ROS by activated phagocyte (Arouma, et al., 1996). In present study, to assess their antioxidant potential of PDB and PDW were evaluated using various in vitro antioxidant activities.

The free radical scavenging activity of the PDB and PDW was evaluated based on their ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet colour). As the electron becomes paired in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with respect to the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge free radicals independently. In present study, we found that both the fractions of P. daemia showed scavenging activity against DPPH with significant activity shown of PDW followed by PDB. The antioxidant activities of PDB and PDW may probably be due to the presence of compounds with hydroxyl group.
Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess of NO to generate nitrite and peroxynitrite anions, which act as free radicals (Ganesh et al., 2004). Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine is used as the marker for NO scavenging activity (Mukherjee, 1989). The chromophore formation was not complete in the presence of P. daemia fractions (PDB and PDW), which scavenge the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentrations of the PDB and PDW increase in a dose dependent manner.

It is well known that superoxide anions damage biomolecules directly or indirectly by forming H$_2$O$_2$, 'OH, peroxynitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation (Yen and Duh, 1994). Superoxides are produced from molecular oxygen due to oxidative enzymes (Sainani et al., 1997) and via non-enzymatic reaction such as autoxidation by catecholamines (Hemmani and Parihar, 1998). In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Both the fractions of P. daemia were found to possess the scavenging effect on superoxide anions, in concentration dependent manner. However, the activity was less than that of BHT. The probable mechanism of scavenging the super oxide anions may be due to the inhibitory effect of PDB and PDW towards generation of superoxides in the in vitro reaction mixture.

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly, and once inside the cell, H$_2$O$_2$ probably reacts with Fe$^{2+}$, and possibly Cu$^{2+}$ ions to form hydroxyl radical which may be the origin of many of its toxic effects (Miller et al., 1993). Hydroxyl radical is highly reactive oxygen centered radical formed from the reaction of various hydroperoxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acid in membranes, and most
biological molecule it contacts (Aruoma et al., 1999) and is known to be capable of abstracting hydrogen atoms from membrane lipids (Yen and Duh, 1994) and brings about peroxidic reaction of lipids. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The PDB and PDW scavenged H\(_2\)O\(_2\) and this may be attributed to the presence of function groups, which could donate electrons thereby neutralizing it by converting into water.

For the measurements of the reducing ability, the Fe\(^{3+}\)–Fe\(^{2+}\) transformation was investigated in the presence of PDB and PDW. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). The reducing ability of a compound generally depends on the presence of reductants which exhibit antioxidative potential by breaking the free radical chain, donating a hydrogen atom. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim et al., 2000). The presence of reductants (i.e. antioxidants) in PDB and PDW causes the reduction of the Fe\(^{3+}\)/ferricyanide complex to the ferrous form. Similar to the antioxidant activity, the reducing power of PDB and PDW increased with increasing dosage. The result shows that both the fractions consist of compounds that cause the greater reducing power.

Phytochemical analysis of *P. daemia* showed presence of number of phytoconstituents viz. alkaloids, phytosterols, terpenoids, glycosides, flavonoids, phenolic acids, saponins and sugars. In pharmacological evaluation the n-butanol (PDB) and water fraction (PDW) of *P. daemia* showed potent activity as compared to other fractions. Phytochemical analysis of PDB showed presence of steroids and triterpenoids like α-amyrin, β-amyrin, lupeol, β-sitosterol, stigmasterol and ursolic acid (Anjaneyulu et al., 1998; Seshadri and Vydeeswaran, 1971), while PDW showed presence of alkaloids, flavonoids and phenolic compounds (Sinha and Dogra, 1985). UV spectrum provides a useful means of detecting conjugated unsaturated chromophores within a molecule such as polyenes, α-, β-unsaturated ketones and aromatic compounds. Within particular families of compounds, the position of maximum absorption can reflect the degree of substitution of the chromophore. UV absorption is associated with the chromophore and not the whole molecule, thus it
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would not distinguish between the compounds which often co-occur. The spectrum may be caused by a summation of chromophores from different parts of a polyfunctional molecule and hence it will only provide a rough estimate of the contents (Agrawal and Paridhavi, 2007). In the present study, UV – visible spectrophotometric analysis revealed peaks in the region 240 to 280 nm which probably were due to presence of flavonoid and some of the phenolic class of compounds. This data supports the qualitative phytochemical analysis of water fraction of *P. daemia* which confirmed the presence of phenolics compounds and flavonoids.

Triterpenoids have been studied for their anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory and tonic effects. They are used in the prevention and treatment of hepatitis, parasitic and protozoal infections and above all, for their cytostatic effects (Petr et al., 2006). The anti-inflammatory effects of triterpenoids are largely ascribed to their ability to inhibit arachidonate 5-lipoxygenase (5-LO) and human leukocyte elastase (HLE) as well as their potential for modulating the immune response by affecting complement and antibody production. 5-LO is a pivotal enzyme in the synthesis of leukotrienes, signal molecules in disorders that are distinguished by the inflammatory response and hypersensitivity, such as asthma, arthritis, ulcerative colitis, Crohn’s disease and disorders of the cardiovascular system *e.g.* shock and ischemia of the myocardium (Wasserman, 1988). The complement is another system which may be influenced by triterpenoids (Kapil and Sharma, 1995). Lupeol, betulin, uvaol, α-amyrin, ursolic acid, 19a-hydroxyursolic acid and 19α, 24-dihydroxyursolic acid shows suppressive effects on the induction of ROS. It is commonly known that production of ROS by neutrophils closely correlates to various inflammatory conditions, especially in dermatology (Geetha and Varalakshmi, 1998; Chen et al., 2002; Yamashita et al., 2002). Lowering the production of prostaglandin PGE2 by inhibition of the activity of cyclooxygenase-2 (COX2) is another therapeutic approach to suppression of the inflammatory response by triterpenoids. This inhibitory effect is accomplished by the triterpenoid glycoside (Ringbom et al., 1998; Kim et al., 2001). Geetha and Varalakshmi (1998) reported that lupeol and lupeol linoleate, whose spectrum of anti-inflammatory activity appears to be different from classical NSAIDs, with the distinct
advantage of its freedom from gastric ulcerogenic effects (Albiero et al., 2002). Lupeol and its derivatives possess antihyperoxaluric and antihypercalciuric activity (Anand et al., 1995). Another factor, which plays a part in the inflammatory response, is increased activity of HLE, which can be part of the pathogenesis of pulmonary emphysema, acute respiratory distress syndrome, glomerulonephritis and rheumatoid arthritis (Bernstein et al., 1994). Ursolic acid produces the inhibition of HLE, which may account for its broad anti-inflammatory, antiarthritic and antiulcerous properties. (Gupta et al., 1981; Hirota et al., 1990). α, β-amyrin modulate acute periodontal inflammation by reducing neutrophil infiltration, oxidative stress and the production of proinflammatory cytokine TNF-α (Pinto et al., 2008).

Flavonoids, also known as nature’s tender drugs, possess various biological/pharmacological activities including anticancer, antimicrobial, antiviral, anti-inflammatory, immunomodulatory, and antithrombotic activities (Havsteen, 1983) Many investigations have proven that varieties of flavonoid molecules possess anti-inflammatory activity on various animal models of inflammation. Especially, some flavonoids have been reported to inhibit chronic inflammation of several experimental animal models (Kim et al., 2004). Flavonoids show in vitro as well as in vivo anti-inflammatory activity. Several cellular action mechanisms are proposed to explain their anti-inflammatory activity like antioxidative activity and inhibition eicosanoid generating enzymes. Certain flavonoids, mainly flavone derivatives, modulate the expression of proinflammatory molecules via inhibition of transcription factor activation (Kim et al., 2004). Flavonoids probably have multiple cellular mechanisms acting on multiple sites of cellular machinery, but the most important contributors to anti-inflammation by flavonoids seem to be the effect on eicosanoids generating enzymes and the effect on the expression of proinflammatory molecules. Flavonoids have different action mechanisms depending on their chemical structures. The important moieties are the C-2, 3- double bond, A-ring 5, 7-hydroxyl groups, and B-ring 4′- or 3′, 4′-hydroxyl groups. The C-3 hydroxyl group as in flavonols is favorable for LOX inhibition and oral anti-inflammatory activity. Flavones (without C-3-hydroxyl group) more strongly down-regulate proinflammatory gene expression. Flavonoids having these chemical structures are apigenin, luteolin, kaempferol, and quercetin. The C-6 or C-8 substituted flavones /flavonols such as baicalein and wogonin are also favorable structures. While these flavonoids may not be suitable for
acute disorders, they have potentials to treat chronic inflammatory disorders due to unique cellular action mechanisms with less adverse effects. Particularly, several phenylated flavonoids show higher activity among the flavonoids examined. They possess potent inhibitory activity against COXs and 5-LOX. Some of them down-regulate proinflamatory gene expression (Kim et al., 1993; Lee et al., 1993; Kim et al., 2004).

In conclusion, present finding demonstrate that *P. daemia* exhibits anti-inflammatory and diuretic activity. Among the various fractions, the n-butanol (PDB) and water (PDW) fraction with 100 and 200 mg/kg have maximum anti-inflammatory activity, while petroleum ether fraction of *P. daemia* is only effective in acute inflammatory animal model and not effective in chronic inflammatory animal model. Anti-inflammatory effect of PDB and PDW associated with their ability to reduce the amount of AA transformed to PGs and LTs by suppressing TNFα level, cleaning away free radicals by increasing the activity of anti-oxidant enzymes, such as SOD and GPx, suppressing the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation, inhibiting chemotaxis, inhibiting mediators leading to inhibition of vascular permeability, by reducing the number of neutrophils at site of inflammation, immunological properties and antioxidative activity. Further, study also signifies that anti-inflammatory activity of PDB is attributed to its ability to inhibit 5-LO, which might be owing to presence of triterpenoids like α, β-amyrin, lupeol, β-sitosterol, β-stigmasterol and ursolic acid, while PDW caused gastrointestinal irritation typical of anti-inflammatory prostaglandin inhibitors (COX inhibition) such as NASIDs, which ascribe to presence of flavonoids and phenolic compounds. Between different fractions, PDB and PDW as maximum oral diuretic activity at 200 and 400 mg/kg. This is mediated via increased urinary Na⁺ output and through loop diuretic action i.e. inhibition of the Na⁺/K⁺/Cl⁻ co-transporter system. This study also serves as a possible rationale for the use of *P. daemia* in traditional medicine for inflammatory conditions.