

## POTENTIATING EFFECT OF 1, 2 - DIAZOLE A PLANT ALKALOID ON CARRAGEENAN AND FORMALIN INDUCED PAW EDEMA IN EXPERIMENTAL MICE

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### ABSTRACT

Inflammation is a response to acute tissue damage due to physical injury, infection, exposure to toxins and ischemic injury. The persistent inflammatory responses in or around developing neoplasm regulate many aspects of tumor progression. Natural plant based alkaloids are often have greater pharmacological effects and are used as medications as local anesthetic, stimulant, analgesic, anti-bacterial, anticancer, anti-arrhythmia, anti-asthma and anti-malarial drug. In the present study, the anti-inflammation activity of 1,2 diazole (pyrazole) a plant alkaloid was examined. In the carrageenan induced mice paw edema model, 1,2 diazole (pyrazole) a plant alkaloid showed significant inhibitory effect on the edema formation. Inflammation induced by formalin results from cell damage, which provokes the production of endogenous mediators such as, histamine, serotonin, prostaglandins and bradykinin. It is then be known from these results that 1,2 diazole (pyrazole) interfere with these inflammatory mediators. Moreover, the high presence of this compound in natural marine mangrove *Rhizophora apiculata* (*R.apiculata*) plant extract added advantage as natural source so that this alkaloid can be easily available and with efficient and potential pharmacological properties to treat variety of diseases without any adverse effect.

**Keywords:** Anti-inflammation, *Rhizophora apiculata*, Alkaloids, 1,2-diazole, Carrageenan, formalin, Paw edema.

### INTRODUCTION

Inflammation is defined as a primary defense mechanism that helps the body to protect itself against allergens, infection, burns and toxic chemicals<sup>1</sup>. Immune cells are invited to the site through the blood stream and the blood vessels near the site will become warm and red due to the increased blood flow. But sometimes inflammation may be act as etiologic factor for all degenerative disease. It is a major response of the immune system to tissue damage and infection, though in most cases it doesn't lead to inflammation. Recent studies reported that unchecked inflammations could lead to cancer development<sup>2</sup>. Inflammation can be acute or chronic where acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. Chronic inflammation is defined as a progressive shift in the type of cells present at the site of the inflammation and it is characterized by simultaneous destruction and recovery of the tissue from the inflammatory process. Acute inflammation has therapeutic consequence whereas chronic inflammation leads to harmful diseases like cancer, alzheimer's disease, diabetes, arthritis and autoimmune diseases<sup>3</sup>. Inflammation is a critical component of tumor progression and It is now becoming clear that the tumor microenvironment, which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration<sup>2</sup>.

The relationship between inflammation, innate immunity and cancer is more widely accepted however, many of the molecular and cellular mechanisms mediating this relationship remain unresolved<sup>2</sup>. Cancer related inflammations include presence of inflammatory cells and inflammatory mediators in new blood vessels and tumor tissues<sup>3</sup> i.e. for inflammatory mediators are chemokines, cytokines, prostaglandines. The inflammatory component of a developing neoplasm may include a diverse leukocyte population - i.e. neutrophils, dendritic cells, macrophages, eosinophils and mast cells, as well as lymphocytes - all of which are capable of producing an assorted array of cytokines, cytotoxic mediators including reactive oxygen species, serine, cysteine proteases, membrane-perforating agents, and soluble mediators of cell killing, such as TNF- $\alpha$ , interleukins and interferons (IFNs)<sup>4,5</sup>.

The strongest association of chronic inflammation with malignant diseases is in colon carcinogenesis arising in individuals with inflammatory bowel diseases i.e. chronic ulcerative colitis and

crohn's disease<sup>6</sup>. An inflammatory cell plays an important role in tumor promotion and producing an attractive environment for tumor growth, facilitating genomic instability and promotes angiogenesis. The inflammatory cells, chemokines and cytokines influence the whole tumor organ by regulating the growth, migration and differentiation of all cell types in the tumor microenvironment including neoplastic cells, fibroblasts and endothelial cells<sup>2</sup>.

Plants have been used as folk medicine to treat inflammation and cancer. Low cost and easy availability has generated new interest in plant medicine which have greater attention due to its little to no side effects<sup>7</sup>. Mangrove plants are widely used in medicine to treat variety of diseases. Some mangrove plants such as *R. mucronata* and *R. mangle* have been screened and known to have anti-bacterial, anti-viral, anti-ulcer and anti-inflammatory properties<sup>8,9</sup>. Though several plants from mangrove are extensively used in traditional medicine only some have been assessed for biological activities. Recent research reveals that mangrove *Rhizophora apiculata* (*R. apiculata*) have high content of pyrazole in the methanolic extract with variety of biological functions<sup>10</sup>. This 1, 2-diazole (pyrazole) is used for wider medicinal activities and it shows lesser adverse effects when compared to the conventional therapy. The 1, 2 diazole refers to both the class of simple aromatic ring organic compounds that is characterized by a ring structure of 3 carbon atoms and 2 nitrogen atoms that are in adjacent positions and also to the unsubstituted parent compound. The structure of 1, 2 diazole (pyrazole) a alkaloid is shown in Figure 1.

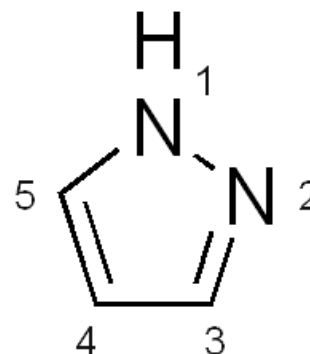


Fig. 1: Structure of 1,2 Diazole (Pyrazole) Alkaloid

This 1,2 diazoles are classified as alkaloid despite them being very rare in nature and It was first isolated from the seeds of watermelon. It is synthesized from acetylene and diazomethane<sup>11</sup>. Pyrazoles and their derivatives are important on account of use in therapy that have variety of properties such as anti-bacterial, -fungal, -diuretic, -cancer, -HIV 38-42 -tumor and - convulsant<sup>12,13,14</sup>. Therefore in this study we evaluate the anti-inflammatory potentiates of 1,2-diazole (pyrazole) a plant alkaloid against carrageenan and formalin induced paw edema in BALB/c mice.

## MATERIALS AND METHOD

### Animals

Male BALB/c mice (4-6-wk-old) were purchased from the Pasteur institute of India, Coonoor, Tamil Nadu, India. All mice were kept in a pathogen-free air-controlled room maintained at 24°C with a ~50% relative humidity and 12-hr light/dark cycle, and provided *ad libitum* access to normal mice chow (Sai Feeds, Bangalore, India) and filtered water. All animal experiments were performed according to the rules and regulations of the Institutional Animal Ethics Committee of the Government of India.

### Chemicals

Gum acacia was purchased from Hi-Media, carrageenan from Hi-Pure Fine Chemical Industries (Chennai, India) and Formaldehyde solution was procured from Universal Laboratories Pvt. Ltd. (Hyderabad, India). 1,2-diazole (pyrazole) from Sigma Aldrich USA.

### Evaluation of anti-inflammatory effect of 1,2-diazole (pyrazole) a plant alkaloid against carrageenan and formalin induced paw edema

#### Carrageenan regimen

BALB/c mice were divided into two groups (n = 6/group). Group I was kept as normal untreated control; mice in Group II received the 1,2-diazole (pyrazole) re-suspended in 1% gum acacia that was injected Intra peritoneal (IP) (10 mg/kg BW) on 10 consecutive days; the last dose was provided 60 min before induction of inflammation. Subsequently, all mice received a subcutaneous

injection of 0.1 ml of a 1% (w/v) carrageenan solution in the plantar region of their right hind paw to induce edema. The paw volume was measured initially and then at 30 min intervals for up to 8 hr and at 12 hr after the injection using a vernier caliper. All data were expressed as mean ( $\pm$  SD).

#### Formalin regimen

An additional set of BALB/c mice was divided into two groups (n = 6/group). Group I was kept as normal untreated control; mice in Group II received the 1,2-diazole (pyrazole) re-suspended in 1% gum acacia that was injected IP (10 mg/kg BW) on 10 consecutive days; the last dose was provided 60 min before induction of inflammation. Subsequently, all mice received a subcutaneous injection of 0.1 ml of a 2% (v/v) formalin solution in the dorsal surface of their right hind paw. Diameters of the hind paw were first measured to obtain the baseline value before the injection; thereafter, measures of dorsal plantar foot thickness (at metatarsal level) were performed on five consecutive days using a vernier caliper. All data were expressed as mean ( $\pm$  SD).

#### Statistical Analysis

The results are expressed as mean ( $\pm$  SD). Statistical evaluation was performed using a one-way analysis of variance (ANOVA) followed by a Dunnett's test using GraphPad InStat (Version 3.0 for Windows 95; GraphPad Software, San Diego, CA).

## RESULTS

### Anti-inflammatory activity of 1,2-diazole (pyrazole) alkaloid against induced edema

#### Carrageenan model

The effect of 1,2-diazole (pyrazole) alkaloid on carrageenan-induced paw edema in mice is shown in Figure 2. The 1,2- diazole (at 10 mg/kg BW, for 10 consecutive days) evinced an anti-inflammatory properties as illustrated by a reduction in paw size during at Fourth hour post-carrageenan injection ( $0.42 \pm 0.01$  mm<sup>3</sup>) relative to that seen in control mice ( $0.47 \pm 0.03$  mm<sup>3</sup>) group animals on the same hour.

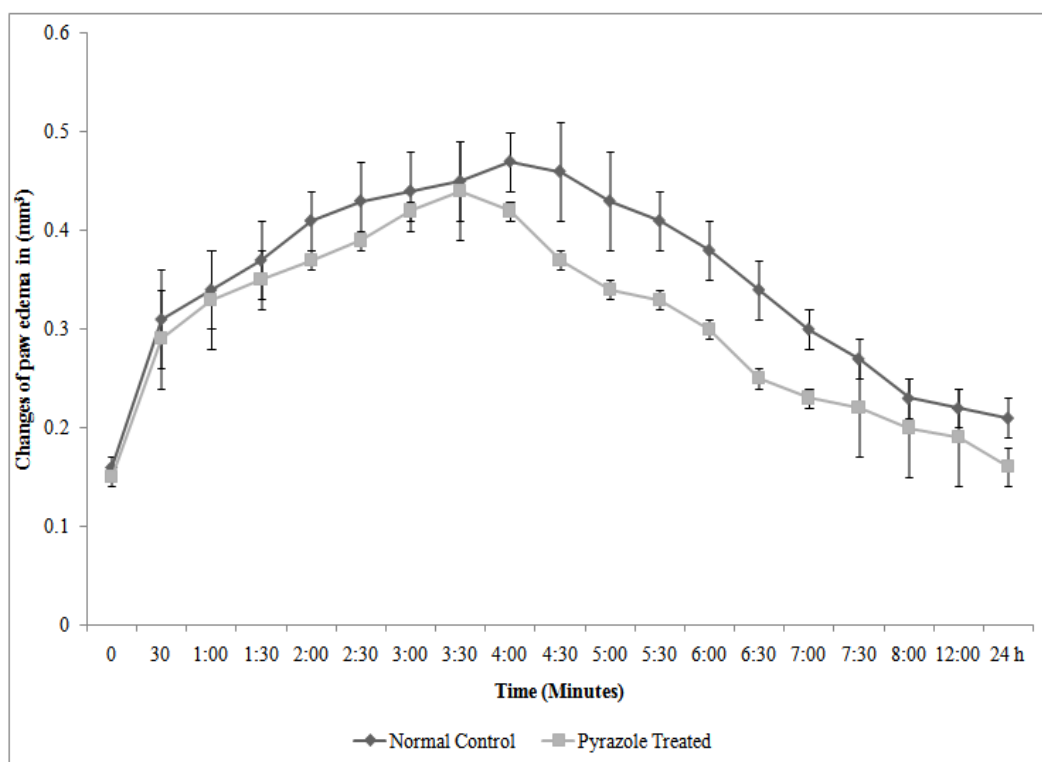


Fig. 2: The effect of 1,2-diazole (pyrazole) alkaloid on carrageenan-induced paw edema in mice.

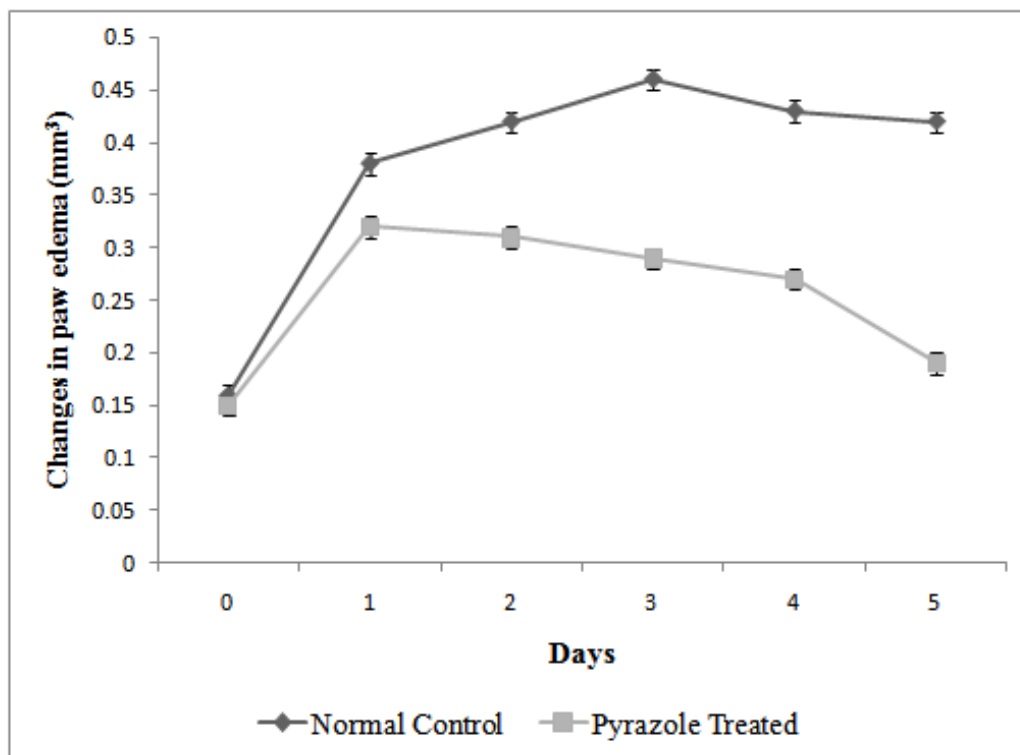


Fig. 3: The effect of the 1,2-diazole (pyrazole) alkaloid on formalin-induced paw edema in mice.

#### Formalin model

The effect of the 1,2-diazole (pyrazole) on formalin-induced paw edema in mice is presented in Figure 3. The 1,2-diazole (again, at 10 mg/kg BW, 10 d) evinced an anti-inflammatory properties as illustrated by a reduction in paw size during at day Second post-formalin injection ( $0.31 \pm 0.01$  mm<sup>3</sup>) relative to that seen in control mice ( $0.42 \pm 0.01$  mm<sup>3</sup>).

#### DISCUSSION

Inflammation is the tissue reaction to infection, irritation or foreign substance. It is a part of the host defense mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin & prostaglandins. The early inflammation changes in damaged tissues releases variety of active materials from polymorph nuclear leukocytes and lysosomal enzymes. The vascular effects are mediated by kinins, prostaglandins and vasoactive amines i.e. histamine, released by mast cells lead to increased in the vascular permeability resulting in plasma exudation. Acute inflammatory ideal are designed to analysis drugs that regulates blood flow (erythema), changes in vascular permeability, chemotaxis, leukocyte migration and other phagocytic cells. Chronic inflammatory ideal are used to find drugs that can modulate the disease process which includes granuloma pouches which deposit granulation tissue and adjuvant induced arthritis<sup>15</sup>. The present clinically used anti-inflammatory drugs have variety of adverse effects and high cost of treatment. Alternative to these drugs are traditional medicines and natural products provides an alternative to conventional therapy in preventing the cells of the body from performing some function that has become hyperactive .and a great hope in the identification of bioactive compounds and their development into drugs for treating inflammatory diseases<sup>16</sup>.

Natural products have long been recognized as an important source of therapeutic and effective medicines. The 1,2 diazoles (pyrazole) are very rare in nature and it was first isolated from the seeds of watermelon. This 1,2 diazole (pyrazole) refers both to the class of simple aromatic ring organic compounds of the heterocyclic diazole series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions

and to the unsubstituted parent compound. Having pharmacological effects on humans they are classified as alkaloids although they are rare in nature.

The alkaloids are a group of naturally occurring chemical compounds of largest single class of secondary plant substances. They have a remarkable and potential range of pharmacological activity. The term alkaloids generally include those basic substances that contain one or more nitrogen atoms, usually in combination as part of a cyclic system<sup>17</sup>. Only colchicine is an established clinical agent for arthritic disease and leukocytoclastic vasculitis. The alkaloid is present in corns and seeds of crocuslike plants. Colchicine is best known for its preventive action against gout, but it also reduces pain and swelling in degenerative and immunological inflammatory disease<sup>18</sup>.

Natural plant based alkaloids are often have greater pharmacological effects and are used as medications, as recreational drugs i.e. the local anesthetic and stimulant cocaine obtained from the leaves of the coca plant, the psychedelic psilocin found in most psychedelic mushrooms; the stimulant caffeine found in varying quantities in the seeds, leaves, and fruit of some plants, the analgesic morphine, the antibacterial berberine found in plant *Berberis aquifolium*, the anticancer compound vincristine from *Catharanthus roseus*, the antihypertension agent reserpine, the cholinomimetic galatamine from *Galanthus Caucasicus*, the spasmolysis agent atropine from *Atropa belladonna*, the vasodilator vincamine from *Catharanthus roseus*, the anti-arhythmia compound quinidine from bark of the cinchona tree, the anti-asthma therapeutic ephedrine from various plants in the genus Ephedra and the antimalarial drug quinine occurs naturally in the bark of the cinchona tree<sup>19</sup>, the diuretic, anti-inflammatory and anti-expectorant from *Terminalia bellirica* Roxb<sup>20</sup> the anti-viral, anti-mutagenic and anti-pyretic from *Glycyrrhiza glabra* Linn<sup>21</sup>. These alkaloids compounds appear to offer the considerable promise for further investigation as anti-inflammatory compounds and some appears to be remarkably active. Around 137 alkaloids presented anti-inflammatory activity among those, the isoquinoline type was the most studied. The Carrageenan-induced pedal edema was the most used model for evaluating the anti-inflammatory activity.

In the present study, the anti-inflammation activity of 1,2 diazole (pyrazole) a plant alkaloid was examined. In the carrageenan induced mice paw edema model, 1,2 diazole (pyrazole) a plant alkaloid showed significant inhibitory effect on the edema formation. This effect started from the Fourth hour and was maintained in all the inflammatory phases, suggesting that the main mechanism of action of the tested alkaloid may involve prostaglandin biosynthesis pathway and may influence other mediators of inflammation. Inflammation induced by formalin results from cell damage which provokes the production of endogenous mediators such as, histamine, serotonin, prostaglandins and bradykinin. It is then known from these results that 1,2 diazole (pyrazole) interfere with these inflammatory mediators. In conclusion, 1,2 diazole (pyrazole) alkaloid can produce significant results against both acute and chronic inflammation. Moreover, the high presence of this compound in marine mangrove *R. apiculata* plant extract added advantage for naturally available plant alkaloid with potential and efficient pharmacological role over inflammation without any adverse effect.

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#### Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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