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In-Vitro Anti Inflammatory Activity Of Pumpkinfruit(Cucurbitamoschata)

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ABSTRACT
Medicinal plants act as an important agent for curing all ailments of mankind. Most of the pharmaceutical industries are now looking towards preparing indigenous plant drugs as they are free of side effects [1]. Pumpkin is one of the important creepers belonging to the family Cucurbitaceae which is used as a vegetable and medicinal agent throughout the world [2]. It was previously reported that various activities like antioxidant, anti-diabetic, anticancer, antimicrobial and hepato protective activity are present in various parts of the plant due to the presence of several components like amino acids and fatty acids [3]. The powder of pumpkin is widely using in bakery products and health supplements as they contain starch, proteins, carbohydrates, dietary fibres and having excellent antioxidant property. Different parts of the plant extract were proved for having anti-inflammatory property.[4] The aim of the study was to find out the in-vitro anti-inflammatory activity of the pumpkin fruit powder.
KEY WORDS: In-vitro anti-inflammatory activity, pumpkin powder, plant extracts.

I. MATERIALS AND METHODS

MATERIALS

Mature pumpkin fruits were collected from the local market. About three pumpkins were purchased having weight about 1-1.5kg respectively. **RAW 264.7 cells** were procured from National Centre for Cell Sciences (NCCS), Pune, Indian and maintained Dulbecco's modified Eagles medium DMEM (Sigma Aldrich, USA).

METHODS

The fruit was washed, peeled and cut into small pieces (1.5×1.5) and placed in a butter paper. The sliced pumpkin was then placed in the hot air oven at 60^0C for 24 hours. Then powdered by a minor grinder then kept in an air tight container.

The cell line was cultured $25cm^2$ tissue cultured flask with DMEM supplemented solution. The cells were grown to 60% together then activate it with 1µL lipopolysaccharide (LPS: 1µg/ml). The activated cells are then exposed with different concentration of sample solutions and incubated for 24 hours. After incubation process, treated cells were used to perform the following anti-inflammatory assay.

CYCLOOXYGENASE (COX) ASSAY

Walker &Gierse method of assay are used here to perform the cyclooxygenase activity. The quantity of 100µL cells from above procedure was incubated with the reagents like Tris-HCl buffer (P^H-8) and 5Mm/L of haemoglobin and glutathione for 1 minute at 25^0C. After addition of 200 Mm/l of arachidonic acid the initiation of reaction started and the termination of reaction takes place after the addition of 200µL of 10% trichloroacetic acid in 1N HCl after 20 minutes of incubation. The tubes were boiled for 20 minutes after centrifugation by the addition of 200µL 1% thiobarbituric acid. After 3 minutes of cooling, the tubes are again centrifuged and COX assay were examined by reading the absorbance at 632nm[5].

Percentage inhibition = (Absorbance of control-Absorbance of test) / Absorbance of control ×100

LIPOOXYGENASE (LOX) ASSAY

Axelrod et.al method was used here to perform the lipooxygenase activity. 2 ml final volume of reaction mixture containing 200µL sodium linoleate, 50µL cell lysate and Tris-HCl buffer at P^H 7.4. The LOX activity was measured as increase in absorbance at 234nm (formation of 5-Hydroxyeicosatetraenoic acid) [6].

Percentage inhibition of enzyme = (Absorbance of control-Absorbance of test)/Absorbance of control×100

MYELOPEROXIDASE (MPO) ASSAY

The cell lysate homogenized with potassium buffer (50mM) and Hexadecyltrimethy

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ammonium bromide (0.57%). 200g of sample were centrifuged for 30 minutes at 4^0C and the supernatant was assayed for MPO activity. By adding 50mM phosphate buffer (P^H-6) containing guaiacol (1.67mg/ml) and H_2O_2 (0.005%) the sample containing MPO was activated. The absorbance was measured at 460nm for determining the activity [7].

U= (ΔOD.4.Vt.dilution factor)/ (L. €470.Δt.V_s)

INDUCIBLE NITRIC OXIDE SYNTHASE

The method described by Salter et.al 1997 was used to determine the nitric oxide synthase activity. 2ml of HEPES buffer was used to homogenise the cell lysate. The assay consists of 0.1ml-2µmol/L L-Arginine, 0.1ml-4µmol/L manganese chloride, 0.1ml-10mmol/L 30µgdithiothreitol (DTT), 0.1ml-1mmol/L NADPH, 0.1ml-4µmol/L tetrahydopterin, 0.1ml-10µmol/L oxygenated haemoglobin and 0.1ml cell lysate. The activity was measured at the absorbance of 471nm[8].

% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

ESTIMATION OF CELLULAR NITRITE LEVELS

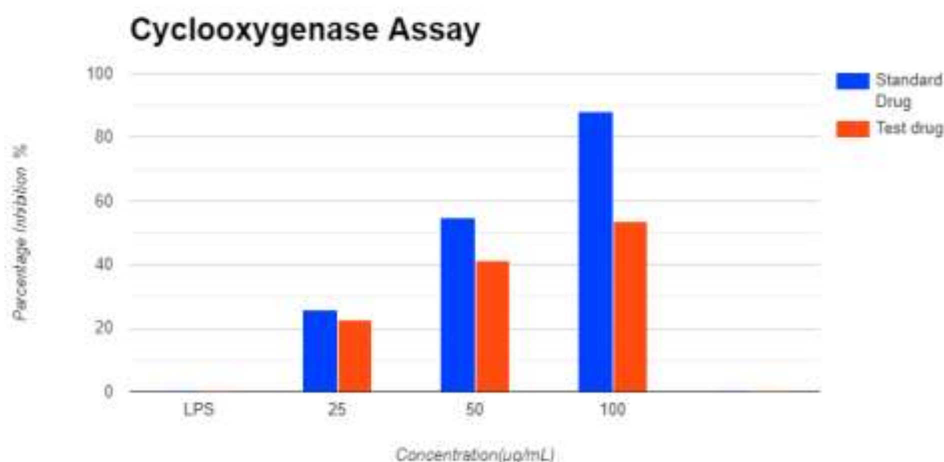
The method of Lepoivre et.al was used to estimate the level of nitrites. 0.1ml of 3% sulphosalicylic acid was added and vortexed in 0.5ml of cell lysate for 30 minutes. At 5,000 rpm sample were centrifuged for 15 minutes and resultant protein free supernatant were used for this estimation. The supernatant (200µL) was incubated in the dark room for 10-15 minutes after the addition of 30µL of 10% NaOH and 300µL of Tris-HCl buffer and 530µL Griess reagent. The activity was measured at the absorbance of 540nm[9].

Percentage inhibition was calculated as follows,
% inhibition = (Absorbance of control-absorbance of test) / (Absorbance of control) ×100

II. RESULTS

ESTIMATION OF CYCLOOXYGENASE ACTIVITY

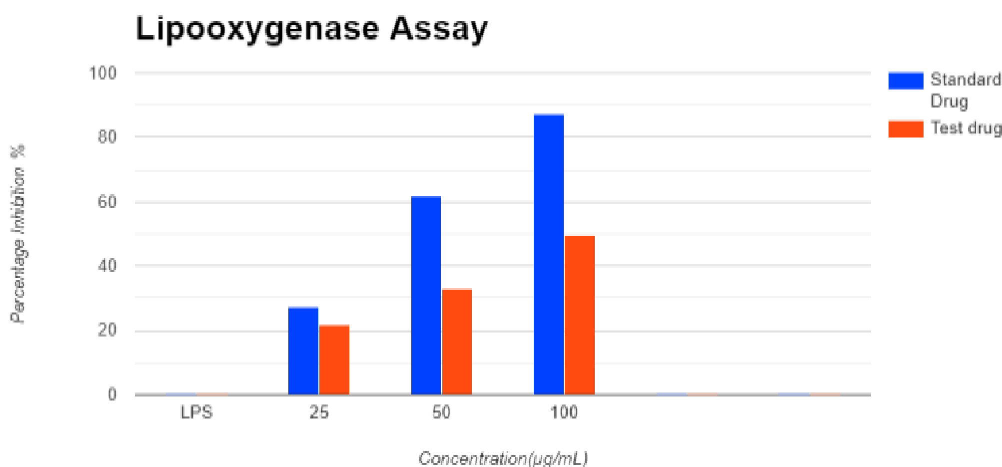
In-vitro anti-inflammatory assay of Cucurbita moschata was evaluated by cyclooxygenase assay. The percentage inhibition of indomethacin increased with increase in concentration and show maximum of 88.253% at concentration of 100µ/ml. The assay shows good inhibition of cyclooxygenase activity in dose dependent manner. The test compound shows 53.53% inhibition at its maximum concentration. The percentage inhibition obtained at different concentrations were seen in the graph (1).



ESTIMATION OF LIPOOXYGENASE ACTIVITY

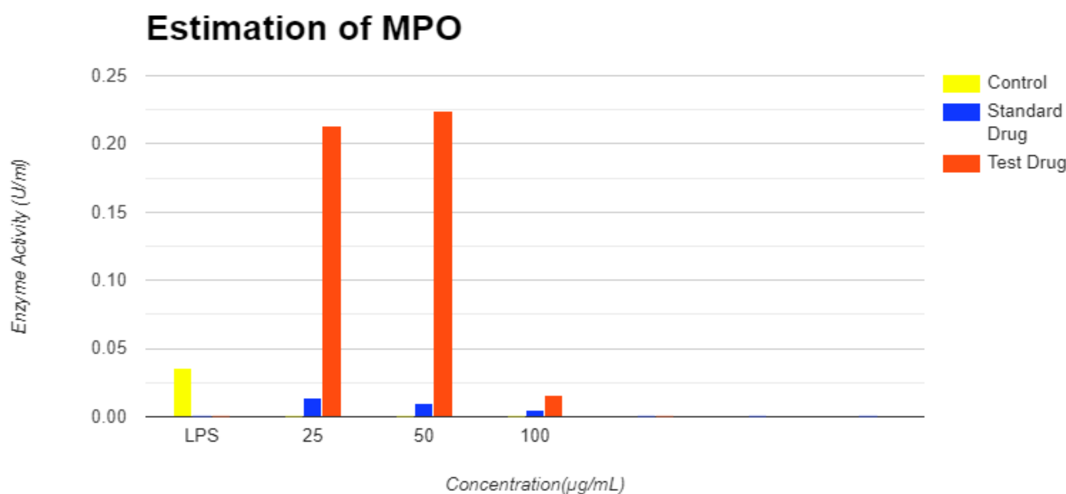
The powder of Cucurbita moschata was found to show good anti-inflammatory activity by acting against lipoxygenase enzyme. The percentage inhibition increased with increasing concentration and Indomethacin showed maximum of 87.428% at a concentration of 100µg/Ml. And at 100µg/ml fruit powder of Cucurbita moschata shows 49.61% lipooxygenase activity The assay

exhibit good inhibition of lipooxygenase activity in dose dependent manner. As the concentration increases the activity also increases, the test compound shows lipooxygenase inhibition activity of 53.53%. The percentage inhibitions obtained from different concentration were depicted in the graph (2).



ESTIMATION OF MYELOPEROXIDASE ACTIVITY

Powder of Cucurbita moschata was found to have very effect in inhibiting myeloperoxidase. Inhibition of MPO,showed that the test drugs are able to prevent the accumulation of neutrophils and thus inhibits inflammation. The reduction in the myeloperoxidase activity by test drug indomethacin andcomparison with control are depicted in graph (3).



INDUCIBLE NITRIC OXIDE SYNTHASE ACTIVITY

Fruit powder of Cucurbita moschatawas found to have very effect in inhibiting inducible nitric oxide synthase which act as a driver of development of inflammation. The test and standard showed reduction in the inducible nitric oxide synthase level in a dose dependant manner. The percentage inhibitions obtained from different concentrations are depicted in the following graph (4).

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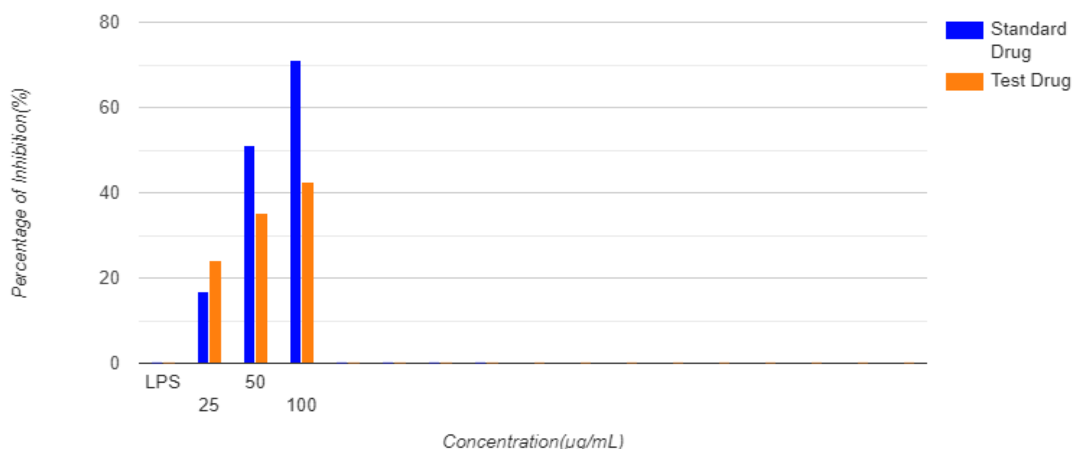
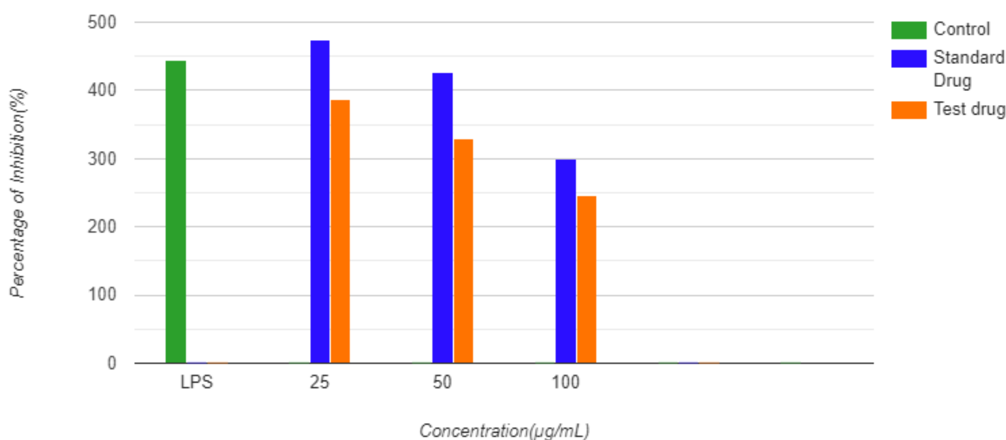


ESTIMATION OF CELLULAR NITRITE LEVEL

Fruit powder of Cucurbitamoschata exhibit good percentage in inhibition of nitrite formation. It was observed that there was dose dependent decrease in the nitrite level in RAW 264.7 medium. Decreased cellular nitrite level is an indication of the capacity to inhibit nitric oxide synthase, thus inhibiting the production of nitric oxide. The absorbance of these compounds was measured at 540nm. The concentration of nitrite produced in different sample concentration was depicted below in the graph (5).



III. DISCUSSION

Inflammation is a healing process which helps in protecting our body from injuries and infections. But sometimes inflammation causes harmful chronic diseases. Arachidonic acid synthesized leukotrienes and prostaglandins which are major mediators of inflammation.Prostaglandins are synthesized through cyclooxygenase pathway and leukotrienes through lipoxygenase pathway. Leukotrienes causes asthma.

Neutrophils and monocytes carriesmyeloperoxidase enzyme, which catalyses the formation of oxidants from H_2O_2 results in phagocytosis of microorganism [10].

Nitric oxide act as a mediator of inflammation. It is produced by Nitric oxide synthase enzyme. Inducible Nitric oxide synthase produce Nitric oxides in inflammatory conditions [11].

In this study it was found that Cucurbita moschata fruit powder has very good anti-inflammatory activity. It was found to be very effective in inhibiting inflammatory mediators like COX, LOX and induce the inhibition of myeloperoxidase and NOS enzymes. Hence it can be used as Anti-inflammatory agents with low side effects.

IV. CONCLUSION

Inflammation is a complex process which is mediated through many mechanisms. In this study the fruit powder of Cucurbita moschataexhibited COX,LOX, myeloperoxidase and inducible nitric oxide synthase inhibitory actions.

Thus powder of Cucurbita moschatacan be used to develop medicines for various inflammatory disorders either as a nutraceutical or as a novel source of new drug in modern medicine after further studies.

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