6. DISCUSSION

In the present study, pharmacognostical, phytochemical and pharmacological studies have been carried out to establish the authenticity of the plant based on morphological, phytochemical and physicochemical data and anti-asthmatic activities in the support of traditional and folkloric use of leaves and seeds of *Cassia occidentalis* Linn, then formulate and developed dosage form of seeds of it.

The macroscopic studies carried out to authenticate the plant *Cassia occidentalis* Linn. revealed that the characteristics of various parts were identical to those reported earlier by Khandelwal KR. Leaves are 4.5-10.5 cm long, ovate to oblong in shape, asymmetric base and greenish in colour, slightly bitter in taste, pubescent surface, and reticulate venations. Yellow petaled flowers are about 2 cm across. Fruits are pod type having green colour when unripe and brown when ripe colored, 5 mm thick, 7 to 12 cm long, straight to slightly curved. Seeds are 40 or more in each pod which are ovoid in shape, compressed at one end and rounded at the another end, 6 mm long, 4 mm broad, hard, smooth, shining, pale brown or dark olive green in colour. [147]

Roasting reduces / removes toxicity of seeds, so seeds were roasted before study. [229]

The leaves and seeds were preliminary evaluated by estimation of proximate analysis. Results of extraction of leaves and seeds indicate maximum extractive values attained with polar solvents like methanol and water whereas with non polar solvents like petroleum ether, benzene, chloroform and acetone were comparatively less. Methanol and aqueous extract was found to be 14.5% and 17.5% for leaves and 23.5% and 30.0% for seeds respectively. Qualitative chemical tests of leaves and seeds extract showed presence of alkaloids, glycosides, tannins, fixed oils, flavonoids, phenolics, triterpenoids, saponins, proteins, carbohydrates, gums and mucilage.

Maximum extractive values and chemical constituents were found in aqueous and alcoholic extracts. Further constituents responsible for antiasthmatic action like phenols, flavanoids, etc found in aqueous and alcoholic extracts. Furthermore, water is non toxic and so no chances of toxic effect due to residual solvent and due to all
these reasons solvent choosed for further biological screening and preparation of formulation was hydroalcoholic mixture of 8 part water and 2 part ethanol.

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. The qualitative analysis of the phytoconstituents of leaves and seeds of the plant *Cassia Occidentalis Linn* showed the presence of tannins and flavonoids in the methanolic and water extract. The peaks obtained in UV-Visible spectra confirm the presence of the same. Consequently the IR spectra of this plant extract shows the presence of OH group and UV-VIS spectrum of this plant extracts has absorption bands at 322 and 265 nm for seeds extract and 342 ans 281 nm for leaves extract. These absorption bands are characteristic for flavonoids and its derivatives. The flavonoids spectra typically consist of two absorption maxima in the ranges 230-285 nm (band I) and 300-350 nm (band II). The precise position and relative intensities of these maxima give valuable information on the nature of the flavonoids.

Acute toxicity studies were performed for hydroalcoholic extract of leaves and seeds of *Cassia occidentalis* L. according to the acute toxic classic method as per guidelines prescribed by OECD. There was not shown any observations like sedation, convulsions, tremors, lethargy, and death etc. for 14 days at the dose of 3000 mg/kg p.o. for leaves and 2000 mg/kg p.o. for seeds.

Since bronchodilators, mediator release inhibitors and anti-inflammatory drugs are the different classes of drugs used conventionally in the treatment of bronchial asthma; various animal models and experimental protocols were used to evaluate anti-asthmatic activity of leaves and seeds of *Cassia occidentalis* Linn. Bronchial asthma is characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of inflammatory mediators (e.g. Histamine, Tryptase, Leukotrienes and prostaglandins). Some of these mediators directly cause acute bronchoconstriction, airway hyperresponsiveness and bronchial airway inflammation. Spasmolytic drugs like beta adrenergic agonists, xanthine derivatives and anticholinergics relax the airway smooth muscles and are used as quick relief medications in acute asthmatic attacks. Beta adrenergic agonists promote bronchodilation by direct stimulation of beta adrenergic receptors in the airway
smooth muscle, that lead to relaxation of bronchial smooth muscle by rapid decrease in airway resistance in vivo. Specific β2 agonists like salbutamol, salmeterol etc. are used since long for symptomatic relief in asthma.

In this study, when the animals were exposed to the aerosol of 0.5 % histamine, there was a bronchospasm seen in the form of Pre-Convulsion Dyspnoea (PCD). Treatment with Ketotifén (1 mg/kg, p.o), as a standard drug; HECL (100 mg/kg and 200 mg/kg, p.o); HECS (30 mg/kg and 60 mg/kg, p.o.) given 7 days before aerosol exposure delayed onset of pre-convulsion dyspnoea (PCD) (73.48 ± 0.53 %, 35.71 ± 0.46 %, 49.30 ± 0.24 %, 40.52 ± 0.47 %, 62.94 ± 0.52 %) respectively in guinea pigs. These significantly and dose dependently increased the onset of convulsion time in guinea pigs. The bronchodilating effect of test drug (HECL and HECS) was comparable to standard control (ketotifen) (1 mg/kg). HECS at 60mg/kg dose showed highest effect among all test solutions.

Significant increase in preconvulsion time was observed due to pretreatment with leaves and seeds of Cassia occidentalis Linn., when the guinea pigs were exposed to either histamine aerosol. This bronchodilating effect of leaves and seeds at high dose was comparable to ketotifen.

When the animals were exposed to the aerosol of 0.5 % histamine, there was a bronchospasm seen in the form of Pre-Convulsion Dyspnoea (PCD). Treatment with Ketotifén (1 mg/kg, p.o), as a standard drug; HECL (100 mg/kg and 200 mg/kg, p.o); HECS (30 mg/kg and 60 mg/kg, p.o.) given 7 days before aerosol exposure delayed onset of pre-convulsion dyspnoea (PCD) (73.48 ± 0.53 %, 35.71 ± 0.46 %, 49.30 ± 0.24 %, 40.52 ± 0.47 %, 62.94 ± 0.52 %) respectively in guinea pigs. These significantly and dose dependently increased the onset of convulsion time in guinea pigs. The bronchodilating effect of test drug (HECL and HECS) was comparable to standard control (ketotifen) (1 mg/kg).

In addition to bronchodilating activity, a significant number of therapeutic approaches for bronchial asthma have been designed based on the antagonism of specific mediators released from mast cells. Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Degranulated cells
liberate mediators of inflammation such as histamine, leukotrienes, platelet activating factors and chemotactic factors for eosinophils, neutrophils etc. from mast cells. The unique mediator profile of mast cells, elicited upon activation through their high-affinity receptors for IgE, include pre-formed granule-associated inflammatory mediators (histamine, neutral proteases, pre-formed cytokines, and proteoglycans) that are released by exocytosis. Finally, activated mast cells synthesize and secrete a host of proinflammatory, chemoattractive, and immunomodulatory cytokines over a period of several hours. \[^{[231-233]}\] The bioactivities of these mediators include brochoconstriction (cys-LTs, histamine, PGD\(_2\)), vasodilation and tissue edema (histamine, cys-LTs), leukocyte infiltration (cys-LTs, PGD\(_2\), tryptases, cytokines and chemokines), collagen matrix turnover and stromal cell growth (tryptases, cytokines), and hyperplasia of bronchial smooth muscle (tryptases, cys-LTs). These properties of mast cells, and their normal residence in bronchi, would seem to position them for a potentially relevant role in the pathophysiology of asthma. \[^{[234]}\] Degranulation of mast cells has been taken as the criteria of positive anaphylaxis. Ketotifen fumarate, a well-known mast cell stabilizer, reduces synthesis of prostaglandins E\(_2\), thromboxane A\(_2\), leukotriene C\(_4\) and B\(_4\). It also inhibits release of histamine, serotonin and other inflammatory mediators from receptors. Cromolyn sodium, which is developed from the structural modification of Khellin is the mast cell stabilizer used in the treatment of mild to moderate asthma by raising cAMP levels due to inhibition of the enzyme phosphodiesterase. \[^{[235]}\]

The hydroalcoholic extract of *Cassia occidentalis* Linn. leaves and seeds was found to inhibit the degranulation of mast cells induced by a non-immunological stimulus. It is known that the physiological stimulus for the release of histamine from mast cells is provided by a combination of antigen with specific antibody fixed on the cell surface. This combination is believed to transiently increase the permeability of membrane to calcium ions showing an absolute requirement for calcium ions for the secretory process to occur. \[^{[236]}\] Anaphylactic and clonidine induced secretion from mast cells share a common requirement as far as the presence of calcium ions is concerned. However, clonidine can utilize intracellular calcium stores to initiate the release process, even in the absence of calcium in the extracellular medium. On the other hand, anaphylactic release requires the presence of calcium in the extracellular medium which moves onto the cell via calcium gates in the membranes. \[^{[235, 238]}\]
significant protection of rat peritoneal mast cells from disruption by clonidine by extract *Cassia occidentalis* Linn. points towards its ability to interfere the release and/or synthesis of mediators of inflammation, indicating its mast cell stabilizing activity. Hence it may be assumed that the cytoprotective effect induced by extract of *Cassia occidentalis* Linn. on mast cell surface could be due to its ability to alter the influx of calcium ions.

In the study of clonidine induced mast cell degranulation, the group of animals pretreated with hydroalcoholic extract of *Cassia occidentalis* leaves and seeds showed significant protection in degranulation of mast cells (32.36 ± 1.98, 39.34 ± 1.8, 54.5 ± 2.01 and 30.0 ± 0.19) at the dose 100 mg/kg, 200 mg/kg for leaves and 30 mg/kg, 60 mg/kg b.w.) when challenged with clonidine.

Further, airway inflammation has been demonstrated in all forms of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, particularly with activated eosinophils and lymphocytes, with neutrophils and mast cells. The degree of bronchial hyperresponsiveness and airway obstruction is closely linked to the extent of inflammation. Anti-inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis, or release of mediators and the effects of inflammatory mediators. Carrageenan induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has been frequently used to assess the anti-edematous effect of natural products.

Carrageenan –induced inflammation is useful in detecting orally active anti-inflammatory agents. Oedema formation due to carrageenan in the rat paw is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The edema produced at the peak (3 h) is thought to be due to the release of kinin-like substances, especially bradykinin. The second phase is sensitive to most clinically effective anti-inflammatory drugs. It is well established fact that non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory activity by inhibition of prostaglandin biosynthesis. The anti-edematogenic mechanism of action of *Cassia occidentalis* leaves and seeds may also be related to prostaglandin synthesis inhibition. Inflammation pain results from the release of hyperalgesic mediators –
prostaglandins and catecholamines – which are supposed to act by regulating the sensitivity of pain receptors. [243-244]

The prevention of degranulation process by the hydroalcoholic extract indicated a possible sensitizing effect on the biomembrane of mast cells. The neutrophil, an end cell unable to divide and with limited capacity for protein synthesis is, nevertheless, capable of a wide range of responses, in particular chemotaxis, phagocytosis, exocytosis and both intracellular and extracellular killing. [245] Neutrophils are present in much larger numbers than any other inflammatory cell in the circulation and in tissue stores, particularly the lung. Neutrophils are one of the first inflammatory cells to be recruited into the airways after either allergen exposure or injury. [246] In acute inflammation, activated neutrophils are the major effector cells of this inflammatory response, releasing interleukins, tumour necrosis factor α, leukotriene B4, platelet activating factor (PAF), proteases, and products of the respiratory burst reaction. [247-249] Mucosal recruitment of neutrophils involves sequential adhesion and transmigration across endothelial, lamina propria and epithelial -integrins, tissue bound factors (IL-8, PAF) and products of the immunoglobulin gene superfamily. [250]

Here, Paw size (cm) of rat was measured at one hour interval for each group in carrageenan –induced edema. In control animals, the sub plantar injection of carrageenan produced a local edema that increased progressively to reach maximal intensity at 5 h after the injection of the phlogistic agent (5.41 ± 0.03). HECL (100 mg/kg and 200 mg/kg, p.o.) and HECS (30mg/kg and 60 mg/kg) showed a significant suppression of carrageenan -induced rat paw edema when compared with the control group.

Mucosal inflammation is associated with an increase in the expression of vascular and leucocyte adhesion molecules. [251-252] A number of cellular adhesion molecules are involved in the adhesion of neutrophils to the site of tissue inflammation. Neutrophils must adhere to the endothelium and subsequently migrate through the vessels before entering the tissue. Neutrophil rolling and arrest on endothelium is mediated through successive interactions of selectins and β-integrins. [246] Neutrophil adhesion to endothelium is enhanced by activation of adenosine A compartments. [253] Subsequent adhesion to apical epithelial membranes results in activated neutrophils persisting in
crypt abscesses with local release of chemotactic and chemoactivating substances.\cite{254-255} In addition to producing a number of functionally diverse substances, polymorphonuclear cells also express receptors for a number of mediators including IL-8, IL-9 and the high-affinity IgE receptor.\cite{256-257} These receptors have been implicated in different inflammatory reactions, including allergic asthma. Neutrophil recruitment from the circulation into the interstitium during inflammation is an extension of a physiological process across an adapted, permissive cell layer. Such transmigration involves the tethering, rolling, tight adhesion, and diapedesis of marginalised flowing cells.\cite{258} Neutrophil adhesion to the vascular endothelium as described in the ‘traffic signal’ paradigm, results from the sequential recruitment of selectins, \(\beta_{221}\) receptors. Binding to the adenosine \(A_2\) receptor results in inhibition of the respiratory burst reaction and decreased binding to fibrinogen.\cite{259-262}

The percentage of neutrophil adhesion was estimated on day 14. Pretreatment with HECL at dose 100 and 200 mg/kg, p.o. and HECS at dose 30 and 60 mg/kg, p.o. induced a significant \((p < 0.001)\) decrease in the \textit{in vitro} neutrophil adhesion to nylon fibers with respect to that of control group, which correlated the decrease in percentage of neutrophils. However, HECL at dose of 100 mg/kg did not show any significant change in neutrophil adhesion when compared with respective control group. Thus hydroalcoholic extract of leaves and seeds of \textit{Cassia occidentalis} Linn. reduced significantly percentage of neutrophil adhesion. This may help in decreasing the release of various cytokines and might be binding to \(A_1\) and/or \(A_2\) receptor on endothelium and results in producing anti-inflammatory action.

With an intension to design a formulation from most biologically active extract that is seed extract of cassia occidentalis, nine formulations of cassia occidentalis seed extract were prepared with varying concentration of three superdisintegrants: Sodium starch glycolate, Kollidone and Crosscarmelloise, Microcrystalline cellulose was used as diluents and stevia powder was used as natural sweetner. For each formulation, blend of extract and excipients were prepared and evaluated for various parameters as explained earlier. The powder blend was compressed using direct compression technique. Bulk density, was found in the range of 0.5439±0.02 and 0.556±0.02 gm/cm\(^3\) and tapped density between 0.491±0.02 and 0.636±0.02 gm/cm\(^3\). Using these
two density data Hausner’s ratio and compressibility index was calculated. The powder blends of all formulations had Hausner’s ratio less than 1.159 indicates better flow property. The carr’s Index was found to be less than 13.675 which indicates a fairly good flowability of the powder blend. The good flowability of the powder blend was also evidenced with angle of repose less than 44 indicating good flow ability. Tablets were prepared using direct compression technique. Since the powder material was free flowing, tablets were obtained of uniform weight due of uniform die fill, with acceptable weight variations as per I.P. The results of post compressional parameters like weight variation, hardness, friability, disintegration and wetting time are shown in Table 5.15. In all the formulations hardness test indicates good mechanical strength, hardness of the tablets was in the range of 3 to 4 kg/cm². Friability was less then 1% in all the formulations, indicated that tablets had a good mechanical resistance. The tablets were subjected for evaluation of in-vitro disintegration time was found to be between 10 to 28 sec. Among all the Formulas, formula no. T1, T2 and T3 containing Kollidone as super disintegrant showed less friability and less disintegration time as compared to others. It may be due to wicking action of Kollidone and swelling nature of the Cassia Occidentalis Linn extract. So further different formulas were prepared using crosspovidone as superdisintigrant in different six concentration to optimize concentration of crosspovidone in which, bulk density was found in the range of 0.447 ± 0.02 to 0.521 ± 0.02 g/cm³ and the tapped density between 0.501 ± 0.03 to 0.602 ± 0.02 g/cm³. The powder blends of all formulations had Hausner’s ratio less than 1.15 indicates better flow property. The compressibility index was found between 10.74 ± 0.52 to 13.19 ± 1.03 which indicates a fairly good flowability of the powder blend. The good flowability of the powder blend was also evidenced with angle of repose (range of 25 to 35) which is below 35° indicating good flowability.

Since the powder material was free flowing, tablets were obtained of uniform weight due to uniform die fill, with acceptable weight variations as per I.P. The hardness of the tablets was found 3 to 4 kg indicating a good mechanical resistance of the tablets, and the parameters were found well within the specified limit for uncoated tablets. The in-vitro disintegration time (DT) of the tablets was found to less than 28 sec. The maximum increase in the dissolution rate was observed with 5% Kollidone amongst the superdisintegrants. The preparation process in direct compression tablets includes
co grinding of all the excipients before compression, resulting the increase in the solubility due to the reduction in the effective particle size of the drug following increase in the wetting of drug particle by the excipients and improved dissolution of drugs.

In all the formulations hardness test indicates good mechanical strength; hardness of the tablets was in the range of $3.33 \pm 0.15$ to $3.53 \pm 0.06$ kg/cm$^2$. Friability was less than 1% in all the formulations, indicated that tablets had a good mechanical resistance. The tablets were subjected for evaluation of in-vitro disintegration time. Formulation F1 containing Kollidone 5% as superdisintigrant showed disintegration time of 10 seconds. It may be due to wicking action of Kollidone and *Cassia occidentalis* Linn extract powder. In all other set of formulations, the disintegration time of tablets was decreased with increased concentration of superdisintegrants. The measurement of wetting time may be used as another confirmative test for the evaluation of fast dissolving tablets. In wetting time study, the wetting time was decreased with increased concentration of superdisintegrants. The least average wetting time and disintegration time of formulation F1 proved the effect of Kollidone. In all the formulations the disintegration may be influenced by the self disintegration property of *Cassia occidentalis* Linn extract powder powder. The stability study for tablets was carried out according to ICH guidelines by storing the tablets in stability chamber (Lab-care Mumbai). No appreciable change in physical characteristics like hardness, disintegration time and wetting time was observed even after the evaluation for 3 months.

As the material was free flowing, tablets were obtained of uniform weight due to uniform die filling. Hardness of tablets was between 3.33 to 3.53 kg/cm$^2$ for all the formulations. Friability was found in between tablets. The wetting time/ dispersion time decreases with increase in the concentration of superdisintegrants.

IR spectroscopy is used to determine the interaction between the drug, polymer and excipients. The drug and polymer must be compatible with one another to produce a product stable, efficacious and safe.
FT-IR results revealed that there was no significant difference in the peaks of Cassia occidentalis seed extract in pure form and the extract in tablets. It was found that there was no interference to the drug with excipients and polymer used in the formulations.

Nowadays, the interest in study of natural products is growing rapidly, especially as a part of drug discovery programs. In our previous study, we have proved that the anti asthmatic activity of Cassia occidentalis seed which is associated with the active constituents of Cassia occidentalis. So its of interest to isolate the pure constituents responsible for the above mentioned pharmacological action. The initial study was carried out with HPTLC and the results showed that there are many compounds in Cassia occidentalis Linn.

Further, the HPTLC chromatogram of cassia occidentalis seed extract and formulation were compared which showed no significant difference in $R_f$ values of spot which indicate no interference of ingredient with extract. The seeds of cassia occidentalis were reported to have toxicity and that’s why the extract was prepared after roasting of seeds. Comparison of chromatogram of roasted seed extract and non roasted seed extract of cassia occidentalis seed revealed that there are some more components present in non roasted seeds which are absent in roasted seed extract. These observation leads to conclude that these extra spots may be of that toxic compound. So it revealed that roasting of seeds decrease/remove toxicity.