5. Discussion
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In the present study, pharmacognostical, phytochemical and pharmacological studies have been carried out to establish the authenticity of the plant based on morphological, physicochemical and phytochemical data and haematinic, analgesic and anti-asthmatic activities in the support of traditional and folkloric use of fruits of *Opuntia elatior* Mill.

The macroscopic studies carried out to authenticate the plant *Opuntia elatior* Mill. revealed that the characteristics of various parts were identical to those reported earlier by Kirtikar & Basu (1999) and The Wealth of India (2001). Phylloclade is an aerial modification of stem, broadly ovate to oblong in shape and greenish in color. Flowers are arising from areoles and contain perianth pink color in centre with yellow at the edge. Fruits are berry pyriform, reddish purple when ripe and greenish when unripe. It is indehiscent, depressed at the apex and many seeded pulpy fruit.

The phytochemical analysis was carried out for standardization of fruit juice contained carbohydrates, flavonoids, phenolics and betalains. Piga (2004) and Moßhammer et al. (2006) reported the presence of color pigment betalains and sugar content in high amount and low acidity of fruit which make it very sweet and delicious. The fruits were preliminary evaluated by estimation of proximate analysis. The average weight of fruit was $24.568 \pm 7.134$ g/unit and among percentage of peel and seed was very low compared to the edible portion. A comparatively low ash value indicates presence of less inorganic matter. The fruits are very good source of watery content as high amount of moisture content and low amount of solid content. The fruit juice can resist microbial growth as low pH and acidic in nature. The density of fruit juice was comparatively near to the water as high moisture content. Viscosity of juice was $53.62 \pm 0.402$ centipoise at 100 rpm.
Discussion

Results of extraction of fruits peel indicate maximum extractive values attained with polar solvents like methanol and water whereas with non polar solvents like petroleum ether, benzene and chloroform were comparatively less. Methanol and aqueous extract of peel was found to be 23.37% and 18.16% respectively.

Qualitative chemical tests of peel extract showed presence of carbohydrate, fats & fixed oil, flavonoids, phenolics, steroids & terpenoids, waxes, saponins and betalains whereas fruit juice showed presence of carbohydrates, flavonoids, phenolics and color pigment betalains.

A comparison of R<sub>f</sub> values and spot color obtained in TLC of carbohydrates, it can be concluded that the fruit juice contained reducing hexose monosaccharides like galactose and glucose (Egon, 2007). TLC of betalain indicates presence of betacyanins type of color pigments as R<sub>f</sub> values was low (0.081) in BAW and high (0.916) in 1% Aq. HCl (Harborne, 2007).

Harborne (2007) reported visible spectrum of betacyanin in methanol-HCl give maximum absorbance in the range of 532 – 554 nm. The spectrophotometric analysis suggests that the external color of prickly pear fruits depends on the relative concentration of betacyanins (red pigments with maximum absorbance at around 535 nm) and betaxanthins (yellow pigments with maximum absorbance at around 480 nm) (Wybraniec et al., 2001; Fernandez-Lopez & Almela, 2001; Stintzing et al., 2003, 2005). The absence of a peak at 484 nm would indicate that in fruits of *Opuntia elatior* Mill., betaxanthins are to be found in a very low level and spectrophotometrically is very difficult to distinguish them from betacyanins, which are present in a much higher concentration at 535 nm in methanol and water extracts of fruit.

Since betacyanins and betaxanthins possess similar spectroscopic and chromatographic properties, HPLC is an invaluable means of separating and analyzing them. Tentative identification of these betalains can be deduced from their chromatographic behavior, and corroborative data may be provided by an analysis of their absorption spectra. HPLC is an excellent means in the
analysis of betalains. The most common support is C_{18}-derivatized silica providing adequate efficiency and retention of betacyanins as well as their sufficient resolution on conventional stationary phases. Because betacyanins exist in aqueous solution in different ionized forms at varying pH values, the use of typical acidic eluents with or without buffers is a useful factor governing their separation (Schliemann et al., 1996, 2000, 2001; Wybraniec et al., 2001, 2006). Fernandez-Lopez et al. (2002) analyzed presence of betalains from the fruits of *Opuntia stricta*, *Opuntia undulata* and *Opuntia ficus-indica* and found HPLC patterns of betalains with retention time at 16.8 min (\(\lambda_{\text{max}} 484\) nm), 19.6 min, and 22.8 min (\(\lambda_{\text{max}} 537\) nm) assigned to indicaxanthin, betanin and isobetanin, respectively. From the respective retention times in comparison to literature, the spectral properties provided by the photodiode array detector and data reported by other researchers peak 1 with retention time 21.76 min at 484 nm was identified as indicaxanthin which present in very low amount and peak 2 with retention time 22.76 min at 535 nm as betanin, present in high amount in fruits of *Opuntia elatior* Mill.

MS provides molecular weight and structural information of the chromatographic bands so that fully-resolved peaks are not required, thus shortening chromatographic runs and reducing sample preparation while ensuring high sensitivity and selectivity. This technique is commonly used in investigations on betalain pigments (Schliemann et al., 1996, 2000, 2001; Wybraniec et al., 2001). Ferndndez-Lopez et al. (2002) screened the presence of betalain pigments in fruits of *Opuntia stricta*, *Opuntia undulata* and *Opuntia ficus-indica*, also Yahia & Castellanos-Santiago (2008) identified betalains from the fruits of 10 Mexican prickly pear cultivars by HPLC and ESI-MS. The presence of betanin was confirmed by its identical spectral properties (maximum absorbance at 535 nm), and the presence of their protonated molecular ions \([\text{M+H}]^+\) with \(m/z 551\), while the prominent secondary ion at \(m/z 390\) due to the presence of protonated aglycones [betanidine + H]^+. From the respective molecular mass in comparison to literature and data reported by other researchers, it was identified as betanin.
Total sugars range from 12 – 17 °Brix and are mainly of the reducing type with glucose being the predominant sugar and fructose being the second sugar thus the fruit pulp is very sweet. Directly absorbable high glucose concentrations in cactus fruits represented as instantly available energy source for brain and nerve cells while being sweeter may enhance the fruit’s flavor. Some authors have also reported the occurrence of galactose and maltose. The total sugar content (11.41 g/100 ml) equivalent to glucose obtained in fruits of *Opuntia elatior* Mill. was low with respect to other *Opuntia* species (Feugang et al., 2006; Piga, 2004; Stintzing et al., 2001).

In fruits belonging to species *Opuntia ficus-indica* and *Opuntia dillenii* contained 117 ± 10 and 45.2 ± 7.4 mg/100 g of total phenolics respectively (Diaz Media et al., 2007). Su Feng Chang et al. (2008) reported total phenolics (91.5 ± 1.5) and flavonoids (29.2 ± 1.5) along with gallic acid (4 ± 0.6), catechin (22.7 ± 0.7) and epicatechin (10.9 ± 0.2) as mg/100 g fresh sample of *Opuntia dillenii* Haw fruits. The total phenolic content (49.82 mg/100 ml) equivalent to gallic acid obtained in fruits of *Opuntia elatior* Mill. was in range of other species.

Citric acid is the major organic acid in cactus pear followed by malic acid, quinic, shikimic and also oxalic acids were found while isocitric, fumaric, glycolic, and succinic acids were only found in traces (Moßhammer et al., 2006; Stintzing et al., 2001). The titratable acidity (0.94%) equivalent to citric acid serves as a low acid food in fruits of *Opuntia elatior* Mill.

The most convenient way to quantify betalains is spectrophotometric method. First, Nelsson (1970) established a method to quantify pigments in beetroot. The total content of betacyanin was determined using the formula reported by Chethana, (2007); Cai et al., (2005); Ferndndez-Lopez et al., (2002). The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from fruits of *Opuntia elatior* Mill. was higher compare to *Opuntia ficus-indica* and *Opuntia undulata* Griff. while lower compare to *Opuntia*
stricta Haw (Fernandez-Lopez & Almela, 2001; Ferndndez-Lopez et al., 2002).

The mineral composition is characterized by high amounts of potassium followed by calcium while other minerals are in the normal range of fruits with respect to reported literature of other Opuntia species (Feugang et al., 2006; Piga, 2004; Stintzing et al., 2001).

This study aimed to evaluate the haematinic effect of Opuntia elatior Mill. fruit on HgCl$_2$ and PHZ–induced anaemia. Before explaining the possible protective role of fruit, it seems essential to describe the mechanism of Hg induced anaemia. Hg ions bind with –SH groups in the bio-membranes, and damage them via lipid peroxidation (Clarkson, 1972). Hg also binds with lysosomal membranes and renders them labile (Lauwery & Buchet, 1972). It inhibits protein synthesis (Nakada et al., 1980), alters the tertiary structure of RNA and DNA (Gruenwedel & Davidson, 1966) and affects their synthesis. Hg disturbs the structure and function of inner mitochondrial (Humes & Weinberg, 1983). All these effects can be held responsible for anaemia due to inorganic Hg–induced cellular damage (EHC-118, 1991).

Iron deficiency is the commonest cause of hypochromic microcytic anaemia. In iron deficiency, the amount of iron lost from the body exceeds the amount absorbed. The physiological demand for iron exceeds iron uptake. First there is depletion of the iron store of the body followed by reduction in the plasma level of iron and development of hypochromic microcytic anaemia. Hypochromic microcytic anaemia can be interpreted based on reduction of haemoglobin content, total RBC count, PCV, MCV, MCH, MCHC and increase in RDW values is the indicator of hypochromic microcytosis (Godkar & Godkar, 2004) and that’s why we precise the estimation of these haematological parameters. The results obtained after 30 days indicated that HgCl$_2$–induced hypochromic microcytic or haemolytic anaemia due to iron loss. The results after treatment indicated that the fruit juice of Opuntia elatior Mill. increased significantly the haemoglobin, total RBC count, RBC indices (MCV, MCH, MCHC), PCV and decreased RDW.
Loss of body weight is a common clinical feature of anaemia. It was observed that there was a remarkable increase in body weight in animals treated with OFJ at dose 10 ml/kg (212.2 g) and 15 ml/kg (227.7 g) after continuous treatment for 30 days which was better than standard treated group. Only OFJ (15 ml/kg) treated rat demonstrated slight increase in body weight (208.7 g) after 30 days but comparatively less than disease treated groups. The reversal of body weight by fruit juice could be considered as a significant effect. It indicates reversal of the toxicant induced tissue degenerative changes. Body weight change is the sum of the effects occurring in different parts of the body and reversal of the toxicant induced decrease is an index of good tissue or cytoprotective activity of the test drugs.

Haemoglobin estimation is considered as the marker for evaluating the correction of anaemia. At the dose of 10 ml/kg and 15 ml/kg of fruit juice showed good percentage of recovering in haemoglobin, 32.99 % and 38.18 %, respectively, which was higher than standard treated group (29.8 %) indicating correction of anaemia. The haematinic action of fruit juice was dose dependant manner. It was observed that there was slight increase in haemoglobin content but not significant in group E higher dose treated rat.

The mean total and differential WBC count give the information regarding defense system of the body. However, fruit juice of *Opuntia elatior* Mill. improved the total and differential WBC count in HgCl$_2$ –induced anaemia. The results were dose dependant and reversed the effect of HgCl$_2$ in rats. Thrombocytopenia (decrease in platelet count) is often associated with prolonged bleeding and poor clot retraction. It also occurs in aplastic, haemolytic, megaloblastic anaemia, hyperplenism, acute leukemia and in immune thrombocytopenia (Godkar & Godkar, 2004). The fruit juice of *Opuntia elatior* Mill. improved the platelet functions and the results was dose dependant in HgCl$_2$ –induced anaemia model.
The increment of blood sugar indicated that the fruit juice was a source of energy due to presence of high amount of carbohydrates. The fruit juice has not major effect on serum cholesterol and triglyceride levels.

Urea is the major nitrogen containing metabolic product of protein catabolism in humans. In leukemia and haemolytic anaemia, release of leukocyte protein contributes to high plasma urea. In gastrointestinal disease, plasma proteins and haemoglobin can be released into the gut and digested. This may contribute to high plasma urea. Creatinine formed as the end product of creatine metabolism is a waste product. The plasma blood urea and creatinine increases in renal diseases (Godkar & Godkar, 2004). Fruit juice of Opuntia elatior Mill. showed tendency towards reversal of these toxicant induced changes. The changes observed after HgCl$_2$ administration can be mainly attributed to the toxicant induced kidney damage. Reversal of most of these changes by fruit juice administration indicates that they do have some element of cytoprotective activity. The kidney is badly damaged by HgCl$_2$ exposure (Rathore and Vaghese, 1994). Fitzhuge et al. (1950) studied Hg-acetate (25 ppm) –induced changes in kidney of rats and reported a dose dependant change in its structure and function. Among human beings, inorganic Hg salt ingestion result in anuria and uraemia from acute tubular necrosis (Kazantzis et al., 1962)

Liver is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver. Therefore, liver can be used as an index for the toxicity of xenobiotics. Total bilirubin may rise in irritation of liver; this reflects liver cell damage or bile duct damage within the liver itself. Proteins are synthesized in liver; low level indicates that the synthetic function of liver has been markedly diminished. Alkaline phosphatase is the marker enzyme produced within the cells of the liver, as the cells are damaged, leaks into the blood stream leading to a rise in the serum levels. It is an enzyme, which is associated with the biliary tract, and it elevated; biliary tract damage and
inflammation should be considered (Godkar & Godkar, 2004). From the bilirubin, alkaline phosphatase and total protein content observations, it seems that fruit juice of *Opuntia elatior* Mill. improves the liver function significantly. The liver showed HgCl$_2$–induced pathological changes (Rathore and Vaghese, 1994). Ashe et al. (1953) had reported severe hepatic effects in rabbits exposed to metallic Hg vapors. Accidental, fatal Hg vapor inhalation exposures in a young child caused hepatocellular damage and biochemical alterations (Jafee et al., 1983).

Spleen is the store house of dead RBC and breakdown of haemoglobin also occurs in the spleen. Hemolytic anaemia leads to accelerated breakdown of haemoglobin causing larger iron deposition in spleen (Chatterjee, 1994). This is likely to be the cause of fibrosis and necrosis observed in the spleen in HgCl$_2$ treated groups. This disturbance in the cytoarchitecture was significantly reversed by test drug administration. In this respect fruit juice was comparatively better because in addition to attenuating the fibrosis, it restored cellularity to moderate level thus inhibiting the toxicant induced cell depletion.

Numerous *in vitro* studies have demonstrated the beneficial effect of phenolics and betalains as antioxidant action. These are generally attributed to the ability of antioxidant to neutralize reactive oxygen species such as singlet oxygen, hydrogen peroxide (H$_2$O$_2$), or suppression of the xanthine/xanthineoxidase system, all of which may induce oxidative injury i.e. lipid peroxidation (Budinsky et al., 2007) and as we know inorganic Hg induced lipid peroxidation, inhibition of protein synthesis and cellular damage which results in anamia.

The fruit juice of *Opuntia elatior* Mill. reversed anaemia induced by HgCl$_2$ in dose dependant manner. The antioxidant phenolics and betanin constituents and mineral compositions appear most likely as the active ingredients responsible for haematinic effect of *Opuntia elatior* Mill. fruits. This results support at least partially the traditional use of fruits in the treatment of anaemia.
Phenylhydrazine produces both aryl and hydroxyl radicals when incubated with rat liver microsomes (Gannett et al., 1997) and oxidised by hydrogen peroxide at pH 7.4 and 37°C (Rehse and Shahrouri, 1998). The radicals induced oxidative stress on the red cell membrane resulting in haemolysis by lipid peroxidation (Cighetti et al., 1999). Sub-chronic intoxication of rats with PHZ (10 mg/kg/day for 8 days) resulted in a marked haemolytic anaemia characterised by decreased RBC, Hb and PCV (Unami et al., 1996). Similar results were obtained in our study when experimental rats were administered PHZ in order to induce anaemia. In addition, Ferrali et al. (1997) observed increased reticulocytosis, methaemoglobinemia and haemocatheresis in PHZ intoxicated rats. The main function of the RBC is the transportation of oxygen in to the tissues of the body. At such, any pathological or physiological condition that affects the RBC alters its function and this may be detrimental to the body. In this study PHZ altered the function of RBC by haemolysis characterised by decreased levels of RBC, Hb and PCV. PHZ increases reactive oxygen species (ROS) and lipid peroxidation, and decreases glutathione (GSH); these effects are reversed by N-acetyl cysteine, a known ROS scavenger (Amer et al., 2004). Thus, PHZ-induced haemolytic injury seems to be derived from oxidative alterations to red blood cell proteins rather than to membrane lipids (McMillan et al., 2005).

Anaemia is a disease characterized by a reduction in the concentration of haemoglobin, circulating red blood cell and pack cell volume per unit of the peripheral blood below the normal for the age and sex of the patient (Aguwa, 1996; Oma, 1991). Blood parasites, bacterial infections, viral infections, drugs/chemical agents and metabolic diseases may result in destruction of red blood cells leading to haemolytic anaemia (Ramzi et al., 1994). Administration of PHZ to rats also resulted in an increase in the MCV and
MCH values which are indicators of macrocytosis thus describing the anaemia as macrocytic. This condition is also common in Vitamin B\textsubscript{12} and folate deficiencies probably as a result of iron deficiency (loss of iron). Macrocytic anaemia has also been reported in rats infected with \textit{Trypanosoma Brucei brucei} (Erah et al., 2003) and this has been linked to iron deficiency anaemia (Mwangi et al., 1995).

In this study, PHZ altered the haematological parameters by haemolysis characterized by decrease in haemoglobin concentration, total RBC counts and PCV on day 3. However, the haematological parameters were restored to normal range after treatment with fruit juice of \textit{Opuntia elatior} Mill. The middle administrated dose of 10 ml/kg reduced the recovery time of the blood parameters from 15 days in the anaemic control to 10 days. Also the recovery was progressive such that after 15 days of continuous treatment, the haemoglobin concentration was higher in group D10, D15 and E treated rats than in the negative control group. It was also observed that the recovery of the treated groups was dose dependant with the highest dose of 15 ml/kg effecting the highest change. Giving the highest dose 15 ml/kg fruit juice to normal rats did not significant alter the haematological parameters.

Leucocytosis with neutrophilia and lymphopenia is at its maximum on day 3, hypersegmented neutrophils were observed rarely, phagocytosing blood lymphocytes are frequent on day 3. The fruit juice normalized the total and differential WBC counts after PHZ administration in rats. The results indicate that fruits of \textit{Opuntia elatior} Mill. improve the host defense mechanism of the body. PHZ treatment induces hypercellularity with erythroid hyperplasia (Criswell et al., 2000). The fruit juice also improved the platelet functions in PHZ treated rats.

This anaemia which resulted from the early lysis of the red blood cells was naturally reversed 7 days later by the regeneration of these blood cells due to the increase of the reticulocytes. Our results indicate that the fruit juice 10 ml/kg and 15 ml/kg dose increased significantly the number of reticulocytes, mainly 7 days after PHZ administration. Moreover, the fruit juice of \textit{Opuntia}
elatior Mill. potentiates the increase of the number of reticulocytes. The fruit juice could stimulate erythropoisis process.

The measurement of serum ferritin level provided a reliable estimate of iron store. The increase of serum ferritin level in group B treated rats indicates that it may be due to liver damage by PHZ. The fruit juice restored the liver functions and did not make significant change in serum ferritin level. Based on the biochemical and histopathological results, Opuntia elatior Mill. fruit juice also reserved the toxic effect of PHZ on liver, kidney and spleen. There is not significant change in cholesterol and triglyceride level in fruit juice treated rats.

The speedy and progressive recovery of anaemic rats responding to treatment of Opuntia elatior Mill. fruits may be due to increased erythropoiesis. The improvement in the haematological indices exhibited by fruit juice might be connected with the minerals, phenolics and betacyanin content of the fruits of Opuntia elatior Mill. The phenolics and betacyanin have remarkable antioxidant activity. These constituents might have direct influence on the protection of haemolysis by reactive oxygen species generated by PHZ. These results support the folkloric use of Opuntia elatior Mill. fruits in the treatment of anaemia.

The potential antinociceptive as central analgesic by using tail immersion test and peripheral analgesic by using acetic acid induced writhing test of the fruits of Opuntia elatior Mill. was investigated. The antinociceptive tests used in the present work were chosen in order to test different nociceptive stimuli, namely cutaneous thermic (tail immersion) and chemical visceral (writhing) stimuli. The results indicate that oral administration of the fruit juice of Opuntia elatior Mill. exhibit central and peripheral analgesic properties, since it exerted a significant and dose-dependent protective effect on chemical (acetic acid injection) and thermic (heat) painful stimuli. Such an efficacy on these two stimuli is characteristic of central analgesics like morphine and tramadol while peripheral analgesics like diclofenac sodium, aspirin are known to be inactive on thermic painful stimuli.
The results demonstrate that the fruits of *Opuntia elatior* Mill. attenuated the nociceptive responses to chemical stimuli in the acetic acid-induced abdominal constriction. The mean number of abdominal contractions was reduced from 25 to 7 at the respective doses of 5 and 15 ml/kg. Diclofenac sodium, the peripheral analgesic drug also produced similar antinociceptive action. It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs and opioids (Collier et al., 1968; Dai et al., 2002). This test is generally used for the screening of central and peripheral analgesic effects (Koster et al., 1959; Vogel and Vogel, 1997). The centrally acting protective effect of the extract was also corroborated in our study by the tail immersion test results.

The analgesic efficacy and potency of acutely administered tramadol is comparable to that of codeine, pentazocine, or dextropropoxyphene (Hennies et al., 1988), while its analgesic and antinociceptive potency is only 5- to 10-fold lower than that of morphine (Lehmann et al., 1990). It is believed that tramadol works by μ-opioid receptors (Raffa et al., 1992) despite its relatively low binding affinity (Hennies et al., 1988). Thus, it is speculated that nonopioid mechanisms are involved in tramadol analgesia. In accordance with the recognized implication of noradrenaline and serotonin in pain modulation, tramadol has been shown to inhibit the re-uptake of noradrenaline and serotonin, thereby increasing the concentration of these two neurotransmitters in selected brain areas, thus raising the pain threshold (Driessen and Reimann, 1992; Raffa et al., 1992).

Lyophilized aqueous extract (100–400 mg/kg, i.p.) of the fruits of *Opuntia dillenii* (Ker-Gawl) Haw was evaluated for analgesic activity using writhing and hot plate test in mice and rat, respectively and also anti-inflammatory activity using carrageenan-induced paw edema in rats, the results exhibited dose dependent action (Loro et al., 1999). Taking this into consideration, it seems that the fruit juice of *Opuntia elatior* Mill. contains morphine and tramadol like components and other peripherally acting...
principles. According to this study, we can concluded that the fruits of *Opuntia elatior* Mill. is endowed with central and peripheral analgesic properties might be due to presence of phenolics and betanin content. In future experiments, studies with purified fractions of fruit will be conducted for further research for deriving mechanism involved in analgesic action.

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 in the present study to evaluate anti-asthmatic activity of fruit of *Opuntia elatior* Mill.

Bronchial asthma is characterized by increased airway reactivity to spasmogens (Cockcroft, 1983). 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 present study, significant increase in preconvulsion time was observed due to pretreatment with fruit juice of *Opuntia elatior* Mill., when the guinea pigs were exposed to either acetylcholine or histamine aerosol. This
bronchodilating effect of fruit juice at high dose was comparable to ketotifen and atropine sulfate. Spasmolytic effect of *Opuntia elatior* Mill. fruit was also evaluated by observing the effect of fruit juice (10, 50 and 100 µl/ml) on acetylcholine and histamine induced ileum contractions to seek for scientific evidence for beneficial use of fruits in spasm produced by any means. The results showed antagonistic effects of the fruit juice against the contraction induced by the standard spasmogens. The results of this study indicated a rightward shift in the log dose-response curve of acetylcholine and histamine in the presence of the fruit juice of *O. elatior* Mill. The maximum effects of acetylcholine and histamine induced contractions were inhibited in the presence of the fruit juice. The non-parallel rightward shift in acetylcholine and histamine log dose-response curves obtained in the presence of the fruit juice, with lowered maximum contraction effect to acetylcholine and histamine would indicate a non-competitive or an irreversible antagonistic effect of *Opuntia elatior* Mill. fruits at muscarinic and histamine H₁ receptors (Linden et al., 1993). In this case the antagonist binds irreversibly to receptor site or to another site that inhibits response to the agonist.

Control of tension in gastrointestinal smooth muscle is dependent on the intracellular Ca²⁺ concentration. In general, there are two types of excitation–contraction coupling based on the type of mechanism responsible for changes in Ca²⁺ concentration. Electromechanical coupling requires changes in membrane potential, which in turn activate the voltage-dependent Ca²⁺ channel to trigger an influx of Ca²⁺ (Sadraei et al., 2003). Acetylcholine and histamine caused contraction via specific receptors and can produce changes in tension (Elorriaga et al., 1996). Both acetylcholine and histamine have functional roles in natural contraction of gastrointestinal tract. Acetylcholine is a neurotransmitter at post-ganglionic parasympathetic neurons that innervate the gut. The response to acetylcholine is mediated by activation of two types (M₂ and M₃) of muscarinic receptors (Goyal, 1988; Levey, 1993). Activation of these receptors results in an increase in intracellular Ca²⁺, an effect mediated by inositol triphosphate acting on internal calcium stores (Caulfield, 1993; Eglen et al., 1996; Elorriaga et al., 1996). Serotonin (5-HT) is also an important substance in the gastrointestinal tract and is present in
both enterochromaffin cells of the mucosa and neurons of the mesenteric plexus; it affects both secretion and motor activity (Briejer et al., 1995; Kunze and Furness, 1999). The histamine contraction is mediated by the release of acetylcholine from the cholinergic neurone as well as activation of serotonergic receptors on the smooth muscles of ileum (Sander-Bush and Mayer, 1996). This experiment showed that *Opuntia elatior* Mill. fruits possess a significant inhibitory effect on rat and guinea pig ileum contraction via antihistaminic and antimuscarinic action.

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, chemotactic, and immunomodulatory cytokines over a period of several hours (Okayama et al., 1995; Toru et al., 1998; Kobayashi et al., 1998). The bioactivities of these mediators include bronchoconstriction (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 (Boyce, 2003). 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
mast cells. Simultaneously it blocks H\textsubscript{1} 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 (Saraf et al., 2000).

In the present study, the fruit juice of \textit{Opuntia elatior} Mill. was found to inhibit the degranulation of mast cells induced by an immunological and 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 (Razin et al., 1995). Anaphylactic and compound 48/80 induced secretion from mast cells share a common requirement as far as the presence of calcium ions is concerned. However, compound 48/80 can utilize intracellular calcium stores to initiate the release process, even in the absence of calcium in the extracellular medium (Burka, 1984). 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 (West, 1983; Saraf et al., 2000). A significant protection of rat peritoneal mast cells from disruption by antigen and compound 48/80 by fruit juice of \textit{Opuntia elatior} Mill. 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 fruit juice of \textit{Opuntia elatior} Mill. on mast cell surface could be due to its ability to alter the influx of calcium ions.

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 (Bousquet et al., 2000). 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 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 (Basu and Nag Chaudhuri, 1991). 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 (Van Arman et al., 1965; Vinegar et al., 1969; DiRosa et al., 1971; Crunkhon and Maecoock, 1971). It is well established fact that non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory activity by inhibition of prostaglandin biosynthesis (Van, 1971). The anti-edematogenic mechanism of action of *Opuntia elatior* Mill. fruit 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 (Ferreira, 1972; Ferreira and Nakamura, 1979).

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 (Dale and Foreman, 1984). 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 (Susan and Qutayba, 2007). 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 (Hayllar and Bjarnason, 1991; Roos and Dolman, 1990; McColl and Showell, 1994). Mucosal recruitment of neutrophils involves sequential adhesion and transmigration across endothelial, lamina propria and epithelial
compartments (Madar, 1994). Subsequent adhesion to apical epithelial membranes results in activated neutrophils persisting in crypt abscesses with local release of chemotactic and chemoactivating substances (Nathan, 1987; Opal et al., 1994). 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 (Soussi-Gounni et al., 2001; 2001a). 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 (Adams and Shaw, 1994). Neutrophil adhesion to the vascular endothelium as described in the 'traffic signal' paradigm, results from the sequential recruitment of selectins, \( \beta_2 \)-integrins, tissue bound factors (IL-8, PAF) and products of the immunoglobulin gene superfamily (Springer, 1994). Mucosal inflammation is associated with an increase in the expression of vascular and leucocyte adhesion molecules (Balsitis et al., 1994; Dippold et al., 1993). 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 \( \beta_2 \)-integrins (Susan and Qutayba, 2007). Neutrophil adhesion to endothelium is enhanced by activation of adenosine A\(_1\) receptors. Binding to the adenosine A\(_2\) receptor results in inhibition of the respiratory burst reaction and decreased binding to fibrinogen (Prescott et al., 1990; Bouma et al., 1994; Dianzani et al., 1994; Cronstein et al., 1992; Meenan et al., 1996). In present study, fruit juice of *Opuntia elatior* Mill. 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.
In conclusion our data suggests that fruits of *Opuntia elatior* Mill. has potential anti-asthmatic activity that may be due to its bronchodilator, mast cell stabilization, anti-inflammatory and reduction of neutrophil adhesion property. The fruit juice was found reddish purple in color due to presence of betanin in higher concentration. So betanin might be responsible for anti-asthmatic action due to its anti-oxidant and/or other property.

The peel of various fruits serves the protective action against microbial invasion. So we aimed to evaluate the antimicrobial activity of fruit peel of *Opuntia elatior* Mill. The peel extracts of fruit exhibited antimicrobial actions in a dose dependant manner against both test bacteria and fungi. Antimicrobial activity of the peel extracts is directly concerning with the components that they contain. Petroleum ether, benzene and methanol extracts showed maximum inhibitory action against gram positive bacteria, gram negative bacteria and fungi, respectively. The *in vitro* antimicrobial activity order of peel extracts summarize in table 4.21. Among constituents of plants, polyphenols have received a great deal of attention, in recent years, due to their diverse biological functions. The antimicrobial activity of the polyphenols, tannins and flavonoids, is well documented. The tannin-rich peels are byproducts of food industry, and are only used in animal feeds in many developed countries. In fact, the antimicrobial activity of peels has been demonstrated against pathogenic bacteria by Ahmad and Beg (2001), Al-Zoreky (2009), Machado et al. (2003), Shan et al. (2007). In future experiments, studies with purified fractions of peel extracts will be conducted for identification of antimicrobial leads.

Taken together, these phytochemical results indicate that the flavonoids betacyanin equivalent to betanin is the active principle in the fruits of *Opuntia elatior* Mill. The fruits are exhibiting haematinic effect against anaemia induced by mercuric chloride and phenylhydrazine while antinociceptive effect against thermal and chemical stimuli. Fruits also appear anti-asthmatic property. Furthermore, peel extracts of fruit appear antimicrobial property against bacteria and fungi. These findings on haematinic, analgesic and anti-asthmatic effects of fruit juice, further add value to the nutritional characteristics of the fruits of *Opuntia elatior* Mill.