EVALUATION OF HYDROETHANOLIC EXTRACT OF _OPUNTIA MONACANTHA_ HAW FOR ANALGESIC ACTIVITY

Muhammad Kifayatullah¹*, Ishrat Waheed ², Sreemoy kanti Das, Mrutyunjay Sisugoswomi, Izharullah

¹Riphah International University Islamabad.
²Lincoln University College, Selangor, Malaysia.

ABSTRACT

_Opuntia monacantha_ Haw belongs to family of Cactaceae, having great potency in treating certain common diseases like fever, urinary problem, tumors, inflammations, piles, anemia, ulcers and bronchitis traditionally. In research papers the cladodes of _Opuntia monacantha_ Haw has shown good effects against Diabetes, Microbes and Oxidative stress; however no work was carried out for in vivo analgesic activity. Here is an attempt was made to investigate the hydroethanolic extract of _Opuntia monacantha_ Haw for analgesic activity compared to that of Aspirin and Diclopenic drug as a reference drug. The experiment was done at the doses of 200mg/kg, 400mg/kg and 800 mg/kg body weight in Balb C mice. The peripheral analgesic activity was studied by acetic acid induced writhing and central analgesic activity was studied by hot plate method. Intraperitonal administration of extract produced significant (p< 0.05) reduction in number of writhing compared to control. In hot plate method the extract significantly prolong paw licking (p< 0.001) in comparison with Diclopenic sodium. Pretreatment with Naloxone decrease reaction time and non significant p> 0.05 while combined treatment prolonged the latency time p< 0.001 showed the involvement of endogenous opioid peptides in the treatment of analgesic response. The systemic pretreatment of mice with Caffeine (10mg/kg) decrease the latency time while combined treatment significantly increased p< 0.001 the analgesic response. Pretreatment with MSG alone decreased the latency time while combined treatment prolonged the latency time significantly p<0.001 showed the involvement of NMDA receptors in analgesic effect.

Keywords:  _Opuntia monacantha_ Haw, Aspirin, Diclopenic sodium Caffeine, MSG, Naloxone.
INTRODUCTION

“Opuntia” is the largest and most attractive member of Cactaceae family, native to Mexico, having approximately 300 to 400 species, the most cultivated cactus, widely distributed in South American continent and Mexico. (El-Samahy, 2006). The genus Opuntia monacantha Haw” commonly known as dropping prickly peer, belongs to family of Cactaceae. (Bari, M.N., Zubair M, Rizwan K., 2012). The plant grows in Europe and Asia. (Hanelt, P., 2001). Chemical compounds such as tannins, phenol, terpenoids and saponins have been isolated from Opuntia monacantha Haw that shows great effects against many diseases. (Bari, M.N., Zubair M, Rizwan K., 2012). The plant grows in Europe and Asia. (Hanelt, P., 2001). The plant is a small tree with fibrous roots that grows upto a height of 5m. The whole plant is about 45cm long, 15cm wide and 1.5cm thick. Flowers are yellow in colour with red spots on the back. The cover of fruits are red and contains seeds that are yellow or pale brown in color. (T.K. Lim., 2012). The ethanolic extract of Opuntia monacantha Haw has significant in vivo hypoglycemic effect in streptozocine induced diabetic rats. (Zhao, M., kuin S, tony E., 2007). In traditional medicine various parts of the plant are used as laxative, carminative and fever. The plant is useful in the treatment of urinary problems and provides an effective treatment for inflammations, tumor, piles, ulcers, anemia and enlargement of the spleen. (Ahmad, S.S., 2007). The juice of the plant is used in ophthalmic, liver complaints, cough and expectorant. (Jabeen, A., et. al. 2010). The cladodes of Opuntia monacantha Haw possess potential antioxidant activity. (Yang., et. al.2010). However no in vivo analgesic activity on Opuntia monacantha Haw has been reported, hence, the present study was undertaken to determine the in vivo analgesic activity of the hydroethanolic extract of Opuntia monacantha Haw in Balb C mice.

MATERIALS AND METHODS

Chemicals

Acetyl salicylic acid (Waku Pure chemical industries. Osaka Japan). Diclofenic sodium and Paracetamol (leads pharma Islamabad). Acetic acid (Sigma Aldrich), Caffeine (Alfa Aesar Karlsruhe, Germany), Stop watch, Digital hot plate (AREC, VELP Scientific), Nalaxone Hcl injection (Nalox, Rotex Medica), Monosodium glutamate (Ajinomoto Co., INC., Tokyo, Japan), Digital thermometer (Holden medical B.V, Netherland)

Test animals

The animals used in the study were Balb C mice (25-35gm), within age 3-4 week of either sex. The animals were kept in cages at animal house, and maintained at room temperature of
25°C±2C with relative humidity (60 ± 10%) under 12 hrs night and light cycle. The animal will kept in fast for 24hr before experiment. (*OECD 425,2001*).

**Collection and Extraction of Plant**
The whole plant of “*Opuntia monacantha Haw*” was collected from District Malakand region of the Khyber Pukhtoon Khwa Province, and was identified by Dr. Ikramullah (Department of Botany Agriculture University, Peshawar) with voucher specimen no. BOT: 335. After washing the cladodes were dried in shade for 20 days at room temperature. The cladodes were grinded and were extracted through maceration technique using 80% ethanol (1:5) as a solvent for 72 hours. After maceration, the mixture was then filter through Whatman #1 paper and was then concentrated under reduce pressure and then air dried.

**Acute toxicity study**
Acute toxicity study of the extract was determined on Balb C mice. The dose of extract was increased one to three folds to determine the safety level of the extract. The mice were divided into three groups each contained two mice. The first group received only normal saline and the second and third groups received i.p injection of tested drug at doses of 1000mg/kg and 3000mg/kg of doses and mice were observed for 72 hrs for any toxic effect. (*OECD 425,2001*).

**Acetic Acid Induced Writhing**
Balb C mice (25-35gm) were divided into five groups of six animals each. The first group used served as negative control received only normal saline solution. The 2nd group was considered as positive control received Aspirin 100mg/kg body weight, while group 3rd group 4th and group 5th received the extract of *Opuntia monacantha Haw* at dose level of 200mg/kg, 400mg/kg and 800mg/kg, respectively. After 1 hr, 0.6% v/v of acetic acid solution (10ml/kg) was administered intraperitonialy for induction of pain to each group of mice individually. The writhing was counted for 20 minutes just 05 minutes after the intraperitonial administration of acetic acid solution. The number of writhing in each treated group was compared to that of a control group. (*Koster R, Anderson M and De Bear EJ (1959)*). The % of analgesic activity (inhibition of writhing) was calculated by following equation (*Sharma, A., et. al., 2011*)

\[
\% \text{age of inhibition} = \frac{N_c - N_t}{N_c} \times 100
\]

Where \(N_c\) = Average number of writhing of negative control per group
And \(N_t\) = Average number of writhing of test per group.
Hot Plate Method

Adult Balb C mice (25-35gm) were divided into five groups of six mice each. Group 1st were considered as negative control, received normal saline solution intraperitonialy. Group 2nd served as positive control received Diclofenic sodium at a dose of 10mg/kg. Group 3rd, 4th and 5th were treated with *Opuntia monacantha Haw* extract at a dose of 200mg/kg, 400mg/kg and 800mg/kg, respectively. Analgesic test was carried out by placing mice on Eddy hot plate. The temperature of hot plate was maintained at 5C°±0.5. The time between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. Response of latency of the animal was noted down in hot plate for three hours with 30-minute’s interval after treatment. *(Okokon, J.E. and P.A. Nwafor, 2011).*

**Hot plate method for Analgesic activity in the presence of Agonist and Antagonists**

The analgesic activity of the hydroethanolic extracts of *Opuntia monacantha Haw* was also determined in the presence of agonist and different antagonist. Adult Balb C mice (25-35gm) were divided into eight groups contained six mice in each. The first group was considered negative control which received only normal saline solution. The 2nd group received *Opuntia monacantha Haw* extract at a dose of 400mg/kg. Group 3rd, 4th, and 5th received Caffeine (Adenosine receptor antagonist), Naloxone (Opioid receptor antagonist) and monosodium glutamate (Monosodium glutamate receptor agonist) at a dose of 10mg/kg, 2mg/kg and 1000mg/kg alone respectively. Group 6th, 7th and 8th were first pretreated with caffeine, naloxone and monosodium glutamate at a dose of 10mg/kg, 2mg/kg and 1000mg/kg, respectively followed by thirty minutes later the extract of *Opuntia monacantha Haw* with a dose of 400mg/kg. The latency time period was recorded before and 30, 60, 90, 120, 150, 180 minutes after administration of drug for group 1- 5th. Similarly, latency time period for group 6th to 8th animals was noted 30 minutes after the administration of caffeine, naloxone and monosodium glutamate separately.

**RESULTS**

The present study was carried out to find out the analgesic effects of the hydroethanolic extract of *Opuntia monacantha Haw*. The methods used in this study were acetic acid induced writhing and hot plate methods.

**Acute toxicity Study**

The hydroethanolic extract of *Opuntia monacantha Haw* was tested at two doses, 1000mg/kg and 3000mg/kg for toxicity and was compared with control (normal saline group). No major
behavioral changes or mortality were noted post administration of the extract at dose of 1000mg/kg for 72 hrs. While death was observed at the dose of 3000mg/kg of extract for 72 hrs.

**Acetic Acid Induced Writhing**

Pretreatment of mice with the extract of *Opuntia monacantha Haw* at a dose of 200, 400 and 800mg/kg body weight produced a very significant reduction in writhing induced by acetic acid p <0.05 when compared to control. The %age of inhibition of writhing in mice were found as 25.21%, 44.26 % and 66.10% for extract at dose of 200, 400 and 800mg/kg body weight, respectively. The positive control group (Aspirin 100mg/kg) was found more potent analgesic compound produced significant effects p < 0.001 in writhing than plant extracts at all dose levels and %age of inhibition was 79%. (Table 1.1).

**Hot Plate Method**

The treatment of mice with extract of *Opuntia monacantha Haw* at a dose of 200, 400 and 800mg/kg body weight (dose dependent) i.p significantly prolonged the latency time as compared to control. The increase in reaction time was significant p < 0.001 as compared to normal control. The significant effect began early 30mintues after i.p administration of the extract and last until following 150 minutes. The increase in reaction time was dose dependent and maximum effects showed by extracts at 150 minutes with latency time for 200mg/kg was 31.66±0.882, for 400mg/kg was 41.83±1.195 and for 800mg/kg latency time was 44.16±1.641. After 150min the latency time began to fall for all doses of extract. The latency time for reference drug was also maximum at 150mint (53.16±0.980) and then start declining. The hydroethanolic extract of *Opuntia Monacantha Haw* shows significant analgesic activity p<0.001 as compared to negative control. (Table 1.2).

**Effect of pretreatment of Caffeine on Opuntia monacantha Haw**

Caffeine (10mg/kg) alone i.p administration into mice group showed non significant p > 0.05 difference in latency time as compared to normal control. However, in another mice group pretreated with caffeine (10mg/kg) decrease latency time and after i.p administration of 400mg/kg of extract the latency time began to increased 30min after caffeine. The increased in latency time continued until 150 minutes and then start decreasing. The increase in latency timed p < 0.001 significant as compared to normal control. It showed that adenosine receptor has involvement in antinociception effect of the hydroethanolic extract of *Opuntia monacantha Haw*. The systemic pretreatment of mice with caffeine (10mg/kg) significantly
reverse the antinociception caused by hydroethanolic extract of *Opuntia monacantha* (400mg/kg). The latency time for adenosine receptor antagonist, *Opuntia monacantha* extract, negative control and combined treatment consisting of caffeine and extract are shown in Table 1.3.

**Effect of pretreatment of Naloxone on *Opuntia monacantha* Haw**

The mice were first pretreated with opioids receptor antagonist (nalaxone 2mg/kg); 30 minutes before the administration of selective best dose of extract 400mg/kg in hot plate. Naloxone 2mg/kg alone group i.p administration decreased the latency time and showed non significant response p >0.05 as compared to normal control. However, nalaxone plus extract treated group showing initially decrease in the latency period; completely reverse the analgesic effect of *Opuntia monacantha* extract (400mg/kg). After 30 min, latency time began to increase in naloxone plus extract group. The increase in latency timed p< 0.001 was significant as compared to normal control. This showed the involvement of endogenous opioid receptors in the medication of analgesic response of *Opuntia monacantha* extract Table 1.4.

**Effect of pretreatment with MSG *Opuntia monacantha* Haw extract**

Mice were first pretreated with glutamate receptor agonist (Monosodium glutamate 1000mg/kg), in hot plate. Monosodium glutamate decrease the analgesic effect of *Opuntia Monacantha* extract (400mg/kg). MSG (1000mg/kg) alone i.p administration into mice group showed non significant p >0.05 difference in latency time as compared to normal control. However, in another group pretreated with MSG (1g/kg) decrease latency time and after i.p administration of 400mg/kg of extract the latency time began to increased 30min after glutamate receptor agonist. The latency time period for MSG and extract treated group increased and level of significance was p < 0.001, which indicates that glutamate receptor also participated in analgesic effect of *Opuntia monacantha Haw* extract (Table 1.5).

### Table 1.1: Analgesic activity in acetic acid induced writhing in Balb C mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of writhing (Count/15min)</th>
<th>% age of inhibition writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(Normal saline)</td>
<td>46.50±1.118</td>
<td>0</td>
</tr>
<tr>
<td>STD(Aspirin)100mg/kg</td>
<td>10 ±0.365 ***</td>
<td>79.41 ±1.862</td>
</tr>
<tr>
<td>Extract 200mg/kg</td>
<td>34.66 ±0.715 ***</td>
<td>25.21 ±2.413</td>
</tr>
<tr>
<td>Extract 400mg/kg</td>
<td>25 ±0.856 ***</td>
<td>44.26 ±2.020</td>
</tr>
<tr>
<td>Extract 800mg/kg</td>
<td>15.66 ±0.803 ***</td>
<td>66.10 ±2.263</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. Significant at * p<0.05, ** P< 0.01 and ***P<0.001 shows significant variation from control
### Table 1.2: Analgesic activity in Hot plate method in Balb C mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Latency time in second</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control Negative</td>
<td>8.16±1.167</td>
</tr>
<tr>
<td>Control Positive</td>
<td>10.16±1.122</td>
</tr>
<tr>
<td>Extract 200mg/kg</td>
<td>8.33±1.498</td>
</tr>
<tr>
<td>Extract 400mg/kg</td>
<td>9.50±0.992</td>
</tr>
<tr>
<td>Extract 800mg/kg</td>
<td>8.66±2.028</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. Significant at * p<0.05, ** P< 0.01 and ***P<0.001 shows significant variation from control

### Table 1.3: Effect of Caffeine on analgesic response of Opuntia Monacantha extract in hot plate test

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Latency time in second</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>11.50±1.335</td>
</tr>
<tr>
<td>Extract 400mg/kg</td>
<td>12.66±1.647</td>
</tr>
<tr>
<td>10mg/kg Caffeine</td>
<td>13.33±0.882</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. Significant at *** P < 0.001 when compared to control N = 6

### Table 1.4: Effect of Naloxone on analgesic response of Opuntia Monacantha Haw

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Latency time in second</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>11.50±1.335</td>
</tr>
<tr>
<td>Extract 400mg/kg</td>
<td>12.66±1.647</td>
</tr>
<tr>
<td>2mg/kg Nalaxone</td>
<td>13±1.1.183</td>
</tr>
<tr>
<td>Naloxone+Extract</td>
<td>10±2.129</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. Significant at *** P < 0.001 when compared to control
### Table 1.5: Effect of Monosodium glutamate (MSG) on analgesic response of Opuntia Monacantha extract in hot plate test

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Normal saline)</td>
<td>11.50±1.335</td>
<td>12.83±1.302</td>
<td>12.50±1.945</td>
<td>14±1.155</td>
<td>11.16±1.662</td>
<td>11.66±1.978</td>
<td>10.83±1.759</td>
</tr>
<tr>
<td>Extract 400mg/kg</td>
<td>12.66±1.647</td>
<td>19.33±1.726</td>
<td>23.83±3.005</td>
<td>28.83±2.197</td>
<td>33±1.183</td>
<td>34.66±1.166</td>
<td>29±2.820</td>
</tr>
<tr>
<td>1000mg/kg MSG</td>
<td>13.16±1.195</td>
<td>12.66±1.054</td>
<td>11±2.129</td>
<td>10±2.066</td>
<td>9.66±1.563</td>
<td>12.16±2.212</td>
<td>11.33±1.606</td>
</tr>
<tr>
<td>Caffeine +Extract</td>
<td>11±1.366</td>
<td>12.83±1.352</td>
<td>15±1.366</td>
<td>20.33±1.801</td>
<td>27.50±2.110***</td>
<td>30.33±1.585***</td>
<td>27.83±2.301***</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. Significant at *** P < 0.001 when compared to Control N = 6

---

**Figure 1.1: Analgesic activity of Opuntia monacantha Haw by acetic acid induced writhing test**

---

**Figure 1.2: Analgesic activity of Opuntia monacantha Haw by Eddy’s Hot plate method**
Data expressed as mean ± SEM. Significant at *** P < 0.001 when compared to control N = 6

Figure 1.3: Analgesic effect of Opuntia Monacantha Extract in the presence of caffeine

Data expressed as mean ± SEM. Significant at *** P < 0.001 when compared to control

Figure 1.4: Analgesic effect of Opuntia Monacantha Extract in the presence opioid receptor antagonist Naloxone

Data expressed as mean ± SEM. Significant at *** P < 0.001 when compared to control

Figure 1.5: Analgesic effect of Opuntia Monacantha Extract in the presence monosodium glutamate receptor agonist (MSG)
DISSCUSSION
The result of the present study showed that the hydroethanolic extract of the cladodes of *Opuntia monacantha* Haw has significant effects against pain in different analgesic models in Balb c mice. The analgesic effect of *Opuntia Monacantha* extracts was determined by hot plate and acetic acid induced writhing method. Acetic acid causes inflammatory pain by increasing the flow of small molecules and large in and out of blood vessel and increasing endogenous substances that causes pain at nerve ending. It has been reported that prostaglandin E1 and PGE2 peripherally, increased with acetic acid. (Abdel-Salam, O.M.E., 2005). Intraperitonal administration of acetic acid releases inflammatory mediators and stimulates nociceptors, increase the release of arachidonic acid from tissue phospholipids and by enzyme cyclooxygenase, converts them into prostaglandins and leads inflammatory pain. The analgesic effect of *Opuntia Monacantha* may be due to the blockade of effects or release of endogenous substances that produces pain in nerve endings in similar mechanism as shown by NSAIDs. The reduction in the number of writhing indicates that *Opuntia Monacantha* might exert analgesic effect by inhibiting the synthesis and release of PGs or blocking their receptors. The acetic acid produced pain effect due to release of mediators such as histamine, serotonin, bradykinine, cytokines and eicosanoids (prostaglandins, prostacyclin, Leukotrine, and thromboxane) from mast cells. (Thamizhselvam, N.S, Soumy., 2012). The hydroethanolic extract of *Opuntia Monacantha* exhibited analgesic activity by significantly (p< 0.05) reducing the number of abdominal contractions in acetic acid induced writhing. At the dose of 200, 400 and 800mg/kg the extract produced the analgesic effects that were comparable to the standard drug (Aspirin), and an increasing in the percent of inhibiting writhing values were noted as 25.21 ±2.413%, 44.26 ±2.020 % and 66.10 ±2.263%, respectively. The Aspirin, produced inhibition of writhing movement inhibiting peripheral pain induced by direct action of acetic acid. The number of inhibition of writhing of standard drug was 79.41%±1.862. Result of writhing test showed that the maximum inhibition effects on writhing of *Opuntia monacantha* was found to be about 66.10% at a dose of 800mg/kg over the control. The highest inhibition effect of the test drug was comparable to the effect of aspirin (79%). In writhing methods, writhing are due to increased level of PGE2α and PGF2α in peritoneal fluid and enzyme lipoxygenase, which is responsible to developed pain. (Dinda, A., et al., 2011). The analgesic effect of *Opuntia Monacantha* extract was also determined by hot plate method. Normally threshold of pain toward heat evaluate by central acting analgesic. The hot plate method was used to evaluate central analgesic activity. (Gunn, A, 2011). The hot plate method is specific for
opoid derived analgesics. (Abbott,F.V. and R. Melzack., 1982). Intraperitonal treatment of the extract produced a potent analgesic effect on thermal stimuli and thus confirmed that the extract also posses central analgesic activity. The extract of the plant administered i.p at different doses (200,400 and 800mg/kg) and Diclofenic sodium (10mg/kg) presented increase in latency time when compared to control group. *Opuntia Monacantha Haw* showed significant p< 0.001 analgesic effect when compared to control group. Maximum analgesic effects of *HEOM* was observed after 90 min of administration and remained upto 150 min. Here, in hot plate method the *HEOM* prolonged the latency time which shows that extract also acts like central acting analgesic.

The analgesic activity of *HEOM* was also checked to investigate involvement of Opioid receptors by using naloxone (opioids receptor antagonist). The participation of the opioid system in antinociception was determined by i.p injection of naloxone (2mg/kg). Animals were pretreated with naloxone. Naloxone being an Opioids receptor’s antagonist is traditionally used to reverse the analgesic effect of Opioids by binding to opioid receptors specially to μ (μ) opioid receptor than delta and kappa receptor (δ,k).( Jordan, B.A.and L.A.Devi.,etal.1999). Early reports revealed that opioids drug like morphine, produces analgesic effect primarily through μ opioid receptor, while the involvement of k receptor in reducing pain was not clear. In fact, the k receptor agonist also produces analgesic effect, because it hyperpolarizes the neuron that are indirectly activated by μ opioid receptor.( Pan, Z., S. Tershner, and H. Fields,2011). Opioid receptors are the member of G protein coupled receptor family and their function is to inhibit Adenyl cyclase(enzyme converting adenosine triphosphate to cyclic AMP), increasing flowing of k⁺ outwards, inhibiting ca⁺⁺ entrance and thus inhibiting neuronal firing and release.( Richard A. Harvey PhD, 2011).The results showed that naloxone (opioid antagonist) reversed the analgesic effect of extract in hot plate test. Pretreatment with nalaxone (2mg/kg), significantly decreased the latency time, while treatment in combination *Opuntia Monacantha* (400mg/kg, and naloxone (2mg/kg, i.p) produced decreased in latency time when compared to test drug alone and increased latency time p<0.001 as compared to normal control. So we concluded that the analgesic effect of tested extract may be due to involvement of opioid receptors like opioids drug.

Adenosine is a purine nucleoside present in all organs and tissues, producing potent physiological action (widening of blood vessels, lowering of blood level and heart stimulants by interacting with A₁ and A₂ adenosine receptors, where AR agonists may cause blockage
and stimulation of Adenyl cyclase (converting ATP to cyclic AMP) (Daly, J., Jacobson, K. and Ukena, D., 1987). Adenosine has the capability of inhibiting Adenyl cyclase enzyme, opening of voltage gated K\(^+\) channels and lowering of extra cellular ca\(^{++}\) channel and thus lowering majority of neurotransmitters released.(Fredholm, B. and T. Dunwiddie, 1988). Four subclasses of adenosine receptor \(A_1\), \(A_3\), \(A_2A\) and \(A_2B\) with variety of role and occurrences in the body have been identified. All belongs to 7 transmembrane (G protein couple receptors). \(A_1\) and \(A_3\) coupled to \(G_i\), inhibiting adenyl cyclase (responsible for cAMP) are inhibited by \(A_1\) and \(A_2\) receptors lowering cAMP and adenyl cyclase enzyme activated through \(G_s\) increasing cyclic AMP.(Kiec-Kononowicz, K., 2001). In peripheral analgesic effect, produced by adenosine \(A_1\) receptor (\(G_i\)) by lowering cAMP level, adenosine \(A_2\) receptor’s activation increased cyclic AMP levels in nerve terminal produced central pain. Adenosine \(A_3\) receptor’s activation produces pain behaviors due to the release of histamine and 5-hydroxytryptamine from mast cells. In the spinal cord, adenosine \(A_1\) receptor’s activation produces antinociceptive effects in acute nociceptive, inflammatory and neurophathic pain tests.(Sawynok, J., 1998). The result showed that pretreatment of mice by i.p routes with caffeine (10mg/kg) non specific adenosine receptors antagonist has significantly blocked the analgesic effect caused by Opuntia Monacantha (400mg/kg) when administered i.p in hot plate test. Pretreatment with caffeine (10mg/kg) alone significantly lowered the latency periods and increase sensitivity to pain (low pain threshold). While the combination of both hydroethanolic extract of Opuntia Monacantha (400mg/kg, i.p) and caffeine (10mg/kg, i.p) produced decreased in latency time when compared to test drug alone because of antagonizing effect extract through Adenosine A1 receptor by caffeine and increased in latency time p<0.001 as compared to normal control. As caffeine decrease the analgesic effect tested extract, so there is possibility that analgesic effect of hydroethanolic extract of Opuntia Monacantha may be through its agonistic action on adenosine \(A_1\) receptor.

Monosodium glutamate (Glutamate receptors agonist) acts on glutamate receptors including, (NMDA, AMPA/kainite, and metabotropic (present on pre and post synaptic membranes) and causes the release of excitatory mediators results in inflammation and nociception. Excessive activation of glutamate receptors can lead to excitotoxicity and neuronal death. (Schousboe,A.,2003). Excitatory aminoacid such as glutamate and NMDA are the main neurotransmitters in nerve ending of primary afferent (A delta and C fiber). From central terminals glutamate released by nociceptor activation caused depolarization in posterior horn neuron end, when excitation reached it causes positive charge ions inside and negative charge...
ion outside the cell. At resting membrane potentials, excitatory amino acid NMDA is closed because magnesium ions block the channels pores. (Woolf, C.J. and R.J. Mannion, 1999). Multiple receptors, especially, NMDA receptor are activated by release of peptides and glutamates in spinal card which causes spinal hypersensitivity. Blocking of excitability is one access but inhibitions of NMDA receptors may also provide analgesic effect. (Besson, J., 1996).

Studies have shown that administration of Monosodium glutamate (glutamate receptor agonist) causes damages of memory and injuries of hypothalamus neurons. (Park, C.H., et.al. 2011). Pretreatment with MSG (1g/kg) significantly decrease the latency time increased sensitivity to pain (low pain threshold) because glutamate mediates there action through NMDA and AMPA receptor which have role in development of pain. NMDA agonist increase neuron excitability in dorsal horn of brain and release various neurotransmitters like P substances which is important element in perception of pain. While the combination of both hydroethanolic extract of Opuntia Monacantha (400mg/kg, i.p) and MSG(1g/kg, i.p) produced decreased in latency time then compared tested extract and increased in latency time p<0.001 as compared to normal control. Analgesic activities of plant extract declining are due to agonist of monosodium glutamate through NMDA receptors. So we concluded that HEOM produced analgesic effect through antagonism of NMDA receptor. In near future NMDA antagonist may be emerge a new class of analgesic drug with least side effect.

CONCLUSION
The results of the present study showed that hydroethanolic extract of Opuntia monacantha Haw posses both peripheral and central analgesic activity and justify its use in traditional medicine as analgesic agent.

REFERENCES