Study comparison analgesic, antipyretic and anti-inflammatory activity of aqueous and alcoholic leaves extract of Lawsonia inermis L. (Henna) with ketoprofen in male albino rats

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Abstract

The pain considered is an important signs for many medical condition which is reduced by using analgesic drugs, that act either locally or systemically via central nervous system, that may have many harmful side effects. This study was carried out to evaluate the analgesic, antipyretic and anti-inflammatory effect of both aqueous and alcoholic extract of Lawsonia inermis L. and comparing with one of non-steroidal anti-inflammatory drugs (NSAID) Ketoprofen by using hot plate test, tail immersion method, and study ability of aqueous and alcoholic extract to reduce writhing induced by acetic acid injection. Also, study the antipyretic and anti-inflammatory activity of henna extract in formalin-induced paw edema. Twenty four Wister rats were used which divided randomly into four equal groups, first group served as control negative treated with distal water, second, control positive, injected with ketoprofen (8 mg/kg B.W.) third group treated with aqueous extract and fourth group treated with methanolic extract (200 mg/kg B.W.) in addition to the ketoprofen. At the end of experiment, the results clarified that alcoholic extract of henna has similar effect of ketoprofen via significant increase (P<0.05) in reaction time to hot plate and tail withdrawal from hot water. Moreover, the results explained significant decrease (P<0.05) in the writhes count( abdominal constriction). As well as, the ability of alcoholic extract to significant (P<0.05 ) reduce body temperature comparing with control group. Likewise, the result revealed that alcoholic extract of Lawsonia inermis has anti-inflammatory activity by significant (P<0.05 ) reduction in edema size induced by formalin injection in paw skin, and Tumor necrosis factor alpha. (TNF-α). Thus, the results of current study illustrated that methanolic extract of Lawsonia inermis has central and peripheral analgesic, antipyretic and anti-inflammatory activity comparing with control group.

Key words: Lawsonia inermis.ketoprofen, analgesic, antipyretic, anti-inflammatory.
Introduction

Pain is unpleasant sensation may associated with actual tissue injury and many medical conditions (1).Physiologically it's very important to mammalian, because act as a protective mechanism for the body especially to human being that indicate to internal problem. Pain modulated either centrally via dopaminergic, noradrenergic and serotonergic pathways (2,3) , or peripherally by inhibition of pain mediators such as prostaglandins, leukotrienes and other endogenous mediators that play important role in create pain (4).Non-steroidal anti-inflammatory drugs are analgesic drug used commonly in treatment fever and analgesic of pain and inflammation, which is thought relive 50% of pain (5). But, like other synthetic nature drugs NSAIDs has many side effect such as gastric ulcer, renal damage, and cardiovascular problem (6,7) The pharmacological action and medicinal properties of the most plants and herbs, as well as the higher safety capacity and low costs , render the herbal medicine are greater demand in the developing countries for primary health care (8). Previously, the natural products derived from plants are an important source for treatment of many disease worldwide (9). Thus, recent medicine convince that natural products is more efficient from synthetic origin drug, because of the synthetic agents are toxic to the hepatocyte, glomeruli and other side effect , while the agents derived from natural sources have fewer side effect (10).therefore, more than 50% of modern drugs are natural origin.

Lawsonia inermis L. which is commonly known as henna is a plant which has been used for staining hand and hair dye (11) also, it used in folk medicine to treat rheumatoid arthritis, headache , ulcer , diarrhea, fever, diabetic, cardiac disease and other medical cases (12). Lawsonia inermis L. have many phytochemical constituents such as carbohydrate, proteins, flavonoids, tannins, phenolic compounds and fatty acids (13). Many studies have confirmed the beneficial effects of methanolic extract of Lawsonia inermis L such as inhibit the growth of malassezia, act as fungistatic and fungicidal (14,15). Also, methanolic extract of henna leaves stimulate immune system via promotion T lymphocyte proliferation (16). As well as lawsonia inermis L. has been shown reduced
hyperglycemia and inflammation (17). Thus, Henna regarded as a medicinal plant because has many properties such as antibacterial, antifungal, antiamoebiasis, antihemorrhagic, hypotensive and sedation. The present study evaluate the anti-nociception, antipyretic and anti-inflammatory properties of aqueous and methanolic leaves extract Lawsonia inermis L comparing the results with ketoprofen.

Materials and methods

- **Experimental design**
  The study was carried out at the animal house of the kut technical institute in march 2017, twenty four albino rats were used in the current study, which is divided randomly into four equal groups: group 1 served as control group, administrated distal water, second group: control positive, injected with ketoprofen (8 mg/kg B.W.) third group treated with aqueous extract and fourth group treated with methanolic extract (200 mg/kg B.W.) in addition to the ketoprofen (8 mg/kg B.W.)

- **Collection of plants and preparation of extract**
  The leaves of L.inermis L. were purchased from local market and identified by national herbarium at Abu-Ghraib.
  
  o **Preparation of aqueous extract**
    The plant extract was performed according to the method of (18), the leave were washed with water and allowed to dry in open shade area for 2 weeks. The dried leave were powdered by electrical grinder and then mixed 100 gm of powdered leave with 500 ml of distilled water in flask, and then heated the mixture for 24 hours at 45 C° with shaking water path, and then filtered by using Whitman paper No.4, the filtrate was stored at 4 C° until use.
  
  o **Preparation of alcoholic extract**
    The leave were washed with tab water, it left to dried at room temperature and then ground into fine powder by using electrical homogenizer. The extract prepared by add 1000 gm of leave powder to 1 litter of 70% ethanol via soxhlet apparatus for more than 24 hrs. after that, the extract was placed in oven 50 C° for 6 hrs. to be free from solvent, finally the extract becomes readily to use (19).

- **Collection of blood samples**
  Blood samples were collected via direct heart puncture technique by using sterile syringe (2 ml) and placed in sterile tube to prepare the serum by centrifugation, serum was kept in deep freeze until using to determine TNF-α in rat serum.

- **Pytochemical screening of lawsonia inermis L.**
  The essential compounds of aqueous and alcoholic extract of lawsonia inermis L. were determined as follows:
  
  o **Flavonoids**: A mixture of 50% ethanol with 50% potassium hydroxide, 5 ml of solvent mixing with 5 ml of extract, if the color that appear is yellow it mean present of flavonoids (20).
  
  o **Phenolic compound**: Two milliliters of ferric chloride solution solution at concentration 1% were added to 3 ml of extract, the phenolic compound was indicated by appearance the green color in a mixture (21).
  
  o **Tannin**: Added 1 g. of lawsonia inermis L. leave powder to 30 ml of water and boiled the suspension in beaker and then added 0.2 ml of 1% ferric chloride, appear the dark blue color indicate of present tannin (22)
  
  o **Glycosides**: A mixture contain a concentrated HCL with a portion of lawsonia inermis L. was heated in water bath for 3 minutes, then 2 ml of benedict reagent to a mixture with boiling to 5 minutes, leave the mixture
to cooling . formation red precipitate indicate presence of glycosides (23).
- Saponin : 2 ml of mercury chloride added to 5 ml of lawsonia inermis L. aqueous extract , formation of white precipitate indicate presence of Saponin (24).
- Analgesic activity
  - Hot plate test: The analgesic activity of Lawsonia inermis L. was evaluated by method of (25) by using physical stimulus . twenty four albino swiss rats were randomly divided into four equal groups ( 6 animal each group): control negative group, rats administrated distilled water, control positive group were animals injected with Ketoprofen (8 mg/ kg b.W.) (26) , and the 3rd and 4th group rats were treated with aqueous and ethanolic extract respectively (200 mg/kg B.W.)(27). The animals were placed on hot plate and the heat was maintained at constant intensity (55 C° ± 3) and the time for the rat jump or licking the forelimbs was noted as a reaction time. To avoid burn the paw the cut off time at 20 second . the test performed twice, after 30 and 60 minutes of treated . at the end the reaction time of all groups compared with control group.
  - Tail immersion method: This test performed according to method of Turner 1971 (28). The rats of all group were tested, by immersed its tail (the last 5 cm) in the hot water ( 55 C° ± 3).The reaction time represent by the time of tail withdrawal from the water, the reaction time were recorded after 1 hr. of administration.
  - Writhing assay method: Twenty four albino swiss rats (150-200 g) were divided randomly into 4 equal groups to determine analgesic activity of henna via chemical stimulus (acetic acid ). The groups are : Control negative, control positive and treated rats groups were rats administrated the aqueous and ethanolic plant extract . this method based on intraperotional injection of acetic acid 3% (300 mg/Kg. b.w.) to all groups to create pain sensation after 1 hr. of rats administration with NSAID and extracts. The abdominal contraction of the animals were observed to count the number of writhe of all tested groups (29).
  - Antipyretic activity
    - The antipyretic feature of Lawsonia inermis L. has been evaluated depending on method of Grover 1990 (30) That based on induction of fever by injection of boiled caw milk (0.5 ml/kg. b.w.) to all groups and then rectal temperature has been measured to each rat using thermometer after two hours, only rats that temperature arise at least 0.7 C were employed for the experiment. The temperature was measured at 0 time, 1 and 3 hours after treated of rats.
  - Anti-inflammatory
    - Formalin test: The anti-inflammatory effect of henna extract was evaluated according to the (31), injection of formalin s/c in the paw and measurement the size of paw edema to evaluate the extract activity by micrometer (vernier) after 0, 1, 3 hours of injection. The animals in all groups received the ketoprofen (10 mg /kg b.w.) and extract before half hour of formalin injection.
    - Measurement of Tumor necrosis factor-alpha: Measurement of pro-inflammatory TNF-α in rats serum before treatment and after 1 and 2 days
of treatment of animals, this test was carried out by using rat TNF-α ELISA Kit (Thermo scientific, USA) at a wavelength of 450 nm. (32).

![Graph](image)

Standard curve to determined TNF-α in serum of rats

- **Statistical analysis**
  Statistical analysis was performed with the SPSS 18.0 software (SPSS Inc.). Statistical comparisons were made with one-way and two way ANOVA followed by LSD to evaluate significant values. The level of significance was expressed as $P<0.05$. (33).

**Results**

- **Phytochemical screening**
  The extract of *lawsonia inermis* L. revealed positive test for flavonoids, phenolic compound tannin and glycosides while, the result explain negative to saponin as shown in the table (1).

<table>
<thead>
<tr>
<th>Table (1): active ingredient that present in the <em>lawsonia inermis</em> extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>compound</td>
</tr>
<tr>
<td>available</td>
</tr>
<tr>
<td>not available</td>
</tr>
</tbody>
</table>

- **Analgesic activity**
  Analgesic activity of *lawsonia inermis* L. leaf extract explained in the table 1, 2, and 3.

  o **Hot plate test**: Table (2) clarified the results of hot plate test, after 30 minutes of administration the rats in both groups, aqueous and ethanolic extract shown a significant ($P<0.05$) analgesic activity by arising pain threshold as well as the animals in positive control
group that injected ketoprofen (10 mg/kg.b.w.) comparing with negative control group, despite of the ethanolic leaf extract is more better than aqueous extract. While after 60 minutes, of administration, the result revealed that ethanolic leaf extract of henna has been significant (P<0.05) analgesic character similar to the ketoprofen when compared with negative control group. Within the time, the table shown the ethanolic extract at 60th minute significant (P<0.05) increased latency period comparing with 30th minutes at the same group.

**Table (2): Effect of Ketoprofen and aqueous and ethanolic extract of henna leaves on reaction time of hot plate test in rats**

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>Control –Ve</th>
<th>Control+Ve</th>
<th>Aqueous extract</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8.52 ± 0.31</td>
<td>12.93 ± 0.47</td>
<td>10.63 ± 0.48 Ba</td>
<td>11.71 ± 0.36 BCa</td>
</tr>
<tr>
<td>30 minute</td>
<td>Aa</td>
<td>Ca</td>
<td>Ba</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.43 ± 0.24</td>
<td>13.20 ± 0.31</td>
<td>11.33 ± 0.53 Ba</td>
<td>12.76 ± 0.55 Cb</td>
<td></td>
</tr>
<tr>
<td>60 minute</td>
<td>Aa</td>
<td>Ca</td>
<td>Ba</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM ; n = 6 ; Capital letter denote a significant different from control (between group) (P<0.05) ; Small letter denote a significant within a group (within time)

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**Tail immersion method:** Table (3) shown the reaction time depending on tail immersion method. The results explained the ethanolic leaf extract of lawsonia inermis L. is more significant (P<0.05) inhibited the pain response to thermal stimuli and better from aqueous extract, comparing with negative control group after 30 and 60 minutes of treatment. While there were no significant (P>0.05) deference between the mean value of analgesic of aqueous extract and negative control at 30 minutes of administration. Moreover, within the time, at 60 minutes, the result shown significant (P<0.05) increased in the time required to withdraw tail of rats from hot water in both ethanolic leaf extract and positive control groups comparing to result at 30 minutes and with negative control group.

**Table (3): Effect of Ketoprofen and aqueous and ethanolic extract of henna leaves on reaction time of tail immersion test in rats**

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>Control –Ve</th>
<th>Control+Ve</th>
<th>Aqueous extract</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4.02 ± 0.14</td>
<td>8.11 ± 0.13</td>
<td>4.85 ± 0.28 Aa</td>
<td>6.53 ± 0.21 Ba</td>
</tr>
<tr>
<td>30 minute</td>
<td>Aa</td>
<td>Ca</td>
<td>Ba</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.19 ± 0.25</td>
<td>9.12 ± 0.29 Cb</td>
<td>5.37 ± 0.34 Ba</td>
<td>7.26 ± 0.39 Cb</td>
<td></td>
</tr>
<tr>
<td>60 minute</td>
<td>Aa</td>
<td>Ca</td>
<td>Ba</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM ; n = 6 ; Capital letter denote a significant different from control (between group) (P<0.05) ; Small letter denote a significant within a group (within time)

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**Writhing assay method:** Table (4) clarified the writhing movement induced by injection of formalin. The aqueous and ethanolic leaf extract exhibited significant (P<0.05) analgesic effect by reduction in the writhing count (% inhibition) comparing with negative control group, despite of ethanolic extract is more better in the reducing the abdominal constriction than aqueous extract. Therefore, the effect of ethanolic extract is comparable to that of
standard drug, ketoprofen 10mg/kg.b.w. (positive control). Therefore, alcoholic extract of henna was increased percent of writhing inhibition more than aqueous extract.

Table (4): Effect of Ketoprofen and aqueous and ethanolic extract of henna leaves on Acetic Acid induced writhing in rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Control –Ve</th>
<th>Control +Ve</th>
<th>Aqueous extract</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Writhe (T/S)</td>
<td>22.83 ± 1.27 A</td>
<td>10.16 ± 0.78 C</td>
<td>16.83 ± 1.19 B</td>
<td>13.33 ± 0.80 BC</td>
</tr>
<tr>
<td>% inhibition</td>
<td>-----------</td>
<td>55.49</td>
<td>26.28</td>
<td>41.61</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM ; n = 6 ; Capital letter denote a significant different from control (between group) (P<0.05) ; Small letter denote a significant within a group (within time)

- Antipyretic activity

Treatment of rats in group 3 and 4 with aqueous and ethanolic extract respectively shown a significant (P<0.05) reversed hyperthermia induced by injection of boiled cow milk,(table 5) compared with negative control group after 1 hour of administration. While after 3 hours, the alcoholic extract show a significant (P<0.05) antipyretic activity similar to ketoprofen comparing with –ve control group. Within the time, the result shown ethanolic extract and keoprofen groups exhibited a significant (P<0.05) suppressed hyperthermia, and the time of peak is 3 hours of administration comparing with control group. While there were no significant (P>0.05) deference in aqueous extract after 3 hours comparing to 1 hour of administration.

Table (5): Antipyretic activity of Ketoprofen and aqueous and ethanolic extract of henna leaves against experimental pyrexia of rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Control –Ve</th>
<th>Control +Ve</th>
<th>Aqueous extract</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td>39.73 ± 0.142 Aa</td>
<td>39.69 ± 0.128 Aa</td>
<td>39.74 ± 0.168 Aa</td>
<td>39.70 ± 0.154 Aa</td>
</tr>
<tr>
<td>1 hour</td>
<td>40.03 ± 0.137 Aa</td>
<td>38.98 ± 0.146 Ba</td>
<td>39.56 ± 0.162 ABa</td>
<td>39.22 ± 0.135 BCa</td>
</tr>
<tr>
<td>3 hours</td>
<td>40.24 ± 0.163 Aa</td>
<td>38.36 ± 0.154 Bb</td>
<td>39.23 ± 0.144 Ca</td>
<td>38.58 ± 0.160 BCb</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM ; n = 6 ; Capital letter denote a significant different from control (between group) (P<0.05) ; Small letter denote a significant within a group (within time)

- Anti-inflammatory activity

The anti-inflammatory effect of ethanolic leaf extract of L. inermis L. and ketoprofen against formalin induced paw edema was shown in table (6a & b). The results of table (6 a) explained a significant (P<0.05) suppression of paw edema in both ethanolic leaf extract and
standard drug after 1 hour from injection of formalin comparing with negative control group. While there was no significant (P>0.05) reduction in paw edema of rats in aqueous leaf extract when compared with −ve control. On the other hand, ethanolic leaf extract of L.inermis L. exerted highest anti-inflammatory effect comparable to ketoprofen after 3 hours of formalin-induced inflammatory paw edema comparing with negative control group. Depending on the results within the group, after 3 hours of formalin injection, the ethanolic leaf extract has been shown possessed potent anti-inflammatory activity by preventing increase paw edema comparing with edema after 1 hour of injection.

On other hand, table (5 b) clarified the mean values of TNF-α in serum rats , the result have shown a significant (P<0.05) reduction in proinflammatory TNF-α in both Ketoprofen and ethanolic extract groups comparing with control negative group after 1 and 2 days of treatment, also, aqueous extract was showed significant (P<0.05) reduction in TNF-α when compared with control negative after 2 days of treatment. Within the time , the result demonstrated that there were no significant (P>0.05) differences in both ketoprofen and alcoholic extract comparing with pretreated values (with in group). As well as disappear of difference in the serum TNF-α value of aqueous extract when comparing the value after 2 days with value after 1 days of treatment . while significant increase (P<0.05) in TNF-α in control group within the time.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control –Ve</th>
<th>Control +Ve</th>
<th>Aqueous extract</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td>3.68 ± 0.13</td>
<td>3.77 ± 0.20</td>
<td>3.76 ± 0.18</td>
<td>3.83 ± 0.15</td>
</tr>
<tr>
<td>1 hour</td>
<td>5.43 ± 0.23</td>
<td>4.48 ± 0.19</td>
<td>4.94 ± 0.26</td>
<td>4.55 ± 0.16</td>
</tr>
<tr>
<td>3 hours</td>
<td>6.18 ± 0.15</td>
<td>5.17 ± 0.26</td>
<td>5.77 ± 0.17</td>
<td>4.83 ± 0.24</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM ;  n = 6 ; Capital letter denote a significant different from control (between group) (P<0.05) ; Small letter denote a significant within a group (within time)

Table (6-b ) : Effect of ketoprofen, aqueous extract and alcohol extract of lawsonia inermis L. in pro-inflammatory Cytokine (Tumor necrosis factor-alpha) in rats serum

<table>
<thead>
<tr>
<th>Time</th>
<th>Control –Ve</th>
<th>Control +Ve (ketoprofen)</th>
<th>Aqueous extract</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>48.43 ± 5.23</td>
<td>46.67 ± 4.19</td>
<td>46.20 ± 3.47</td>
<td>45.33 ± 4.73</td>
</tr>
</tbody>
</table>

95
After 1 day of treatment  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Ab</td>
</tr>
<tr>
<td>65.16 ± 4.45</td>
<td>Ab</td>
<td></td>
</tr>
<tr>
<td>44.98 ± 3.09</td>
<td>Ba</td>
<td></td>
</tr>
<tr>
<td>58.76 ± 3.87</td>
<td>Ab</td>
<td></td>
</tr>
<tr>
<td>42.25 ± 2.89</td>
<td>Ba</td>
<td></td>
</tr>
</tbody>
</table>

After 2 days of treatment  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Ac</td>
</tr>
<tr>
<td>88.12 ± 3.54</td>
<td>Ac</td>
<td></td>
</tr>
<tr>
<td>50.37 ± 5.32</td>
<td>Ba</td>
<td></td>
</tr>
<tr>
<td>62.25 ± 4.55</td>
<td>Cb</td>
<td></td>
</tr>
<tr>
<td>47.78 ± 4.14</td>
<td>Ba</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE.M ; n = 6 ; Capital letter denote a significant different from control (between group) (P<0.05) ; Small letter denote a significant within a group (within time)

**Discussion**

Pain is a major symptom of many medical condition and the most indicator of many medical cases, the pain either locally by release of pain mediators such as prostaglandin, interleukin and histamine or centrally through CNS via sensory nerve impulses. Analgesic Agents that exhibit their action at spinal cord nociceptive reflex is called central analgesic agents (34). The hot plate test and tail immersion method widely used as neurologic pain model to determine the central analgesic activity of analgesic agents (35, 36). Acetic acid injection induce abdominal withes due to liberating the endogenous substances such prostaglandins, bradykinine and substance P (37).therefore, writhing assay method used to assess the peripheral analgesic (38). The ethanolic extract of Lawsonia inermis L. revealed significant increase in latency time in second ( table 1 and 2) more than aqueous plant extract, comparing with negative control groups, and has same effect of ketoprofen. As well as significant inhibiting number of withes (Table 3) comparing with control.

Ketoprofen is one of non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit synthesis of endogenous pain mediators such as prostaglandin, bradykinine etc. prostaglandin and bradykinine were exhibited play essential role in the pain process (39). The NSAIDs possessing analgesic, antipyretic and anti-inflammatory activity by inhibiting prostaglandin liberating via cyclooxygenase pathway (40). Therefore, ethanolic leaves extract of L. inermis clarified both central and peripheral analgesic activity, similar to ketoprofen (10 mg/kg b.w) by increase reaction time via thermal stimulus and decrease abdominal constriction via chemical stimulus (acetic acid) by inhibition of the local endogenous pain mediator such as prostaglandin and bradyginin. Lawsonia inermis L. contain many phytochemical compound such as tannin, alkaloid, flavonoids and steroids which have been possess potent analgesic activity (41, 42). The mechanism of analgesic may due to inhibition of local peritoneal receptors via inhibition cyclooxygenase activity. the result of the current study agree with (43) who explain that isolated compound (Lawsonone) of henna has potent anti-inflammatory, analgesic and antipyretic action.

The body defence against infectious agents by increase body temperature, this occur by formation of pro-inflammatory mediators such as cytokines (interleukins and tumor necrotic factor alpha(TNF-α)) and prostaglandin E2 near to the hypothalamus (44), subsequently increase body temperature. so most antipyretic agents inhibit formation of PGE2 to exhibit their effect to reduce body temperature. The results of current study revealed that L. A. L. (henna) extract reduce body temperature comparing to the control group, this is due to reducing of pro-inflammatory mediators such bradykinin and PGE2 (45). In addition to the ability of NSAID (Ketoprofen) in
reducing temperature in rats-induced pyrexia, this is due to direct effect of drugs on reduction pro-inflammatory mediators in hypothalamus (46).

The result has been shown the aqueous and alcoholic extract has anti-inflammatory effect at 3 hours of injection formalin comparing to control group and better effect in reducing paw edema than ketoprofen. Similar effect explained by (47) who found the ethylacetate leave extract of henna possess anti-inflammatory properties. The lawsonia inermis contain several compound like naphthaquinones which has many biological activity such as antibacterial and anti-inflammatory (48). Many studies reported that L. inermis L. has antinociceptive, antipyretic and anti-inflammatory activities (49, 50). Henna clarified inhibition of second phase of formalin induced nociception by reducing hyper-nociception induced bradykinin, cytokine and decrease release of IL.1B and PGE2 (45). Many researcher have demonstrated that phyto-constituents of many plants flavonoids , phenolic compound etc.. act as Gama amino butyric acid (GABA) receptor ligand in the central nervous system that works as benzodiazepine like molecules via CNS depression activity (51).

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