ANALGESIC ACTIVITY OF EXTRACTS OF MALUS DOMESTICA (APPLE)

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ABSTRACT

Malus domestica (Apple) a traditional plant widely used since Iron Age and has multiple benefits. In the present study extracted & isolated pectin (MDP) from pulp and extracted quercetin (MDQ) was evaluated for analgesic action by using various animal models. MDP and MDQ extracted and identified by various phytochemical analysis method. The analgesic effect of MDP and MDQ was assessed by using differential pain models such as acetic acid induced writhing and formalin induced paw licking model. In acetic acid induced writhing, both MDP & MDQ showed inhibitory effect on writhing. In formalin induced paw licking model, MDP and MDQ inhibit both the phases, reduction in the licking time significantly increased in the late phase which indicates the action of drug more in peripheral pain pathway. The result of the present study demonstrates the marked analgesic effect of extracted compound from apple peels and pulp.

KEYWORDS: analgesic, malus domestica, pectin, quercetin.

INTRODUCTION

Over 7,500 types of apples (Malus domestica), a majority of them have been cultivated since the Iron Age, but historians have found evidence that apples were first farmed by humans in ancient Egypt. It is the pomaceous fruit of the apple tree, species Malus domestica in the rose family (Rosaceae). It is one of the most widely cultivated tree fruits, and the most widely known of the many members of genus Malus that are used by humans. Apples grow on small, deciduous trees. The tree originated in Central Asia, where its wild ancestor, Malus sieversii, is still found today. Apples can be consumed fresh, but they are also a valuable raw material
for processing into apple juice, concentrates, canned, frozen, dried, and stewed fruits, jellies, purée and cider.\cite{1}

Today, it is known through different studies that the fruit fiber or pectin offers many healthy substances, which may combat cancer and inflammation in the body, and fiber, which helps regulate blood sugar, lower cholesterol and promotes digestive health also pectin is widely used as a functional ingredient in the food industry due to its ability to form aqueous gels and has been used in jams and jellies, fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products. Apple pomace is rich in pectic substances and important raw materials for pectin production all around the world. Apple pomace is produced as a byproduct of the juice factories and it is either used for animal feeding or is disposed of as an industrial waste. Pectin is a family of complex variable polysaccharides extracted from the primary cell wall of higher plants. Chemically, pectin consists of linear polymers of D-\(\alpha\)-(1 \(\alpha\) 4) anhydrogalacturonic acid. Part of the carboxyl groups of the anhydro-galacturonic acid is esterified with methanol. Pectin in apple pomace is mainly present in the form of protopectin, an acid soluble polysaccharide, so extraction is done by acidic solution in presence of controlled temperature.\cite{2}

Apples contain many flavonoids and phenolic acids. Apples constitute one of the basic sources of antioxidants. Epidemiological studies have linked the consumption of apples with reduced risk of some major diseases such as cancers, cardiovascular disease, asthma, and diabetes. \textit{In vitro} studies show that apples have strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation, and lower cholesterol. Dietary plant polyphenolic molecules, specifically flavonoids, have become of great interest researchers due to their widely reported potential health benefits. Quercetin is one of the most studied plant flavonoids and has been reported to have antioxidant, anticarcinogenic, antiinflammatory, antiaggregatory, antihypertensive and neuroprotective effects. Apple peels, one of the major dietary sources for flavonols, \textit{i.e.}, quercetins, is also a byproduct of apple processing industry.\cite{3}

**MATERIALS AND METHODS**

Fresh fruits of \textit{Malus domestica}, common name called apple, were collected from local fruit shop at kalyan. The fruit is identified and authenticated by Agharkar Research Institute, Pune. The 10.0 kg of fruits of \textit{Malus domestica} was cleaned by washing with water to remove any contaminant and allowed to air dried. After drying the peel was removed by peeler and air
dried at room temperature for 10 days. This dried apple peel was grind in mixer grinder to get coarse powder. Peel removed apples were crushed in grinder, crushed pulp was then pressed in muslin cloth and apple pomace air dried for 7 days. This dry apple pomace pool was then crushed and mixed the product was called apple flour.

**Extraction & isolation of pectin**[4]

Pectin was extracted under reflux in a condensation system at 97°C for 30 min (solute/solvent 1:50), using water acidified with citric acid to pH 2.5, using apple flour (pool) as raw material. Hot acid extract was pressed in a cheese cloth bag and the concentrated “juice” was cooled to 4°C. The apple pectin was precipitated by alcohol-juice treatment 2:1 (v/v). The mixture of solvent and precipitate was stirred for ten minutes and then left to rest for one hour in order to allow pectin flotation. With this procedure the pectic substances remain at the surface of the alcohol/water mixture and thus it is easier to remove them in a quantitative way. The floating pectin was filtered through cheesecloth, rinsed with 95° GL alcohol and then pressed. The pressed pectin was dried to constant weight at 55°C, cooled in a dessicator. The hard pectin cake was broken up, ground and sieved in order to obtain powdered pectin.

**Extraction of quercetin**[5]

The dried and coarsely powdered peels of fruits Malus domestica 5 gm mixed with 250 ml methanol (0.01% HCl) in iodinated flask mixed well, placed in the ultrasonic bath and exposed for 30 minutes at temperature 20 to 28°C. The methanolic mixture was filtered and then concentrated under reduce pressure using rotaevaporator. Mixed concentrate with 30 ml of ethyl acetate and filtered it and poured in to evaporating dish, allowed to evaporate all ethyl acetate and light green colored compound containing quercetin (MDQ) was obtained.

**Phytochemical study for qualitative analysis of active principles**[6-9]

Qualitative analysis of MDP & MDQ for the presence of various medicinally important active phytochemicals such as pectin, alkaloids, anthraquinones, flavonoids, saponins, tannins, sterols, reducing sugars, glycosides, resins and triterpenes was carried out as per the methods described earlier.

**Experimental animals**[10]

Healthy Swiss Albino mice 20-30 gm were used for the study. Acute toxicity study of test drug was performed on Swiss albino mice of either sex according to OECD guidelines 423.
They were group into 5 groups per 6 animals. These animals were acclimatized in animal house of Dr. L.H. Hiranandani college of Pharmacy under standard husbandry conditions. The animals were housed in standard polypropylene cages with wire mesh top and husk as bedding. The animals had free access for food and water supplied *ad libitum* under strict hygienic conditions. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental groups

*Group I: Control (normal saline 0.5 ml/kg, oral)*

*Group II: MDP1 (10 mg, oral)*

*Group III: MDP 2 (100 mg, oral)*

*Group IV: MDQ 1 (25 mg, oral)*

*Group V: Etoricoxib (10 mg/kg, oral)*

Experimental models

**Acetic acid induced writhing**[^11]

This experiment was carried out in mice (20-30 g) using 6 animals in each group, according to the method described by Witkin et al. They were divided into 5 groups, where Groups II to III received MDP at the dose rate of 10, 100 mg/kg & group IV received MDQ 25mg/kg, respectively (orally) while Group V was administered with the standard drug etoricoxib (10 mg/kg, orally) 1 h prior to the injection of acetic acid. The control group received the vehicle. The effect of the extract and etoricoxib on acetic acid-induced writhing was observed in comparison to control. Writhing in animals was produced by i.p. administration of 300 mg/kg acetic acid (3%) solution. Each mouse was then put into a big glass cylinder and the total number of writhing episodes for a period of 20 min after the injection of acetic acid was counted. The percent inhibition of writhing count of the treated group was calculated from the mean writhing count of the control group.

**Formalin induced paw licking**[^11]

This experiment was carried out in mice (20-30 g) using 6 animals in each group, according to the method described by Witkin et al. They were divided into 5 groups, where Groups II to III received MDP at the dose rate of 10, 100 mg/kg & group IV received MDQ 25mg/kg, respectively (orally) while Group V was administered with the standard drug etoricoxib (10 mg/kg, orally) 1 h prior to the injection of acetic acid. The control group received the vehicle.
The effect of the extract and etoricoxib on acetic acid-induced writhing was observed in comparison to control. Briefly, 60 min after subcutaneous injection of 20 µl of 2.5% formalin into the dorsal surface of the right hind paw was applied. Animals were observed in the chambers. Animals were observed from 0 to 5 min (Early phase) and from 15 to 30 min (Late phase) and the time that they spent licking the injected paw was recorded and it was considered as indicative of nociception.

RESULTS

Phytochemical analysis
The phytochemical of MDQ showed presence of carbohydrate, flavonoids, quercetin and MDP showed presence of pectin.

Acetic acid induced writhing
Table 1: Effects of MDP1, MDP2 and MDQ on Acetic acid induced writhing.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg</th>
<th>No. of writhing Mean ± SEM</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>51.33 ± 1.308</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>10</td>
<td>19 ± 2.671***</td>
<td>62.98%</td>
</tr>
<tr>
<td>MDP1</td>
<td>100</td>
<td>31.83 ± 9.097*</td>
<td>37.99%</td>
</tr>
<tr>
<td>MDP2</td>
<td>15</td>
<td>15 ± 3.759***</td>
<td>70.77%</td>
</tr>
<tr>
<td>MDQ</td>
<td>25</td>
<td>19.50 ± 6.043**</td>
<td>62.01%</td>
</tr>
</tbody>
</table>

Graph 1: Effects of MDP1, MDP2 and MDQ on Acetic acid induced writhing.

Values are expressed as mean ± SEM (n = 6), *p<0.05. **p<0.01, ***p<0.001; ns = non significant, compared with Disease Control Group (one-way ANOVA followed by Dunnett’s Multiple Comparisons test). Writhing in animals was produced by i.p. administration of 300 mg/kg acetic acid (3%) solution. In this test, vehicle treated groups showed 51.33 (± 1.308) writhes. Standard drug significantly inhibited the number of writhes in mice (P<0.001).
Treatment of animals with MDP1, MDP2 (10 and 100 mg/kg) shows significant inhibit writhing in mice in dose dependent manner 62.98 and 37.99% respectively while MDQ (25 mg/kg) significantly (p<0.01) reduced the writhing. These results showed that MDP1, MDP2 and MDQ possessed analgesic activities against acetic acid induced writhing and were comparable to standard drug etoricoxib.

Formalin induced paw licking

Table 2: Effects of MDP1, MDP2 and MDQ on Formalin induced paw licking

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of paw licking</th>
<th>Early phase</th>
<th>Late phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.66 ± 6.72</td>
<td>102.17 ± 13.20</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>25.83 ±1.01***</td>
<td>18.00 ± 8.30***</td>
<td></td>
</tr>
<tr>
<td>MDP1</td>
<td>62.17 ± 7.67</td>
<td>38.33 ± 17.80**</td>
<td></td>
</tr>
<tr>
<td>MDP2</td>
<td>52.17 ± 1.44*</td>
<td>14.50 ± 3.78***</td>
<td></td>
</tr>
<tr>
<td>MDQ1</td>
<td>41.33 ± 7.74**</td>
<td>16.16 ± 2.07***</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 6), *p<0.05. **p<0.01, ***p<0.001; ns = non significant, compared with Disease Control Group (one-way ANOVA followed by Dunnett’s Multiple Comparisons test). The results of orally administered MDP1, MDP2, MDQ on the formalin induced paw licking are presented in Graph 5.5. In this model, MDP at 10 mg, showed a non significant as mean time in sec reduction but MDP2, MDQ1 shows significant reduction P<0.05 and p<0.01 respectively in the licking time suggesting analgesic activity in the early phase (0-5 min). MDP1 showed significant reduction (p<0.01) where as MDP2 & MDQ1 shows significant reduction (P<0.001) in the licking time at late phase (15-30 min) with respect to control.
DISCUSSION

The present study was undertaken with the objective of exploring analgesic activity of extract by isolation and identification of active constituents from Apple (*Malus domestica*). Apple peel was separated from pomace to get different active constituents from peel and pomace. Apple peel extract (MDQ) showed presence of quercetin by different identification method such as flavonoids test, quercetin test. Whereas, apple pomace extract showed presence of pectin (MDP).

Pain is a sensorial modality, which in many cases represents the only symptom for the diagnosis of several diseases, and often has a protective function. It is a common and distressing feature of many diseases and analgesics relieve pain by acting in the central nervous system or on peripheral pain mechanisms, without significantly altering consciousness. Throughout history, man has used many different forms of therapy for the relief of pain, and medicinal herbs are highlighted due to their popular use. Acetic acid induced writhing and Formalin induced paw licking typically resembles to human clinical pain conditions and is sensitive to peripheral acting analgesics.

The acetic acid-induced abdominal constriction is a standard, simple and sensitive test for measuring analgesia induced by both opioids and peripherally acting analgesics. This test, besides being used as antinociceptive model for opioids, is also commonly employed as a visceral inflammatory pain model. In acetic acid-induced abdominal constriction, pain is elicited by the injection of an irritant such as acetic acid into the peritoneal cavity which induces a stereotypical behavior in mice, which is characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles and a reduction in motor activity and coordination. Inhibition of number of episodes by analgesics is easily quantifiable. The algesic mechanism of abdominal writhing involves the release of arachidonic acid via prostaglandin biosynthesis and sympathetic nervous system mediators. However, it was known that constriction induced by acetic acid was considered to be a non-selective anti-nociceptive model; since acetic acid indirectly induced the release of endogenous mediators stimulated the nociceptive neurons that were sensitive to non steroidal anti-inflammatory drugs (NSAIDs).

In acetic acid induced writhing, MDP1, MDP2 & MDQ showed inhibitory effect on writhing. Standard drug and MDP2 significantly inhibited the number of writhes in mice (P<0.001). Treatment of animals with MDP1, MDP2 (10 and 100 mg/kg) showed significant inhibited
writhing in mice in dose dependent manner 62.98 (p<0.05) and 37.99% (p<0.01) respectively. Abdominal constriction responses are found to partly involve local peritoneal receptors. This method has been associated with prostanoids in general, e.g. increased levels of PGE2α and PGF2α in peritoneal fluids, as well as lipoxygenase products by some researchers. Therefore, the results of the acetic acid-induced writhing strongly suggest that the mechanism of action MDP & MDQ may be linked partly to lipoxygenase and or cyclooxygenase inhibition. However, the results of this writhing test alone did not ascertain whether the anti nociceptive effect was central or peripheral. In order to confirm it, the formalin test was carried out.

The formalin induced paw licking test was carried out to further strengthen the evidence of the anti-nociceptive activity of the extract seen in the abdominal writhing. Formalin induced model was performed to distinguish between central and peripheral anti nociceptive action. The formalin test is considered as a valid and reliable model of persistent nociception and involves 2 distinct phases, a neurogenic pain that corresponds to the early phase (0-5 min), followed by an inflammatory pain that is accompanied by the release of inflammatory mediators designated as the late phase (15-30 min). The first phase of pain (lasting the first 5 min) corresponding to the acute neurogenic pain, is attributed to direct activation of nociceptors and primary afferent fibers by formalin causing the release of bradykinin and tachykinins and the activation of the transient receptor potential vanilloid 1 (TRPV1) channel. This phase is inhibited by opioid analgesics. The second phase (lasting from 15 to 30 min after injection of formalin) is due to an inflammatory reaction caused by tissue injury leading to the release of histamine, serotonin, prostaglandin and excitatory amino acids. This late phase is inhibited by NSAIDs and opioid analgesics. A slight delay was observed in the onset of paw licking in the entire therapeutic dose and the double dose levels, but in early phase onset was early with all test compounds in different concentration as compare to standard. Further observation indicated was reduction in the licking time at the late phase by MDP1 (P<0.01), MDP2 & MDQ (P<0.001) was significant. Thus concluded that the MDP1, MDP2 & MDQ1 inhibit both the phases, reduction in the licking time significantly increased in the late phase which indicates the action of drug more in peripheral pain pathway. The mechanism for the analgesic activity of the test drug may be exhibited through the inhibition of PG synthesis.
CONCLUSION
The present study undertaken gave us a very good potential to explore the analgesic activity of different extracts of *Malus domestica* (Apple). The extracts of apple MDP and MDQ on isolation of biologically active constituents like pectin and quercetin respectively were assessed for their potential analgesic activities. In vivo studies performed confirmed with analgesic activity of MDP and MDQ extracts of *Malus domestica* (Apple), which may due to the presence of isolated active constituents like pectin and quercetin. An intensive study on molecular pathways, mediators and the enzymes is required for the potential use of MDP and MDQ extracts of Malus domestica (Apple) as analgesic drugs.

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