Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 2, 2013

Research Article

# EFFECT OF MIRABILIS JALAPA LINN FLOWERS IN EXPERIMENTALLY INDUCED ARTHRITIS AND CONSECUTIVE OXIDATIVE STRESS

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*Received: 25 Dec 2012, Revised and Accepted: 16 Feb 2013*

**ABSTRACT**

The aim of the present study was to investigate the effect of *Mirabilis jalapa* Linn flowers in formaldehyde and Freund's adjuvant induced arthritis. Hydro ethanolic extract of *Mirabilis jalapa* flowers (HEMJ) were prepared and subjected to preliminary phytochemical investigations. Anti-arthritic activity of HEMJ was evaluated in formaldehyde and Complete Freund's adjuvant (CFA) induced arthritic model in wistar rats. Body weight changes, haematological and antioxidant parameters were evaluated in CFA model. The phytochemical study revealed the presence of flavonoids, saponins, tannins and steroids. HEMJ significantly suppressed the paw edema in both formaldehyde and CFA models ($P<0.001$). Body weight, haematological and antioxidant changes in the CFA rats are restored to normal. Our findings provide evidence that *Mirabilis jalapa* flowers possess significant anti-arthritic activity.

**Keywords:** *Mirabilis jalapa*, Arthritis, Formaldehyde, Complete Freund's adjuvant, Antioxidant

## INTRODUCTION

Rheumatoid arthritis is a chronic autoimmune inflammatory disease that affects the joints and other tissues in the body [1]. Millions of peoples are living with arthritis and related disorders worldwide [2]. Currently available drugs include steroids, NSAID's, TNF-α and IL-1β antagonists, has shown only limited success against arthritis [3]. Moreover the serious side effects produced by these drugs make them unfavourable for patients. It is well known that the ingestion of natural antioxidants reduces the risk of arthritis, cardiovascular diseases, cancer and other diseases associated with ageing [4]. Recently, researchers are directed towards traditional system of medicine for the discovery of drugs that have antioxidant and anti-inflammatory potential with minimum side effects.

*Mirabilis jalapa* belongs to the family *Nyctaginaceae* and is commonly known as 'four o clock' plant or 'marvel of Peru'. It is a perennial herb, that produces flowers all over the season usually open around four o clock evening and so the name 'four o clock plant'. Phytochemical studies have revealed that the plant is rich in sitosterol, stigmasterol, ursolic acid, oleanolic acid, and brassicasterol [5, 6]. *Mirabilis jalapa* have been reported for various biological activities like anti-spasmodic, anti-bacterial, and anti-inflammatory by scientific community [7-9].

*Mirabilis jalapa* have been used in traditional folk medicine to treat inflammatory diseases, and arthritic conditions [10, 11]. However no such study on the anti-arthritic activity of *Mirabilis jalapa* has been reported. Taking these facts into considerations, an attempt has been made in this study to evaluate the anti-arthritic and antioxidant potentiality of HEMJ using selected experimental arthritis models.

## MATERIALS AND METHODS

### Drugs and chemicals

CFA, Indomethacin, Glutathione peroxidase (GPX; 010M4061) assay kit, Catalase assay kit (111M4120V), Superoxide dismutase (SOD; BCBC2239) assay kit were purchased from Sigma Aldrich. Formaldehyde was obtained from Merck and all other chemicals used were of analytical grade.

### Plant material collection and Identification

Fresh flowers of *Mirabilis jalapa* was collected in the month of August from Ernakulum district, Kerala. The plant material was authentified by G Valsala Devi, Curator, Department of Botany, Kariavattom, University of kerala, where a voucher specimen no KUBH 5799 was deposited.

### Preparation of plant extract

The flowers were collected, washed with water and shade dried in open air, then pulverized to dry powder using electric grinder. About 500 gm of the powder was extracted with 4 litres of ethyl alcohol (70%) by cold maceration for 7 days. The extract was filtered, evaporated using vacuum rotary evaporator (Buchi) and heated on water bath at 45 ± 5°C to obtain HEMJ (8.4% yield w/w). Carboxy methyl cellulose (0.5%) was used as solvent to prepare different doses of HEMJ.

### Animals

Wistar albino rats of either sex (150-200gm) were used for present investigation and they were obtained from Central Animal Facility, NIPER Guwahati. Animals were housed under standard environmental conditions of temperature (25±2°C) and light & dark cycle (12:12 h). Rats were fed with standard pellet diet and water ad libitum. All experimental studies were done after getting permission from the Institutional Animal Ethics Committee, Gauhati Medical College, Guwahati.

### Preliminary phytochemical investigation [12, 13]

Preliminary phytochemical screening of extract was performed using standard procedures and tests with little modifications.

### Acute toxicity study [14]

The acute toxicity study was carried out as per the OECD guidelines 425. Initially HEMJ was administered orally at a limit dose of 2000 mg/kg to a single female rat. The rat was observed closely for the first 4 h and then periodically up to 24 h for any toxic symptoms and mortality. After 24 h same dose was administered to four more female rats.

### Formaldehyde induced arthritis in rats [15]

Rats were randomly divided into five groups consisting of six animals: normal control (formaldehyde not injected), arthritic control, HEMJ 100 mg/kg, HEMJ 200 mg/kg, and Indomethacin 3 mg/kg. Arthritis was induced by injecting 0.1 ml of a 2% formaldehyde solution into the plantar aponeurosis of right hind leg on the first and third day. All treatments were given orally for 10 consecutive days and the mean increase in the paw volume of each group was measured on 0, 7, and 10$^{th}$ day using digital plathysmograph (Ugobasile, Italy).

### CFA induced arthritis in rats [16]

Each treatment group contained six wistar rats. The rats were randomly divided in to five groups: normal control (no CFA

injection), arthritic control, HEMJ 100 mg/kg, HEMJ 200mg/kg, and Indomethacin 3 mg/kg. Arthritis was induced by sub plantar injection of 0.1 ml of CFA in to the right hind leg. CFA contains heat killed and freeze dried *Mycobacterium tuberculosis* (strain H37Ra, ATCC-25177) suspended in mineral oil. All treatments were given orally for 21 days and the paw volume was measured at 8th, 14th, and 21st day using digital plathysmograph.

Body weight was noted daily and on the 22nd day, animals were sacrificed. Blood was collected for hematological studies, liver excised and homogenate was prepared in phosphate buffer for antioxidant study.

**Hematological parameters [17]**

Hematological parameters like RBC, WBC, hemoglobin counts, and erythrocyte sedimentation rate (ESR) were estimated using standard laboratory methods.

**Antioxidant parameters**

SOD, GPX and Catalase levels were measured using assay kits supplied by Sigma Aldrich, according to manufacturer's instruction. Lipid peroxidation (LPO) [18], Reduced glutathione (GSH) [19], and tissue protein [20] were estimated by standard methods.

**Statistical analysis**

Values were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA (Graph pad prism version 6) followed by Dunnets post hoc test and values of P<0.05 were considered to be statistically significant.

**RESULTS**

Preliminary Phytochemical investigation of HEMJ showed the presence of steroids, tannins, flavonoids and saponins.

**Acute toxicity study**

The HEMJ did not show any toxic reactions and mortality up to a dose of 2000 mg/kg. Hence, HEMJ 100 mg/kg and 200 mg/kg were taken as treatment dose for this study.

**Formaldehyde induced arthritis**

Administration of 2% formaldehyde on days 1 and 3 produced paw swelling in all injected animals. This swelling was sustained throughout the observation period of 10 days (Table 1). The increase in paw volume was less in the indomethacin and HEMJ treated groups as compared to the arthritic control, and this difference was significant (P<0.01) on all observed days. The percentage of edema inhibition shown by HEMJ 100 mg/kg, 200 mg/kg and indomethacin 3 mg/kg on the 10th day was 56.8%, 59.52%, and 61.94% respectively.

**CFA induced arthritis**

Paw volume was measured at 0, 8, 14 and 21 days after CFA inoculation. There was a significant increase of paw volume in arthritic control rats when compared to normal group. Both doses of HEMJ and indomethacin 3 mg/kg showed significant reduction of paw volume when compared with the arthritic group (Table 2). The percentage edema inhibition exhibited by HEMJ 100mg/kg and 200 mg/kg on 21st day as compared to arthritic control was 46.51% and 49.16% respectively. The average gain in body weight on day 21 was compared to day 0 (Table 3).The animals in the arthritic control group gain less body weight as compared to both doses of HEMJ and indomethacin 3 mg/kg group.

**Table 1: Effect of HEMJ on paw volume and edema inhibition in formaldehyde induced arthritis**

| Group | Paw volume in ml | | |
|---|---|---|---|
| | 0th day | 7th day | 10th day |
| Normal control | 0.89±0.08 | 0.89±0.06 | 0.9±0.08 |
| Arthritic control | 0.88±0.06 | 2.82±0.42 | 2.94±0.54 |
| HEMJ 100 mg/kg | 0.90±0.01 | 1.36±0.25** (51.77%) | 1.27±0.29*** (56.8%) |
| HEMJ 200 mg/kg | 0.91±0.04 | 1.24±0.26** (56.02%) | 1.19±0.30*** (59.52%) |
| Indomethacin 3 mg/kg | 0.89±0.07 | 1.20±0.22*** (57.44%) | 1.12±0.34*** (61.94%) |

Values are expressed as mean ± SEM (n=6). **P<0.01, ***P<0.001 as compared with arthritic control. Percentage inhibition of edema shown in parentheses.

**Table 2: Effect of HEMJ on paw volume and edema inhibition in CFA induced arthritis**

| Groups | Change in paw volume | | | | % inhibition on 21st day |
|---|---|---|---|---|---|
| | 0th day | 8th day | 14th day | 21st day | |
| Normal control | 0.850±0.06 | 0.855±0.04 | 0.857±0.08 | 0.861±0.06 | - |
| Arthritic control | 0.87±0.08 | 2.55±0.23 | 2.84±0.27 | 3.01±0.29 | - |
| HEMJ 100 | 0.87±0.12 | 1.49±0.24* | 1.55±0.26** | 1.61±0.22*** | 46.51 |
| HEMJ 200 | 0.85±0.11 | 1.41±0.24** | 1.48±0.27*** | 1.53±0.36*** | 49.16 |
| Indomethacin 3 | 0.84±0.06 | 1.32±0.31** | 1.41±0.28*** | 1.44±0.34*** | 52.15 |

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared with arthritic control.

**Table 3: Change in body weight in adjuvant induced arthritis in rats**

| Groups | Mean body wt in grams | | Mean difference in body wt |
|---|---|---|---|
| | 0th day | 21st day | |
| Normal control | 172.36±0.54 | 195.28±0.69 | 22.92±0.17 |
| Arthritic control | 175.47±0.71 | 171.63±4.31 | -3.84±3.25 |
| HEMJ 100 mg/kg | 178.21±0.93 | 189.34±4.12** | 11.13±3.26 |
| HEMJ 200 mg/kg | 174.95±0.87 | 191.15±4.09*** | 16.2±3.19 |
| Indomethacin 3 mg/kg | 177.49±0.93 | 192.64±4.36*** | 15.15±3.41 |

Values are expressed as mean ± SEM (n=6). **P<0.01, ***p<0.001 as compared with arthritic control.

The changes in hematological parameters of CFA rats are shown in (Table 4).There was decrease in RBC count and hemoglobin; while increase in WBC count and ESR in arthritic group, as compared to the control group. Both doses of HEMJ treatment significantly improved the altered hematological parameters (P<0.01).

Table 5 depicts the liver anti-oxidant levels of control and arthritic groups. There was a significant decrease of SOD, Catalase, GSH, and GPX in arthritic groups as compared to the control rats. It was shown that HEMJ in both the doses significantly elevated the anti-oxidant levels in liver. Furthermore HEMJ treatment significantly reduced the LPO level (P<0.1) in arthritic rats.

**Table 4: Changes in haematological parameters in adjuvant induced arthritis**

| Groups | RBC count (x$10^6$ cells/$mm^3$) | WBC count (x$10^3$ cells/$mm^3$) | Haemoglobin (%) | ESR |
|---|---|---|---|---|
| Normal control | 6.81±0.09 | 6.64±0.12 | 14.92±0.10 | 12.91±0.15 |
| Arthritic control | 5.74±0.11 | 7.85±0.27 | 13.81±0.24 | 13.97±0.21 |
| HEMJ 100 | 6.67±0.22** | 7.05±0.24* | 14.79±0.17** | 13.08±0.14** |
| HEMJ 200 | 6.71±0.15** | 6.95±0.20** | 14.84±0.22** | 13.03±0.25** |
| Indomethacin | 6.75±0.21** | 6.90±0.14** | 14.87±0.11** | 12.96±0.14** |

Values are expressed as mean ± SEM (n=6). *P<0.5, **P<0.01, as compared with arthritic control.

**Table 5: Changes in anti oxidant parameters in adjuvant induced arthritis**

| Group | LPO (nM/mg of protein) | Catalase (mM/min/mg of protein) | SOD (units/mg of protein) | GSH (µg/mg of protein) | GpX (nM/min/mg of protein) |
|---|---|---|---|---|---|
| Normal control | 0.031±0.001 | 0.170±.040 | 0.06±0.020 | 1.32±0.19 | 0.18±0.030 |
| Arthritic control | 0.281±0.002 | 0.04±0.001 | 0.004±0.001 | 0.702±0.100 | 0.020±0.01 |
| HEMJ 100 mg/kg | 0.033±0.001* | 0.210±0.07** | 0.06±0.03* | 1.360±0.12*** | 0.24±0.06 |
| HEMJ 200 mg/kg | 0.026±0.001* | 0.24±0.041*** | 0.095±0.04** | 1.69±0.14*** | 0.28±0.04* |
| Indomethacin 3 mg/kg | 0.035±0.003* | 0.14±0.039 | 0.046±0.01 | 1.14±0.12*** | 0.12±.02 |

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***p<0.001 as compared with arthritic control.

## DISCUSSIONS

Several studies have shown that flavonoids, tannins and saponins are useful in the treatment of inflammation and arthritis [21-23]. Phytochemical analysis of HEMJ revealed the presence of tannins, flavonoids and saponins. Thus, Mirabilis *jalapa* may serve as a potential source of bioactive compounds in the treatment of arthritis. In this study HEMJ did not show any signs of acute toxicity up to a dose of 2000 mg/kg body weight, so the $LD_{50}$ value of HEMJ must be greater than 2000 mg/kg body weight.

Formaldehyde induced arthritis is one of the most commonly used model for assessing anti-arthritic potential of plant extract. Formaldehyde injection precipitates the proteins, present in the site of injection and body starts an immune reaction against the denatured proteins which leads to arthritis [24]. Moreover inflammatory mediators like histamine, serotonin and prostaglandin like substances are also contributed to the edema formation. So the suppression of edema by HEMJ in formaldehyde model shows its anti-inflammatory and immuno-modulatory effect.

The CFA model was developed in albino rats by sub plantar injection of heat killed *Mycobacterium tuberculosis*. CFA induced arthritis is manifested mainly due to cell mediated immune reaction and rat is an ideal animal in developing arthritis [25]. In CFA model, the edema caused in the first phase is due to the irritant action of adjuvant and immunological events leads to late phase arthritis [26-27]. The inhibition of paw edema showed by graded doses of HEMJ in the late phase of arthritis may be due to its suppression of cell mediated immunity.

The decrease in the body weight during arthritis is due to deficient absorption of nutrients through the intestine and treatment with anti-inflammatory drugs normalizes the process of absorption [28]. The restoration of body weight by HEMJ and indomethacin may be due to the inhibition of inflammation associated with arthritis and this leads to improved intestinal absorption of nutrients.

From the results it is clear that the decrease in RBC count and hemoglobin level represents the anemic condition in arthritic rats. This is due to the abnormal storage of iron in the reticulo endothelial system and synovial tissue and the failure of bone marrow to respond to anemia [29]. The significant increase of WBC count in arthritic rats may be due to the stimulation of immune system against the invading antigens and the respective decrease in HEMJ treated groups showed its immunomodulation effect. ESR, another important parameter which elevated in arthritis is due to the accelerated formation of endogenous proteins such as globulin and fibrinogen [30]. HEMJ in both doses and standard drug indomethacin restored the ESR back to normal level thus justifying its significant role in arthritic conditions. Furthermore, HEMJ in both doses restored the antioxidant levels in the liver which was disrupted by CFA induced arthritis. This may be due to the simultaneous anti-arthritic and antioxidant potential of HEMJ.

## CONCLUSION

In conclusion, the anti-arthritic and anti-oxidant activity exhibited by the HEMJ is a result of the synergistic action of the bioactive compounds present in the flowers.The results contribute towards validating the traditional use of *Mirabilis jalapa* flowers in the treatment of rheumatoid arthritis. However, no animal model completely depicts the pathophysiology and disease progression in this debilitating disease. Therefore, further investigational studies are required to elucidate the exact mechanism of anti-arthritic activity.

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