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Research Article

Dipsacus asperoides Extract Improves Physiological Behaviour and Controls Oxidative Stress Produced by a Rat Model of Osteoarthritis

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Abstract

Background and Objective: Osteoarthritis (OA) is the most complex joint condition in the world. It affects many joints in the body and is a leading cause of disability. The objective of this study was to evaluate the efficiency of *Dipsacus asperoides* (DA) extract in improving physical performance and regulating oxidative stress in an animal model of monosodium iodoacetate-induced Osteoarthritis (OA). **Materials and Methods:** To study the physical performance, rats were tested for the wire hang test, von frey test, open field test, rotarod test and hotplate assay were tested. After the examination period, the drug-administered animal's tissue homogenate was analysed for the biochemical estimations. **Results:** In this investigation, group VI exhibited the longest PWT time duration in the DA-administered groups in the von frey assay. Group VI achieved the longest hanging time (68 sec) in the hang test. Group VI exhibited a minimum of 51 movements in 5 min in an open-field test. In the rotarod experiment, group VI animals took a longer time (168 sec) to fall off the rotarod. Groups V and VI as well as group II, had a strong withdrawal response to heat stimulation in a hotplate test. In the biochemical analysis, DA administrated animals exhibited significant outputs compared to the other tested animals. **Conclusion:** The present study demonstrated that the phytochemical components that engage in physical performance improve osteoprotection and cure OA-related diseases.

Key words: *Dipsacus asperoides*, osteoarthritis, physiological behaviour, oxidative stress, animal model

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Osteoarthritis (OA) is the world's most complicated joint disease. This illness has so far claimed more than 100 million lives all over the world[1]. It affects a variety of joints in the body and is a major source of disability, which is becoming more prevalent as the population ages. The OA is also known as musculoskeletal system disease, degenerative bone disease and degenerative arthritis. The OA is caused by a modification in the mechanical and biological mechanisms that occur as a result of the ordinary coupling of articular cartilage breakdown and production[2]. It is distinguished by systemic, biological, genetic and morphological changes in cells and the Extracellular Matrix (ECM), which result in softening, fibrillation, ulceration, loss of articular cartilage, synovial inflammation, sclerosis of subchondral bone, osteophyte formation and subchondral cyst formation[3]. Although the specific mechanism of OA is unknown, it is assumed to be mediated by a variety of factors including Tumour Necrosis Factor (TNF), Interleukin (IL), Matrix Metalloproteinases (MMP), inducible nitric oxide synthase and C reactive protein[2]. Researchers believe that age, gender, obesity and genetic factors all have a role in the development of OA[4]. However, the physiological mechanism of the structural alterations associated with this disease, on the other hand, is complicated and the relationship to pain is unclear[5,6]. The primary goals of modern arthritis treatment are to relieve pain, reduce inflammation and improve joint performance[7].

Currently, Synthetic therapies using Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are widely used to treat OA inflammation and pain. But the long-term use of NSAIDs can cause a severe negative impact due to toxicity, side-effects and reappearing of symptoms in the gastrointestinal and cardiovascular systems in the body[8,9]. Even now, the origin of OA is unknown and no successful therapeutic cure for OA has been discovered[10]. Due to the restrictions of pharmaceutical therapy, alternative medicine and treatment would be quite essential to diagnosis in the early stages of treating OA. Currently, more investigations are being conducted into pain management and joint function improvement using natural bioactive substances[11,12].

Herbal medications and other therapeutic approaches are the mainstays of this alternative therapy. The vast majority of herbal bioactive compounds or phytochemical components are beneficial in disease prevention with few or no side effects. The DA is one of the well-known medicinal plants in traditional Chinese medicine. The root of the plant is commonly used to treat joint pain, limb paralysis, flutter injuries, tendon injuries and fractures. Recent investigations show that *D. asperoides* contains antioxidant and anti-inflammatory properties[13] as well as antiasthmatic properties[14].

The phytochemical components that engage in the molecular mechanism and gene expression changes to improve osteoprotection and cure OA-related diseases. A recent investigation found that DA paste relieved osteoarthritic knee pain in rats[15]. However, the ethnomedicinal benefits of DA on pain management and joint function have yet to be documented, even though natural product research for OA is an active field of study. Considering the efficiency of the medicinal plant, this investigation was carried to determine whether it improves pain management and joint function in rats by inducing Monosodium Iodoacetate in them.

MATERIALS AND METHODS

Plant material collection: Fresh DA plant material was obtained during January, 2020 from a private botanical garden in Hefeng, Hubei Province, China. The plant specimen was submitted to the institution herbarium (Herbarium no. of the plant is Ch10213\58294\2020).

Extraction of plant material: The extraction procedure was followed according to the method of Chun *et al.*[16]. The DA plant material (850 g d.wt.) was crushed using a blender and extracted with ethanolic solvent (70%) for 24 hrs. The solvent was filtered out using Whatman® No. 1 Filter paper. To concentrate the collected solvent, a rotary evaporator (R300BuchiRotavapor® at 28°C) with decreased pressure was used. The resulting residue was kept at 4°C for further examination.

Partial purification using column chromatography: Column chromatography was carried out on a standard 120×2 cm glass column filled with 15 g of silica gel (200-400 mesh). The plant residue on top of the silica gel bed and the column were eluted successively with ethanol.

High-performance liquid chromatography (HPLC): The chemical constituents present in the ethanol extract of DA were analysed using an LC 300 HPLC system interfaced with Simplicity Chrom™ CDS software.

Identification of compounds: The ethanolic extract of DA was performed on a chromatographic system. HPLC solution software was used to acquire and analyse the obtained spectral data (Santa Clara, CA). The unknown compound's spectra were cross-checked with the spectrum of known components recorded in the HPLC library.

Animals: Male Lewis rats (160-180 g) were procured from the Institution's Central Animal House for this investigation. Throughout the testing period, the rats were maintained in separate and wide and sanitary cages. The animals were fed and watered and kept in 12 hrs light-dim cycles at 22°C and 40-60% humidity. All investigations were conducted following the National Institutes of Health Guidelines for the Protection and Handling of Laboratory Animals (USA), as approved by the institute's local ethics board Reg. No: (61432/2020/CPCSEA/12.01.2020), as well as animal care and practise requirements.

Chemicals and reagents: All chemical reagents and solutions are freshly prepared at the beginning of this study in double-distilled water as stock solutions. Hyaluronic acid (HC) and Monosodium Iodoacetate (MIA) were purchased from Sigma Aldrich Chemical Co.

Acute toxicity: The LD_{50} value was determined using rats following Li *et al.*[17] DA plant extract at a logarithmic concentration was administered orally to 5 groups of 6 rats (100, 200, 300, 400 and 500 mg kg^{-1}). The toxicological impacts were assessed in terms of mortality from 1 hr after the dose to 24 hrs and the recorded effect will be considered as LD_{50}.

Induction of osteoarthritis: The MIA-induced animal model of OA was executed according to the modified descriptions of Combe *et al.*[18]. The test rodents were injected MIA (1 mg/30 L) in the tibiotarsal joint of the right hind paw for 2 days in a row as described earlier by Jimbo *et al.*[19].

Experimental protocol: The MIA induction rats were randomized and partitioned into 6 groups consisting of 6 rats (n = 6). These experiments were carried for 7, 14, 21 and 28 days:

Group 1 : Saline (10 mL kg^{-1} (i.p.)), served as control
Group 2 : 30 μL MIA (1 mg/30 μL saline) (i.p.)
Group 3 : 30 μL of HA (15 mg kg^{-1} orally) as a standard
Group 4 : 30 μL of DA extract (100 mg kg^{-1} orally)
Group 5 : 30 μL of DA extract (200 mg kg^{-1} orally)
Group 6 : 30 μL of DA extract (300 mg kg^{-1} orally)

All rats were evaluated for behavioural evaluations such as the wire hang test, von frey test, open field test, rotarod test and hotplate test on the 1st, 7th, 14th, 21st and 28th days after MIA induction.

Ankle measurement: Ankle diameters were measured following the descriptions of Jimbo *et al.*[20]. The measurements were taken by Anteroposterior on the 7th, 14th, 21st and 28th days after the MIA was administered.

Behavioural assessment

Wire hang test: The Wire Hang test was carried out following the instructions provided by Patel *et al.*[2]. The physical pain of animals with OA was assessed using a wire hang test utilising a wire screen.

Von frey test: The Von Frey test was performed based on the method of Ziaei *et al.*[21]. Paw Withdrawal Threshold (PWT) was measured by von Frey monofilaments.

Open field test: The open-field assay was carried out according to the method of Choudhary *et al.*[22]. The square arena was used to analyse the locomotor activity of the test animals.

Rotarod test: The rotarod test was performed by the method of Singh *et al.*[23]. A rotarod performance test was used to assess motor coordination and balance. An accelerating rotarod performance test was used to determine the time to fall.

Hotplate test: The Hotplate assay was conducted by the modified method of Tsai *et al.*[24]. The hot plate experiment was performed to assess the time to paw licking by using a hot plate analgesia metre. The reaction time was measured by the tested animals' licking their fore or hind paws or jumping before and 15, 30, 45, 60, 90 and 120 min after drug treatment.

Biochemical analysis

Dissection and homogenization: After a behaviour evaluation, the rats were decapitated on the 28th day. The tissue homogenate (10%) was prepared in 0.1 M PBS solution (pH 7.4) and centrifuged at 10,000 rpm for 20 min. The supernatant was collected and utilised for biochemical analysis.

TBARS assay: The TBARS test has been carried out using a modified method of Gateva *et al.*[25].

GSH assay: The reduced glutathione level analysis was investigated by the modified method described by Wei *et al.*[26].

Catalase assay: The catalase assay was tested according to the modified method described by Chen *et al.*[27].

Superoxide dismutase assay (SOD): The SOD assay was examined using a modified method depicted by Wang *et al.*[28].

Glutathione peroxidase assay: Determination of GPx activity was conducted by the modified method described by Wang *et al.*[28].

Data analysis: All data collection and analysis were expressed as mean SD in terms of the number of tests (n = 6). Individual correlations and Duncan's Multiple Range Test (DMRT) were used to examine the statistical outputs, which were evaluated using one-way analysis of variance (ANOVA) in SPSS version 17 software. The data was found statistically significant.

RESULTS

In the current study, ethanolic DA extract was concentrated under decreased pressure to yield a 12.23 g dark green sticky mass. There was no mortality in the animal bioassay in Table 1. In the toxicological investigation, various dose levels such as 100, 200, 300, 400 and 500 mg kg^{-1} were employed. During the 24 hrs evaluation period, the test animals were confirmed to be normal and no mortality was observed. Three dose levels (100, 200 and 300 mg kg^{-1}) were chosen for further investigation in this study.

Figure 1 shows the change in edema size of the tested animals. In the study, group II (only MIA-induced) showed high edema (0.7 mm) on the 7th day and 0.66 mm on the 28th day. In DA-administered test group IV, edema of 0.69 mm on the 7th day gradually reduced to 0.6 mm on the 28th day. In group V, 0.69 mm of edema was shown on the 7th day and it was reduced to 0.6 mm on the 28th day. On the 7th day, 0.68 mm of edema was observed in group VI, which was reduced to 0.5 mm on the 28th day. The results demonstrated that after administering DA extract, the edema level was dramatically decreased ($p<0.01$) dependent on the dosage.

Figure 2 shows the hanging time duration of the tested animals. In this experiment, the wire hand test was used to estimate the physical pain of the tested animals. In this study, group III (HA injected group) animals showed maximum hanging time (55 sec) on the 28th day of the administration compared to other tested groups. In DA-administered groups, group IV showed a hanging time of 21 sec in 1st of administration but on the 28th day hanging time was showed 29 sec. The hanging time in group V was 19 sec on the 1st day and it was increased to 39 sec on the 28th day. In group VI, a hanging time of 26 sec was shown on the 1st day and it was increased up to 39 sec on the 28th day.

In the present study, the Paw Withdrawal Threshold (PWT) of the test animals was measured by the von frey test in Fig. 3. PWT time duration in group III (HA injected group) was 23 sec on the 1st day, 21 sec on the 7th day and 27 sec on the 28th day.

Group IV in the DA-administered group exhibited 17 sec on the 1st day, 8 sec on the 7th day and 13 sec on the 28th day. Group V showed 19 sec on the 1st day, 9 sec on the 7th day and 18 sec on the 28th day. In group VI, the time was 17 sec on the 1st day, 13 sec on the 7th day and 26 sec on the 28th day. This experiment proved that DA administration significantly ($p<0.05$) increased the Paw Withdrawal Threshold (PWT) of the test animals.

The open-field test is concluded with an analysis of the behavioural activities associated with locomotion. The open-field test is expressed in Fig. 4 and 5. The peripheral movements of the tested animals are expressed in Fig. 4. In this assay, group III animals showed maximum peripheral movement compared to other tested groups. In DA administrated groups, group VI showed a minimum of 51 moves in 5 min. It is almost similar to the standard group (group III). Figure 5 expressed the central movements in tested animals. The maximum number of central movements was found in group III (26 movements) and group VI (25 movements). This study's findings revealed a significant reduction ($p<0.05$) in behaviour analysis, such as peripheral and central movements, in the examined animal groups (IV and V), when compared to the HA Injected group (group III).

The rotarod analysis was used to measure the locomotor function and motor coordination of the drug administrated animals. The duration of the mice to drop off the rod was measured in this study during a maximum observation time of 5 min. When compared to the other tested groups, group VI animals took the longest time duration (168 sec) to fall from the rotarod. This experiment proved that the latency to fall in the rotarod test increased significantly ($p<0.05$) from the 1st day to the 28th day according to the dosage given to the tested animals Fig. 6.

The hotplate test is conducted to analyze the thermal hyperalgesia of the tested animals. In this experiment, the low withdrawal response to heat stimulation was noted in III, V and group VI. Group II showed a high withdrawal response to heat stimulation in Fig. 7. This study's result proved that DA administration significantly improved ($p<0.05$) the heat tolerance according to the increasing dose level in the OA-induced groups of animals.

The biochemical changes of the DA administrated animals were expressed in Table 2. In this study, TBARS, GSH, SOD, CAT and GPx levels in tissue cells were examined in

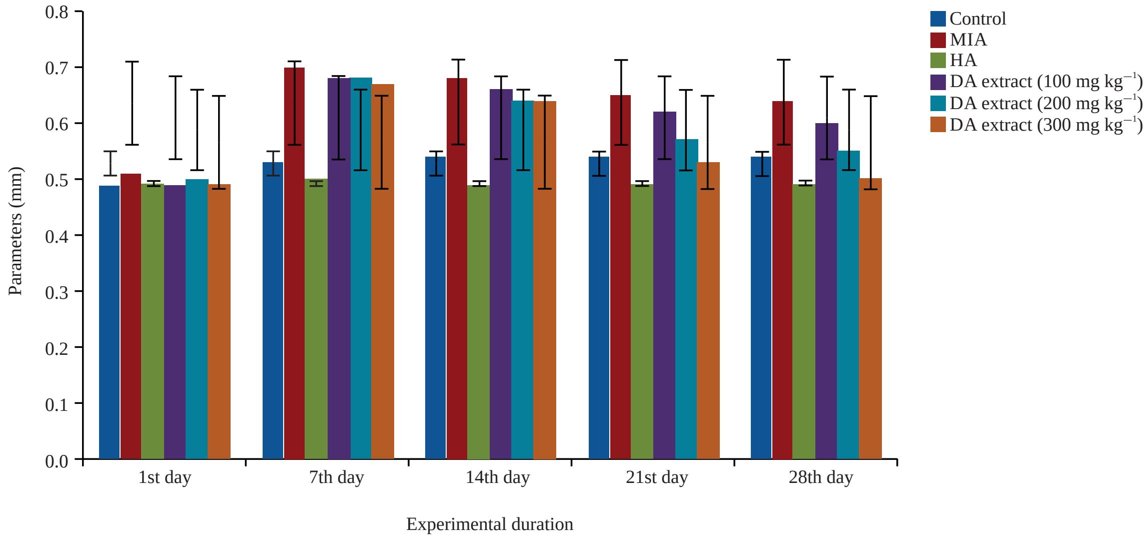

Fig. 1: Changes in edema size of the tested animals
Data were represented as Mean±SEM and p = 0.05

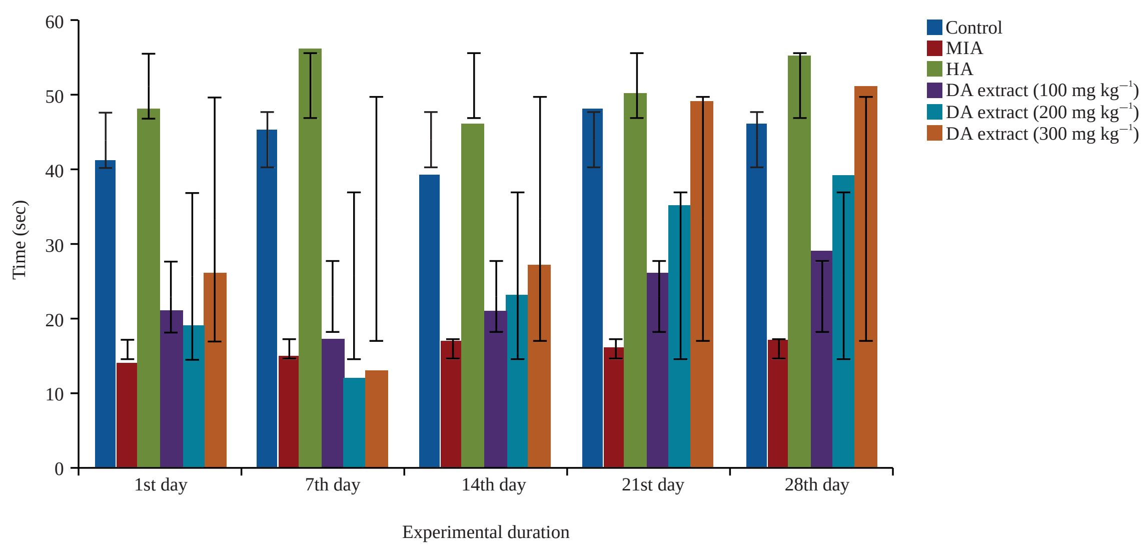

Fig. 2: Hanging time duration by the tested animals
Data were represented as Mean±SEM and p = 0.05

Table 1: LD_{50} results of DA extract on rats

Group	Extract	Dose (mg kg^{-1}) (i.p.)	Log dose	Dead/total	Dead (%)	Corrected (%)	Probit
1	DA extract	100	2	0/6	0	2.5	3.04
		200	2.3	0/6	0	2.5	3.04
		300	2.47	0/6	0	2.5	3.04
		400	2.60	0/6	0	2.5	3.04
		500	2.7	0/6	0	2.5	3.04

tested group animals. After MIA treatment, lipid peroxidation levels increased in group II animals compared to control group but decreased significantly in DA-treated groups (group IV (5.1±0.11 μmol L^{-1}), group V (4.9±0.1911 μmol L^{-1}) and group VI (4.7±0.1111 μmol L^{-1}). In the reduced glutathione test, the level of reduction was observed in group II animals compared to group I animals but considerably increased in DA-treated groups (group IV (0.42±0.004 nM mL^{-1}), group V (0.65±0.11 nM mL^{-1}) and group VI (0.85±0.33 nM mL^{-1}). Group II exhibited a significant

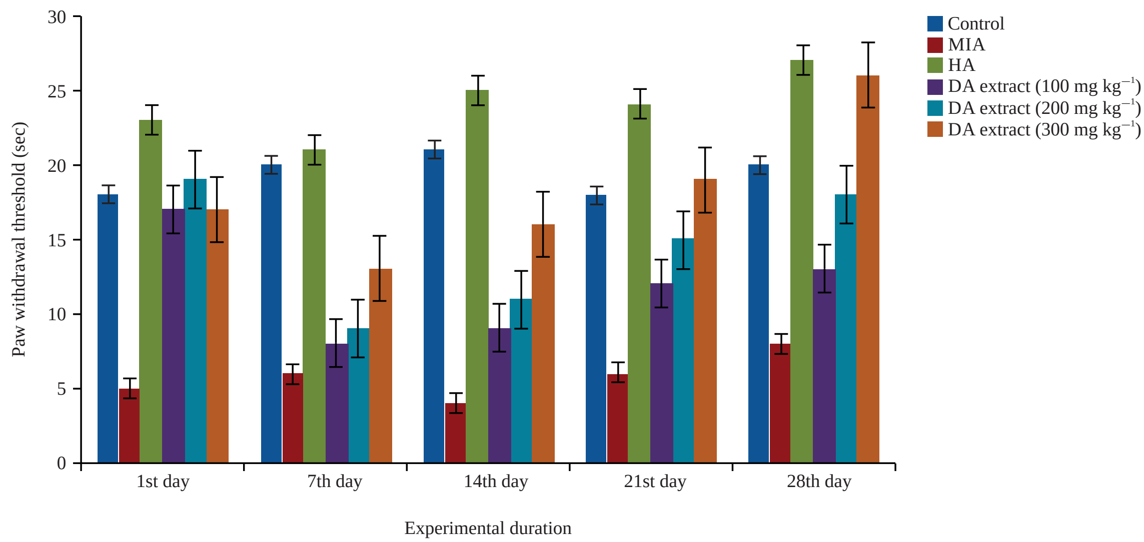

Fig. 3: Paw withdrawal threshold (PWT) of the tested animals
Data were represented as Mean±SEM and $p = 0.05$

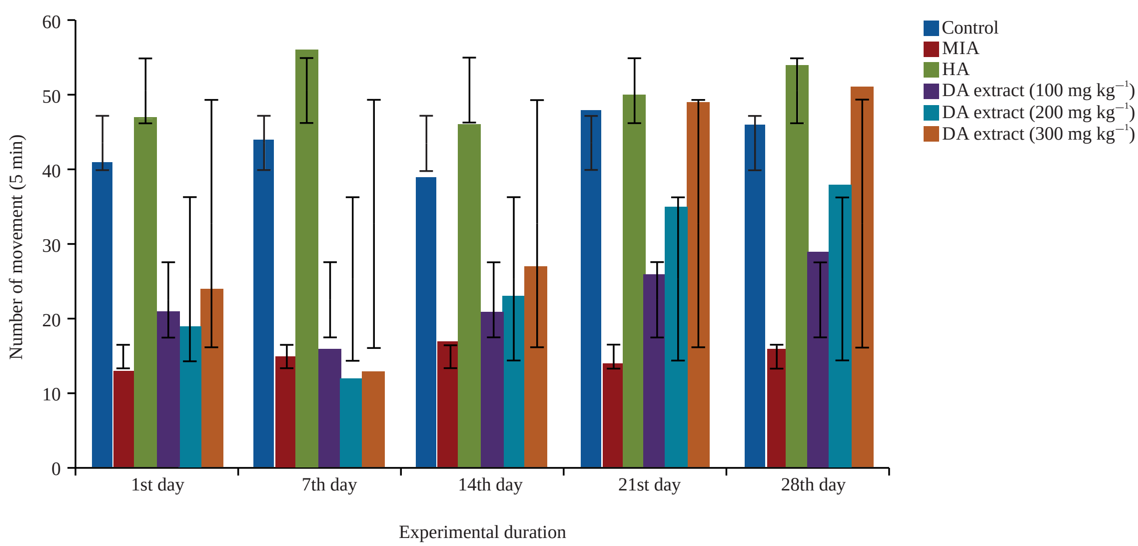

Fig. 4: Peripheral movements of the tested animals
Data were represented as Mean±SEM and $p = 0.05$

Table 2: TBARS, GSH, SOD, CAT and GPx levels in tissue cells

Groups	TBARS (μmol L^{-1})	GSH (nM mL^{-1})	SOD (U^A mg^{-1} protein)	CAT (U^B mg^{-1} protein)	GPx(U^c mg^{-1} protein)
Control	4.1 ± 0.11^a	0.89 ± 0.33^b	11.84 ± 0.33^b	1.62 ± 0.01^c	0.89 ± 0.06^b
MIA	6.02 ± 0.33^a	0.26 ± 0.03^a	5.16 ± 0.01^a	0.38 ± 0.13^b	0.12 ± 0.01^c
HA	4.6 ± 0.66^b	0.92 ± 0.01^b	10.14 ± 0.19^c	1.2 ± 0.11^b	0.81 ± 0.13^a
DA extract (100 mg kg^{-1})	5.1 ± 0.11^c	0.42 ± 0.004^c	6.12 ± 0.11^b	0.49 ± 0.66^c	0.82 ± 0.33^a
DA extract (200 mg kg^{-1})	4.9 ± 0.19^b	0.65 ± 0.11^b	8.37 ± 0.13^a	0.67 ± 0.06^a	0.48 ± 0.13^c
DA extract (300 mg kg^{-1})	4.7 ± 0.11^c	0.85 ± 0.33^a	9.82 ± 0.03^c	0.99 ± 0.17^c	034 ± 0.11^b

a: Quantity of enzyme necessary to occupy 50% of the NBT reduction, b: Mmles of H_2O_2 consumed/min/mg/protein, c: Quantity of glutathione utilized/min, the values that do not share a common superscript letter differ substantially at $p<0.005$ (DMRT), SOD: Superoxide dismutase assay, GPx: Glutathione peroxidase, assay, CAT: Catalase assay, GSH: Glutathione level and TBARS: Thiobarbituric acid reactive substances

decrease in superoxide dismutase activity as compared to group I (control group). However, group IV (6.12 ± 0.11 U^A mg^{-1}), group V (8.37 ± 0.13 U^A mg^{-1}) and group VI (9.82 ± 0.03 U^A mg^{-1}) showed significant increases. In glutathione peroxidase assay, MIA only treated group expressed a significant increase compared to the control

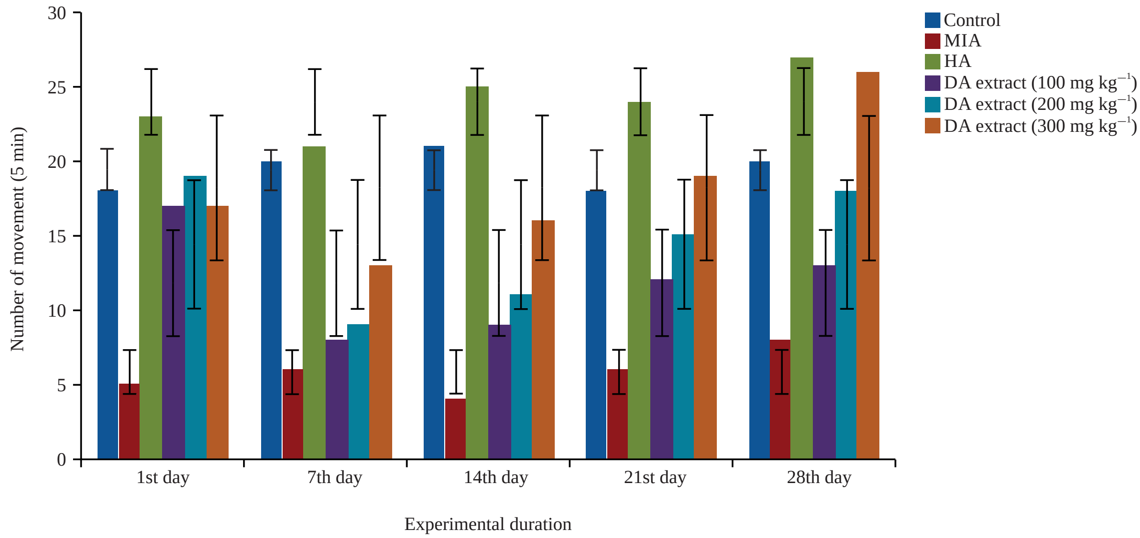

Fig. 5: Central movements of the tested animals
Data were represented as Mean±SEM and $p = 0.05$

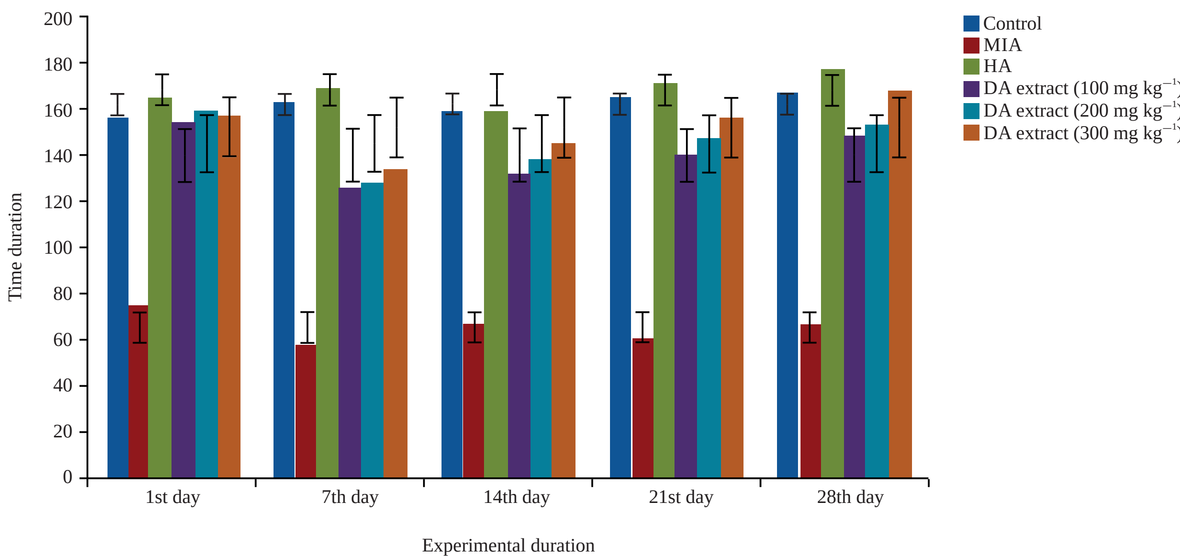

Fig. 6: Latency to fall in rotarod analysis by the tested animals
Data were represented as Mean±SEM and $p = 0.05$

group but dropped considerably in DA-treated animals (group IV (0.49±0.66 U^B mg^{-1}), group V (0.67±0.06 U^B mg^{-1}) and group VI (0.99±0.17 U^B mg^{-1}). In the peroxidase assay, MIA-treated rats (group II) exhibited a significant increase in comparison to the control group, whereas DA-treated animals showed a significant decrease (group IV (0.82±0.33 U^C mg^{-1}), group V (0.48±0.13 U^C mg^{-1}) and group VI (0.34±0.11 U^C mg^{-1}). In these anti-oxidant assays, both group IV and group I exhibited no significant changes in SOD, CAT or GPX levels.

Figure 8 express the HPLC chromatogram of the tested ethanolic DA extract. These HPLC spectra represent the presence of several chemical compounds which were conformed to known compound spectra, previous references and the HPLC library. The compounds and their peaks are as follows: Limonene (1.534, 2.841), sesquiterpenoids (24.16, 25.22), caryophyllene (14.25), akebia saponin D (6.112), cubebene (9.549), isochlorogenic acid (27.59), eugenol (7.141), Loganin/Loganic acid (14.25), chlorogenic acid (31.23, 22.76), etc.

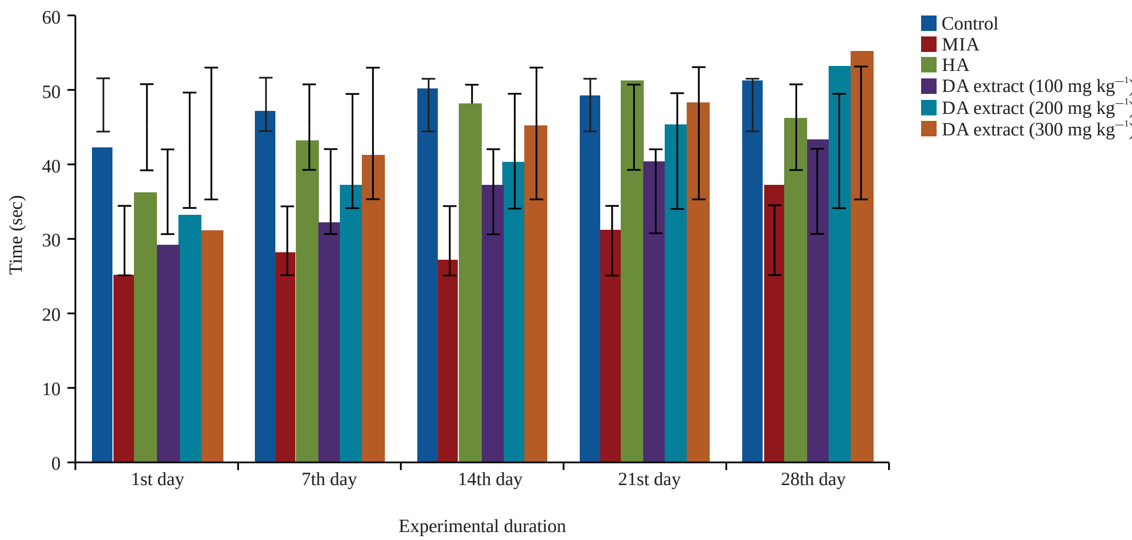

Fig. 7: Response latency time of the paws to heat stimulation in hotplate test
Data were represented as Mean±SEM and p = 0.05

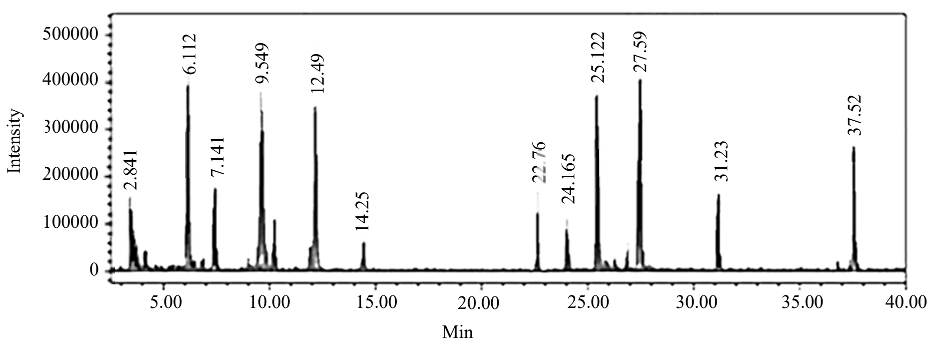

Fig. 8: HPLC chromatogram of ethanolic DA extract

DISCUSSION

The pharmacological challenge today is to characterise and comprehend the variety of bioactive chemicals derived from plants. These bioactive chemicals derived from plants are significant in the treatment of a wide range of diseases. The modes of action are quite distinct and they can be expressed independently or in conjunction with other medicinal substances. Another fascinating challenge is determining which plants have been used in traditional medicinal systems all over the world, investigating their phytochemical components and explaining whether and how their bioactive metabolites may impact the known therapeutic potential. Nowadays, several ancient remedies have been developed into contemporary licensed medications that have undergone clinical testing. Structured clinical studies have demonstrated the effectiveness of a number of these plant-derived medicines, allowing them to be recommended in evidence-based clinical therapy[29].

The primary goal of this study was to determine the efficacy of the Chinese traditional medicinal plant DA and how its extract improves physiological behaviour and regulates oxidative stress in an osteoarthritis rat model. Fresh DA plant materials were obtained from a nearby private botanical garden and the entire plants were chopped into small pieces and extracted with ethanol. The ethanolic DA extraction was then condensed under reduced pressure to produce a 12.23 g dark green sticky mass. The DA extract was partly purified using column chromatography and the eluted extract was analysed for the presence of chemical components using an HPLC system. Based on the retention durations, the peaks were identified as the following chemical components: Sesquiterpenoids, caryophyllene, akebia saponin D, cubebene, isochlorogenic acid, eugenol, loganic acid and chlorogenic acid. Several chemical components, including triterpenoids, triterpene saponins, iridoids, iridoid glucosides, lignans, phenolics and alkaloids, have been discovered from the roots of DA in earlier research[30]. Wink[29], discovered around

15 phenolic compounds, three of which had not before been discovered in DA. Sun *et al.*[30] studied the chemical composition of dipsacus asper oil. Li *et al.*[31] conducted extensive research on comparative proteomic analysis of DA roots from China.

According to the results of the investigations, the behavioural evaluation produced overwhelming outcomes. During the investigation period, no mortality was found in the animal bioassay. The ankle assessment procedure is one of the most significant assessments used to detect ankle joint swelling (edema) in drug-administered test animals.

In this study, the MIA-induced group had significantly more edema (0.7 mm) than the other examined groups. Simultaneously, DA-treated test groups exhibited very low edema development. The results of this study revealed that the edema level was considerably reduced (p<0.01) based on the dose. These findings are consistent with earlier research that found MIA injection enhanced ankle anteroposterior and transverse dimensions[19]. The intra-articular injection of HA substantially increased knee joint diameter, resulting in knee swelling[32,33]. Halfaya *et al.*[34], observed substantial increases in both the right ankle anteroposterior and transverse dimensions in all osteoarthritic OA rats compared to the normal and control group at all periods of observation. Ragab *et al.*[35], discovered substantial increases in the right ankle anteroposterior and transverse diameters in the MIA group at all time points following MIA treatment compared to the normal control group. But Jimbo *et al.*[20], discovered no significant changes in knee anteroposterior or transverse width among MIA and MIA-HA animals at any time point. Several studies and articles have indicated that some polyphenols and flavonoids have a significant role in the therapy of inflammation and arthritic problems. For example, Vitexin demonstrated substantial analgesic, anti-inflammatory and immunologic properties by considerably increasing the heat threshold and reducing the paw edema caused by carrageenan[36]. Galuteolin exhibited potent anti-inflammatory action in animals, reducing inflammatory swelling produced by DMB[37]. Furthermore, luteolin can regulate inflammatory reactions[38] and apigenin is a strong anti-inflammatory medicine[39]. The therapy of osteoarthritic animals with HA resulted in a reduction in the rising levels of the right ankle anteroposterior and transverse widths after the test when compared to the osteoarthritic animals.

The von frey test was used to determine the Paw Withdrawal Threshold (PWT) of the test animals in this investigation[40]. Compared to the other groups, the HA-injected group (group III) had the longest PWT time duration. As compared to group III, group VI had the longest PWT time length in the DA-administered group, whereas group III had the shortest PWT time duration. Choi *et al.*[9] previously used the von frey test to investigate the analgesic properties of ALM16 against secondary mechanical hypersensitivity in OA. In their investigation, treatment of ALM16 at 200 and 400 mg kg^{-1} b.wt., in the MIA-induced OA pain model substantially enhanced the lowering of PWT levels and became more successful at raising PWT levels than the other treated groups. Several investigations have revealed that JOINSTM, a therapy that combines with extracts of oriental herbs that are frequently and traditionally used to treat OA individuals in Korea, has cartilage protective properties by preventing ECM constituent breakdown via MMP expression levels[41].

The hang test is used in animal behaviour evaluation to measure the physical pain of the animals[42]. Because of the extreme pain induced by the intra-articular injection, the animals' grip strength, hanging duration and locomotor activity were considerably decreased. In this study, when compared to the other examined groups, the animals in the HA-injected group had the longest hanging period (80 sec). On the 28th day, group VI had the longest hanging time in the DA-administered groups, with a maximum hanging duration of 68 sec. This finding indicates that DA treatment resulted in a substantial (p<0.05) increase in hanging time from 14-28 days. There is significant evidence that some cytokines are involved not only in the beginning but also in maintaining chronic pain by direct stimulation of nociceptive sensory neurons. As previously mentioned, phytochemicals found in DA may block peripheral inflammatory processes, compromising the production of cytokines and prostaglandins and therefore decreasing inflammation pain in animals. The activation of opioids receptors may also contribute to the role of peripheral pathways. Moreover, more research is needed to investigate the role of phytochemicals in the regulation of analgesia via central pathways.

The open-field test is concluded with an analysis of the behavioural activities associated with locomotion such as crossing the central squares, indicating exploratory behaviour such as mental pressure, adaptability and mobility[43]. In this experiment, group III animals showed the highest peripheral movement when compared to the other examined groups. Group VI demonstrated a minimum of 51 movements in 5 min. It is nearly identical to the standard group. Other DA-administered test groups did not exhibit significant movements, which may be attributed to the observed articular joint inflammation and degeneration, or due to osteoarthritic pain. In the central movement study, group III and group VI showed a high number of central movements. The ongoing therapy with DA helped to prevent the impediment caused by MIA by promoting a considerable reduction in behaviour and simply extending movement activities. Our findings are consistent with Binfare *et al.*[44], who discovered a reduction in behavioural activity, rearing and all other behaviours in haloperidol-treated rats. The rotarod experiment for rodents is an excellent technique to assess strength and endurance. It can also be used as a corollary test in conjunction with other rodent behavioural tests. The rotarod analysis was performed to assess the drug-administered animals' locomotor activity and motor function[45]. In this experiment, group VI animals took a longer duration (168 sec) to fall from the rotarod than the other examined groups. The latency to fall in the rotarod test increased significantly ($p<0.05$) from the 1st day to the 28th day according to the dosage given to the tested animals. The hotplate test is conducted to analyze the thermal hyperalgesia of the tested animals. In this experiment, the low withdrawal response to heat stimulation was noted in group III, group V and group VI. Group II showed a high withdrawal response to heat stimulation. The presence of phytochemicals may influence tested animals. Several studies have shown that steroids are powerful sedative drugs that decrease spontaneous motor activity in mice[46]. Moreover, flavonoids have been shown to have anxiolytic[47] and antidepressant properties[48]. As an outcome, the phytochemical composition of DA extract is believed to have a role in the reported medicinal and therapeutic activities.

The aetiology of several disorders is entwined with oxidative pressure and the consequences of lipid peroxidation. Cell reinforcement's agents such as melatonin, quercetin, ebselen and vitamin E are effective in the treatment of a wide range of diseases. Oxidative stress aggravates cancer, diabetes, arthritis, rheumatoid arthritis, neurological diseases, hypertension, atherosclerosis and chronic inflammatory disorders (OS). Recent research has suggested that OS plays a role in the development of OA. The OS is continually generated, damaging cells and the extracellular matrix proteins. Increased production of ROS with a decrease in antioxidants suggests disease progression[49]. The majority of antioxidant reaction elements are responses to OS inducers.

It responds mostly to oxidative stress inducers. When exposed to oxidative stress, Nrf2 attaches to the antioxidant reaction elements and activates antioxidant genes[50]. Under pathological conditions of OA, the Nrf2/ARE signalling system is primarily important for cellular defences against OS. The aforementioned impact can be associated with a change in specific endogenous antioxidant characteristics, such as a drop in glutathione levels and a decrease in antioxidant defence enzyme activity, such as superoxide dismutase and catalase[51]. Catalase is a key antioxidant that assists in the neutralisation of H_2O_2's negative effects. The catalase enzyme converts H_2O_2 to water and non-reactive oxygen species, reducing the accumulation of free radical precursors. Catalase activity decreases due to OS[52].

The levels of TBARS, GSH, SOD, CAT and GPx in tissue cells of tested animals were explored in this research. Lipid peroxidation levels increased in group II animals after MIA treatment compared to the control group but decreased considerably in DA-treated groups. In the reduced glutathione assay, glutathione levels in DA-treated groups were significantly higher than in the control group. Groups VI, V and VI demonstrated substantial increases in superoxide dismutase activity as compared to the other examined groups. In glutathione peroxidase tests, group IV, V and VI showed significantly lower levels than the other groups. In the peroxidase assay, the DA-tested group exhibited a substantial reduction compared to the other group. Both group IV and group I showed no significant increases in SOD, CAT or GPX levels in these anti-oxidant tests. The current findings demonstrate that the movements promoted by DA extract in the OA animal model were associated with substantial improvements in the behavioural activities displayed by the recovered OA.

CONCLUSION

The phytochemical constituent's presence in the DA extract plays a significant role in the molecular mechanism and protection against exposure in models and it has been attributed to its potent ethnopharmacological effects. The outcomes of this study showed that DA extracts have a positive effect on the experimental animal model. However, more investigation is necessary to completely understand the gene expression and molecular mechanism involved in the osteoprotective effect.

SIGNIFICANCE STATEMENT

The DA is a well-known medicinal herb in traditional Chinese medicine. It was used to treat joint pain, limb paralysis, flutter injuries, tendon injuries and fractures. However, no appropriate studies have been conducted to determine the characteristics of DA. Therefore, the current study was conducted to prove that the presence of phytochemical ingredients in DA extract plays a critical role in the molecular mechanism and protection against exposure in models and this has been connected to its substantial ethnopharmacological effects.

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REFERENCES

1. Gelber, A.C., 2014. Osteoarthritis. Ann. Intern. Med., Vol. 161. 10.7326/0003-4819-161-1-201407010-01001.
2. Patel, D.V., M.G. Sawant and G. Kaur, 2015. Evaluation of anti-osteoarthritic activity of *Vigna mungo* in papain induced osteoarthritis model. Indian J. Pharmacol., 47: 59-64.
3. Xia, B., D. Chen, J. Zhang, S. Hu, H. Jin and P. Tong, 2014. Osteoarthritis pathogenesis: A review of molecular mechanisms. Calcif. Tissue Int., 95: 495-505.
4. Wu, Y., L. Chen, Y. Wang, W. Li and Y. Lin *et al*., 2015. Overexpression of sirtuin 6 suppresses cellular senescence and NF-κB mediated inflammatory responses in osteoarthritis development. Sci. Rep., Vol. 5. 10.1038/srep17602.
5. Felson, D.T., 2005. The sources of pain in knee osteoarthritis. Curr. Opin. Rheumatol., 17: 624-628.
6. Beyreuther, B., N. Callizot and T. Stohr, 2007. Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain. Arthritis Res. Ther., Vol. 9. doi.org/10.1186/ar2121.
7. Sokolove, J. and C.M. Lepus, 2013. Role of inflammation in the pathogenesis of osteoarthritis: Latest findings and interpretations. Ther. Adv. Musculoskeletal Dis., 5: 77-94.
8. Yang, H.J., M.J. Kim, J.Y. Qiu, T. Zhang, X. Wu, D.J. Jang and S. Park, 2019. Rice porridge containing welsh onion root water extract alleviates osteoarthritis-related pain behaviors, glucose levels and bone metabolism in osteoarthritis-induced ovariectomized rats. Nutrients, Vol. 11. 10.3390/nu11071503.
9. Choi, D.J., S.I. Choi, B.R. Choi, Y.S. Lee, D.Y. Lee and G.S. Kim, 2019. Cartilage protective and anti-analgesic effects of ALM16 on monosodium iodoacetate induced osteoarthritis in rats. BMC Complementary Altern. Med., Vol. 19. 10.1186/s12906-019-2746-7.
10. Lane, N.E., K. Shidara and B.L. Wise, 2017. Osteoarthritis year in review 2016: Clinical. Osteoarthr. Cartil., 25: 209-215.
11. Cameron, M. and S. Chrubasik, 2013. Topical herbal therapies for treating osteoarthritis. Cochrane Database Syst. Rev., Vol. 5. 10.1002/14651858.cd010538.
12. Dhippayom, T., C. Kongkaew, N. Chaiyakunapruk, P. Dilokthornsakul, R. Sruamsiri, S. Saokaew and A. Chuthaputti, 2015. Clinical effects of Thai herbal compress: A systematic review and meta-analysis. Evidence-Based Complementary Altern. Med., Vol. 2015. 10.1155/2015/942378.
13. Park, J.Y., S.D. Park, Y.J. Koh, D.I. Kim and J.H. Lee, 2019. Aqueous extract of *Dipsacus asperoides* suppresses lipopolysaccharide-stimulated inflammatory responses by inhibiting the ERK1/2 signaling pathway in RAW 264.7 macrophages. J. Ethnopharmacol., 231: 253-261.
14. Shin, N.R., A.Y. Lee, G. Park, J.W. Ko, J.C. Kim, I.S. Shin and J.S. Kim, 2019. Therapeutic effect of *Dipsacus asperoides* C. Y. cheng et T. M. Ai in ovalbumin-induced murine model of asthma. Int. J. Mol. Sci., Vol. 20. 10.3390/ijms20081855.
15. Siu, W.S., W.T. Shum, W. Cheng, C.W. Wong and H.T. Shiu *et al*., 2019. Topical application of Chinese herbal medicine DAEP relieves the osteoarthritic knee pain in rats. Chin. Med., Vol. 14. 10.1186/s13020-019-0278-1.
16. Chun, J.M., A.Y. Lee, J.Y. Nam, K.S. Lim and M.S. Choe *et al*., 2021. Effects of *Dipsacus asperoides* extract on monosodium iodoacetate-induced osteoarthritis in rats based on gene expression profiling. Front. Pharmacol., Vol. 12. 10.3389/fphar.2021.615157.
17. Li, L.Y., X. Wang, T.C. Zhang, Z.J. Liu and J.Q. Gao, 2021. Cardioprotective effects of omega 3 fatty acids from fish oil and it enhances autoimmunity in porcine cardiac myosin-induced myocarditis in the rat model. Z. Naturforsch. C., 76: 407-415.
18. Combe, R., S. Bramwell and M.J. Field, 2004. The monosodium iodoacetate model of osteoarthritis: A model of chronic nociceptive pain in rats?. Neurosci. Lett., 370: 236-240.
19. Jimbo, S., Y. Terashima, A. Teramoto, T. Takebayashi and I. Ogon *et al*., 2019. Antinociceptive effects of hyaluronic acid on monoiodoacetate-induced ankle osteoarthritis in rats. J. Pain Res., 12: 191-200.
20. Jimbo, S., Y. Terashima, T. Takebayashi, A. Teramoto and I. Ogon *et al*., 2017. A novel rat model of ankle osteoarthritis induced by the application of monoiodoacetate. J. Arthritis, Vol. 6. 10.4172/2167-7921.1000260.
21. Ziaei, A., S. Sahranavard, M.J. Gharagozlou and M. Faizi, 2016. Preliminary investigation of the effects of topical mixture of *Lawsonia inermis* L. and *Ricinus communis* L. leaves extract in treatment of osteoarthritis using MIA model in rats. DARU J. Pharm. Sci., Vol. 24. 10.1186/s40199-016-0152-y.
22. Choudhary, D., P. Kothari, A.K. Tripathi, S. Singh and S. Adhikary *et al*., 2018. *Spinacia oleracea* extract attenuates disease progression and sub-chondral bone changes in monosodium iodoacetate-induced osteoarthritis in rats. BMC Complementary Altern. Med., Vol. 18. 10.1186/s12906-018-2117-9.
23. Singh, S., A. Mishra and S. Shukla, 2016. ALCAR exerts neuroprotective and pro-neurogenic effects by inhibition of glial activation and oxidative stress via activation of the Wnt/β-catenin signaling in parkinsonian rats. Mol. Neurobiol., 53: 4286-4301.

24. Tsai, H.C., T.L. Chen, Y.P. Chen and R.M. Chen, 2018. Traumatic osteoarthritis-induced persistent mechanical hyperalgesia in a rat model of anterior cruciate ligament transection plus a medial meniscectomy. J. Pain Res., 11: 41-50.
25. Andreeva-Gateva, P., L. Traikov, Z. Sabit, D. Bakalov and R. Tafradjiiska-Hadjiolova, 2020. Antioxidant effect of alpha-lipoic acid in 6-hydroxydopamine unilateral intrastriatal injected rats. Antioxidants., Vol. 122. 10.3390/antiox9020122.
26. Wei, H.J., M. Du and H.Y. Bai, 2019. Correlations of melatonin and glutathione levels with oxidative stress mechanism in Parkinson's disease. Zhongguo Yi Xue Ke Xue Yuan Xue Bao., 41: 183-187.
27. Chen, D., Y. Zhou, K.E. Lyons, R. Pahwa and M.B. Reddy, 2015. Green tea consumption reduces oxidative stress in Parkinson's disease patients. J. Behav. Brain Sci., 5: 194-202.
28. Wang, H., Y. Pan, B. Xue, X. Wang and F. Zhao *et al*., 2011. The antioxidative effect of electro-acupuncture in a mouse model of Parkinson's disease. PLoS ONE, Vol. 6. 10.1371/journal.pone.0019790.
29. Wink, M., 2015. Modes of action of herbal medicines and plant secondary metabolites. Medicines, 2: 251-286.
30. Sun, X., G. Ma, D. Zhang, W. Huang and G. Ding *et al*., 2015. New lignans and iridoid glycosides from *Dipsacus asper* Wall. Molecules, 20: 2165-2175.
31. Li, H., D. Zhang, L.H. Tan, B. Yu, S.P. Zhao and W.G. Cao, 2017. Comparison of the antioxidant properties of various solvent extracts from *Dipsacus asperoides* and identification of phenolic compounds by LC-ESI-QTOF-MS-MS. South Afr. J. Bot., 109: 1-8.
32. Lu, H.Y., X.C. Liu, Q.Z. Liu and Z.L. Liu, 2017. Chemical composition of *Dipsacus asper* Wallich ex candolle (Dipsacaceae) essential oil and its activity against mosquito larvae of *Aedes aegypti* and *Culex pipiens* pallens. Trop. J. Pharm. Res., 16: 179-184.
33. Jin, H., H. Yu, H. Wang and J. Zhang, 2020. Comparative proteomic analysis of *Dipsacus asperoides* roots from different habitats in China. Molecules, Vol. 25. 10.3390/molecules25163605.
34. Halfaya, F.M., G.H. Ragab, U. Hagag, O.M. Ahmed and W.A. Elkheir, 2020. Efficacy of hyaluronic acid in the treatment of MIA-induced ankle osteoarthritis in rats and its effect on antioxidant response element. J. Vet. Med. Res., 27: 119-127.
35. Ragab, G.H., F.M. Halfaya, O.M. Ahmed, W.A. El-Kheir and E.A. Mahdi *et al*., 2021. Platelet-rich plasma ameliorates monosodium iodoacetate-induced ankle osteoarthritis in the rat model via suppression of inflammation and oxidative stress. Evidence-Based Complementary Altern. Med., Vol. 2021. 10.1155/2021/6692432.
36. Zhu, Q., L.N. Mao, C.P. Liu, Y.H. Sun, B. Jiang, W. Zhang and J.X. Li, 2016. Antinociceptive effects of vitexin in a mouse model of postoperative pain. Sci. Rep., Vol. 6. 10.1038/srep19266.
37. Cheng, X., F. Zhang, J. Li and G. Wang, 2019. Galuteolin attenuates cerebral ischemia/reperfusion injury in rats via anti-apoptotic, anti-oxidant and anti-inflammatory mechanisms. Neuropsychiatr. Dis. Treat., 15: 2671-2680.
38. Wang, S., M. Cao, S. Xu, J. Zhang and Z. Wang *et al*., 2017. Effect of luteolin on inflammatory responses in RAW264.7 macrophages activated with LPS and IFN-γ. J. Funct. Foods, 32: 123-130.
39. Liang, H., S. Sonego, E. Gyengesi, A. Rangel, G. Niedermayer, T. Karl and G. Münch, 2017. OP-25-anti-inflammatory and neuroprotective effect of apigenin: Studies in the GFAP-IL6 mouse model of chronic neuroinflammation. Free Radic. Biol. Med., Vol. 108. 10.1016/j.freeradbiomed.2017.04.064.
40. Deuis, J.R., L.S. Dvorakova and I. Vetter, 2017. Methods used to evaluate pain behaviors in rodents. Front. Mol. Neurosci., Vol. 10. 10.3389/fnmol.2017.00284.
41. Choi, J.H., J.H. Choi, D.Y. Kim, J.H. Yoon and H.Y. Youn *et al*., 2002. Effects of SKI 306X, a new herbal agent on proteoglycan degradation in cartilage explant culture and collagenase-induced rabbit osteoarthritis model. Osteoarthr. Cartil., 10: 471-478.
42. Rajasankar, S., T. Manivasagam and S. Surendran, 2009. Ashwagandha leaf extract: A potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease. Neurosci. Lett., 454: 11-15.
43. Seibenhener, M.L. and M.C. Wooten, 2015. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. J. Vis. Exp., Vol. 2015. 10.3791/52434.
44. Binfare, R.W., A.O. Rosa, K.R. Lobato, A.R.S. Santos and A.L.S. Rodrigues, 2009. Ascorbic acid administration produces an antidepressant-like effect: Evidence for the involvement of monoaminergic neurotransmission. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, 33: 530-540.
45. Hong, J.I., I.Y. Park and H.A. Kim, 2020. Understanding the molecular mechanisms underlying the pathogenesis of arthritis pain using animal models. Int. J. Mol. Sci., Vol. 21. doi.org/10.3390/ijms21020533.
46. Zhang, L.M., J.Z. Yao, Y. Li, K. Li, H.X. Chen, Y.Z. Zhang and Y.F. Li, 2012. Anxiolytic effects of flavonoids in animal models of posttraumatic stress disorder. Evidence-Based Complementary Altern. Med., Vol. 2012. 10.1155/2012/623753.
47. Herrera-Ruiz, M., R. Roman-Ramos, A. Zamilpa, J. Tortoriello and J.E. Jimenez-Ferrer, 2008. Flavonoids from *Tilia americana* with anxiolytic activity in plus-maze test. J. Ethnopharmacol., 118: 312-317.
48. Butterweck, V., G. Jurgenliemk, A. Nahrstedt and H. Winterhoff, 2000. Flavonoids from *Hypericum perforatum* show antidepressant activity in the forced swimming test. Planta Med., 66: 3-6.

49. Yamada, E.F., A.F. Salgueiro, A. da Silva Goulart, V.P. Mendes, B.L. Anjos, V. Folmer and M.D. da Silva, 2019. Evaluation of monosodium iodoacetate dosage to induce knee osteoarthritis: Relation with oxidative stress and pain. Int. J. Rheum. Dis., 22: 399-410.
50. Kurutas, E.B., 2015. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutr. J., Vol. 15. 10.1186/s12937-016-0186-5.
51. Jürs, A.V., C. Völkner, M. Liedtke, K. Huth, J. Lukas, A. Hermann and M.J. Frech, 2020. Oxidative stress and alterations in the antioxidative defense system in neuronal cells derived from NPC1 patient-specific induced pluripotent stem cells. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21207667.
52. Bhangale, J.O. and S.R. Acharya, 2016. Anti-parkinson activity of petroleum ether extract *Officus religiosa* (L.) leaves. Adv. Pharmacol. Pharm. Sci., Vol. 2016. 10.1155/2016/9436106.