Therapeutic effect of umbelliferon- α -D-glucopyranosyl-(2^I→1^{II})- α -D-glucopyranoside on adjuvant-induced arthritic rats

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Abstract The aim and objective of the present investigation was to evaluate the antiarthritic and antioxidant effect of umbelliferon- α -D-glucopyranosyl-(2I→1II)- α -D-glucopyranoside (UFD) in chemically induced arthritic rats. The different doses of the UFD were tested against the turpentine oil (TO), formaldehyde induced acute arthritis and complete freund's adjuvant (CFA) induced chronic arthritis in Wistar rats. Arthritic assessment and body weight was measured at regular interval till 28 days. On day 28, all the groups animals were anaesthetized, blood were collected from the puncturing the ratro orbital and estimated the hematological parameters. The animals were sacrificed; synovial tissue was extracted and estimated the malonaldehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD). The different doses of the UFD showed the protective effect against turpentine oil, formaldehyde induced acute arthritis and CFA induced chronic arthritis at dose dependent manner. Acute model of arthritis such as TO and formaldehyde induced inflammation due to releasing of the inflammatory mediators; significantly inhibited by the UFD at dose dependent manner. CFA induced arthritic rats treated with the different doses of the UFD showed the inhibitory effect on the delayed increase in joint diameter as seen in

arthritic control group rats. UFD significantly improved the arthritic index, body weight and confirmed the antiarthritic effect. UFD showed the effect on the hematological parameter such as improved the level of the RBC, Hb and decline the level of the EBC, ESR and confirmed the immune suppressive effect. UFD significantly improved the level of the endogenous antioxidant and confirmed the antioxidant effect. This present investigation suggests that the UFD has prominent antiarthritic impact which can be endorsed to its antiarthritic and antioxidant effects.

Keywords UFD · Antiarthritis · Turpentine · Formaldehyde · CFA · Antioxidant

Introduction

Rheumatoid arthritis is a chronic, systematic, autoimmune, progressive, inflammatory disorder which affecting the synovial joints and produced the inflammation in the synovial membrane; that leads to joint destruction, which is responsible for the abnormality, disability and restricted joint movements (Mishra et al. 2011; Gao et al. 2008). The most common effect of the RA is pain, inflammation and tissue damage caused restriction of movement of the limbs (Gomes et al. 2010). Free radical induced oxidative stress is a primary factor in generation of the various degenerative diseases such as arthritis, diabetes, atherosclerosis, inflammation and other condition of the disease. Free radicals and reactive oxygen species are involved in the pathogenesis of RA. During RA, polymorph nuclear leukocytes and macrophages are moved to the inflamed area, which results in an increase, the production of the superoxide and hydrogen peroxide in the inflamed area that can cause significantly impairment and destruction of the cartilage, synovial fluids and other articular constituents (Abotsi et al. 2010). Free radicals such as superoxide ion are thought to cause the cellular disruption due to

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lipid peroxidation in the inflamed area (Lunec et al. 1981; Biemond et al. 1984; Hadjigogos 2003). It is well known that the formation of lipid peroxidation have deleterious effects on bio membranes (Lunec et al. 1981). If free radicals are not scavenged by the endogenous antioxidant mechanisms, they lead to the tissue damage and loss of the function of the several tissue and organs. In RA, failure of the antioxidant defense system has been reported.

Presently, NSAIDS (Non-Steroidal Anti-Inflammatory Drugs) such as Ibuprofen, Phenylbutazone, Aceclofenac etc., DMARDs (Disease Modifying Anti-Rheumatic Drugs) such as Methotrexate, Cyclosporin A etc., Anticytokine therapy such as Adalimumab, Infliximab etc. and immune suppressive drugs are used to control the pain, inflammation during arthritis. But mostly used synthetic drug having the limitation such as increase the cardiovascular risk, gastro intestinal irritation, hematologic toxicity, nephrotoxicity and loss of response with chronic use (Ramani and Pradhan 2012). It is therefore desirable that the potential antiarthritic agents to be investigated for their possible contribution to the antioxidant defense system.

Many of the antioxidant protect molecules exposed the free radical and reactive oxygen species, which have been reduced the oxidative stress and protect the food from the deterioration and spoilage (Koleva et al. 2000). Lots of the synthetic antioxidant available in the market such as BHT (butylated hydroxyl toluene), BHA (butylated hydroxyl anisole) and PG (propyl gallate). But these synthetic antioxidants have side effects such as BHT produces internal and external hemorrhage, BHA is carcinogenic and PG produces the cytotoxic effect (Nakagawa and Tayama 1995; Nawar 1996; Nair et al. 2012). Due to these side effects of synthetic antioxidants we have attempted to find the effective antioxidant from the natural resources which can prevent the free radical formation during the arthritic condition. Antioxidants obtained from natural sources protect human body from the free radical generation and many chronic diseases and their symptoms.

In this study antiarthritic and antioxidant properties of Umbelliferon- α -D-glucopyranosyl-(2^I→1^{II})- α -D-glucopyranoside was evaluated in the CFA induced arthritic rats. This study provides the scientific justification for the use of compound UFD to treat the arthritic condition as well as an alternative drug for control of arthritis.

Umbelliferon- α -D-glucopyranosyl-(2^I→1^{II})- α -D-glucopyranoside

Umbelliferon- α -D-glucopyranosyl-(2^I→1^{II})- α -D-glucopyranoside (1), is a benzopyrone in nature, member of the coumarin family, found in many kinds of medicinal plants, and found in a rich source of human diet. Umbelliferone has been shown effect on lipid peroxidation and chemically induced colon carcinogenesis (Muthu et al.

2013), antidiabetes (Ramesh and Pugalandi 2006; Kumar et al. 2013a), antigenotoxic (Kaur et al. 2012; Rezaee et al. 2014), antifungal (Bai et al. 2012; Islam et al. 2012), modify the structure and pharmacological activities of Bn IV (Toyama Dde et al. 2011). All these effect of UFD showed the antifungal, antibacterial, anti-inflammatory, antidiabetic, antihyperlipidemic, antioxidant, antigenotoxic activities. A great number of the pharmacological uses have been endorsed for UFD, with much interest has been given particularly to its anti-diabetic, anti-hyperlipidemic, anti-oxidant activity (Kumar et al. 2013a). As discussed earlier, the free radical plays an important role in the development of the arthritis and related disorder we investigate the powerful antioxidant to cure the arthritis and related disorder.

Materials and methods

Chemical

Complete Freund's adjuvant (CFA), Indomethacin and other chemicals like Turpentine Oil (TO), Formaldehyde, Carboxyl methyl cellulose and reagent used analytical grade for the study and procured from approved organization.

Plant material and isolation of compound

Umbelliferon- α -D-glucopyranosyl-(2^I→1^{II})- α -D-glucopyranoside (UFD) (Fig. 1) was isolated from the stem bark of *Aegle marmelos* Corr. as previously reported Kumar et al. (Kumar et al. 2013b).

Drugs

Standard drug and test drug was prepared in 2 % CMC (Carboxyl methyl cellulose) solution.

Acute toxicity study

The oral acute toxicity and approximate lethal dose of the isolated compound were determined in the Wistar rats using the reported method Kumar et al. (Kumar et al. 2013b). Before the experimentation all rats were kept starved (16 h) and randomly divided into six groups and each group contains 6 rats. Different doses of UFD were administered to the test groups in graded doses ranging up to 100 mg/kg. The animals were continuously observed for 2 h for the following (Kumar et al. 2014; Ghosh 1984)

1. Behavioral profile: restlessness, irritability, alertness and fearfulness.
2. Neurological profile: spontaneous activity, touch response, reactivity, pain response and gait.

Fig. 1 Structure of umbelliferon- α -D-glucopyranosyl-(2¹→1¹¹)- α -D-glucopyranoside

3. Autonomic profile: urination and defecation.

If any contraindication and death occur after 24 h and 72 h was recorded.

Turpentine oil induced joint edema in rats

The animal was divided into seven groups of six animals each as follows:

- Group I (Non Edema): Served as vehicle control, 2 % CMC, p.o.
- Group II (Non Edema): Treated with UFD 40 mg/kg, p.o.
- Group III (Edema Control): 2 % CMC, p.o.
- Group IV (Edema UFD-I): 10 mg/kg, p.o.
- Group V (Edema UFD-II): 20 mg/kg, p.o.
- Group VI (Edema UFD-III): 40 mg/kg, p.o.
- Group VII (Edema Indomethacin): 10 mg/kg, p.o.

Wister rats were fasted for 24 h before experimentation with access to water. After 30 min administration of tested and standard drugs, acute non-immunological inflammatory joint edema was produced by injecting the 0.02 ml of TO injection into the synovial cavity of the left knee joint of rats (Hanson et al. 1974). The diameter of the joint was monitored by micrometer screw gauge till 6 h at 1 h intervals (Winter et al. 1962).

Formaldehyde induced arthritis in rats

The animal was divided into 7 groups of 6 animals each as follows:

- Group I (Non Edema): Served as vehicle control, 2 % CMC, p.o.
- Group II (Non Edema): Treated with UFD 40 mg/kg, p.o.

- Group III (Edema Control): 2 % CMC, p.o.
- Group IV (Edema UFD-I): 10 mg/kg, p.o.
- Group V (Edema UFD-II): 20 mg/kg, p.o.
- Group VI (Edema UFD-III): 40 mg/kg, p.o.
- Group VII (Edema Indomethacin): 10 mg/kg, p.o.

Formaldehyde induced acute arthritis method was used with minor modification of the reported method (Singh and Majumdar 1996). On day 1, after 30 min drug administration, chronic non immunological arthritis was induced by sub plantar injection of 0.1 ml of 2 % formaldehyde solution and repeated on day 3. The arthritis was measured by increasing the paw volume over a period of 10 days using a micrometer screw gauge (Winter et al. 1962).

Chronic immunological arthritis

Complete Freund's adjuvant induced arthritis in rats

The animal was divided into 7 groups of 6 animals each as follows:

- Group I (Non Edema): Served as vehicle control, 2 % CMC, p.o.
- Group II (Non Edema): Treated with UFD 40 mg/kg, p.o.
- Group III (Edema Control): 2 % CMC, p.o.
- Group IV (Edema UFD-I): 10 mg/kg, p.o.
- Group V (Edema UFD-II): 20 mg/kg, p.o.
- Group VI (Edema UFD-III): 40 mg/kg, p.o.
- Group VII (Edema Indomethacin): 10 mg/kg, p.o.

Arthritis was induced in all groups rats except normal control and normal control treated with UFD (40 mg/kg) by

injecting the 0.05 ml of CFA injection in the sub plantar region of the left paw on day 1. CFA induced arthritic rats treated with the different doses of the tested drug and indomethacin for 28 days (Purnima et al. 2006). The edema of the left hind paws was evaluated at regular interval using the micrometer screw gauge (Winter et al. 1962).

Arthritis assessment in CFA rats

CFA induced arthritic rats showed the clinical symptoms which was evaluated by visual scoring system on scale 0–4, 0: no change, 1: swelling and erythema of the limb, 2: mild swelling and erythema of the limb, 3: gross swelling and erythema of the limb, 4: gross deformity and inability of the limb. A score of the 4 limb was counted and score more than 1 showed the arthritis and the maximum score of the arthritis is 16. The frequency and day of onset of arthritis was also recorded (Kim et al. 2008).

Biochemical analysis

For estimation of different biochemical parameters, blood was withdrawn from the puncturing the retro-orbital of the all group rats under mild anesthesia condition. Blood was collected into the anticoagulation tubes using coagulant, centrifuged the blood sample and the supernatant serum was collected. Different enzyme reagent was added to determine the WBC (White blood cells), RBC (Red blood cells) (Chesbrough and Mc Arthur 1972), ESR (Erythrocyte sedimentation rate) (David and Sykes 1951) and Hb (hemoglobin) (Austin and Drabkin 1935) analyzed.

Antioxidant marker

All group rats were scarified on the day 28, using an excess of diethyl ether. An aliquot of the articular tissue homogenate was used for the determination of antioxidant markers such as Superoxide dismutase (SOD), Glutathione peroxidase (GPx) (Nam et al. 2006), Malondialdehyde (MDA) and Glutathione by the method of (Campo et al. 2003).

Statistical analysis

All the data were expressed as the mean $\pm$ SEM and analysis of variance (ANOVA) was used for the statistical analysis using Graph Pad Prism version 5.0. The values were considered to be significant when the P value was $p < 0.05$.

Result

Effect of UFD on acute toxicity

The tested compound did not showed any toxicity effect and mortality in all groups when given orally at dose 100 mg/kg. There was no lethality or toxicity found at any selected doses until the end of the study. Hence, 10, 20 and 40 mg/kg were taken as therapeutic dose.

Effect of UFD on turpentine oil induced inflammation

The TOinduced rat paw edema method was used for the evaluation of the acute anti-inflammatory study of the test compound. Table 1 shows the inhibitory effect of UFD on TOinduced acute paw edema in the Wistar rats. The UFD at dose 10, 20 and 40 mg/kg and indomethacin 10 mg/kg were administered orally 30 min prior to TOinjection. Different doses of the UFD showed appreciable inhibition of the edema size in comparison with indomethacin at dose dependent manner. The UFD dose 40 mg/kg showed excellent protection against inflammation (82.99 % inhibition, respectively). Another dose of the UFD (10 mg/kg and 20 mg/kg) showed the inhibition 62.19 % and 71.07 %, respectively, in comparison of indomethacin, which has a percent inhibition of 78.26 %.

Effect of UFD on formaldehyde induced arthritis

The acute antiarthritic effect of the UFD was evaluated on the formaldehyde induced acute arthritis model in the Wistar rats. The assessment made on the 10th day showed that, different doses of the UFD significantly inhibited ($P < 0.001$) the swelling in the injected hind paw as compared to the formaldehyde induced acute arthritic group rats. On the 10th day the percentage inhibition of paw edema exhibited by different doses of the UFD were 51.54 %, 65.31 % and 73.26 % respectively; while indomethacin treated animals showed 69.57 % inhibition of paw edema (Table 2).

Effect of UFD on CFA developing arthritis

UFD significantly decline the % increase of paw volume in the injected paw (primary lesions) and non injected paw (secondary lesions) of the rats as compared to the CFA control group rats. CFA induced arthritic rat treated with the UFD and indomethacin showed the significantly inhibitory inflammatory effect on the paw edema. The antiarthritic inflammatory effect of the UFD (10 mg/kg; 66.61), (20 mg/kg; 74.10) and (40 mg/kg; 91.02) and indomethacin (10 mg/kg; 82.78) presented in the Table 3.

Table 1 Effect of UFD on turpentine oil induced rat paw edema

S. No	Treatment	Increase in joint diameter						% inhibition
		1 h	2 h	3 h	4 h	5 h	6 h	
1	Normal Control	0.474±0.009	0.474±0.009	0.474±0.009	0.474±0.009	0.474±0.009	0.474±0.009	–
2	Normal Control + UFD (40 mg/kg)	0.464±0.009	0.464±0.009	0.464±0.009	0.464±0.009	0.462±0.007	0.462±0.007	–
3	Arthritic Control	0.558±0.005	0.658±0.011	0.752±0.013	0.84±0.015	0.944±0.018	1.058±0.019	–
4	UFD I (10 mg/kg)	0.508±0.011	0.55±0.008	0.594±0.009	0.452±0.012	0.484±0.011	0.4±0.007	62.19
5	UFD II (20 mg/kg)	0.492±0.008	0.524±0.005	0.562±0.006	0.508±0.007	0.426±0.005	0.306±0.002	71.07
6	UFD III (40 mg/kg)	0.498±0.011	0.524±0.009	0.552±0.008	0.458±0.13	0.336±0.014	0.18±0.007	82.99
7	Indomethacin (10 mg/kg)	0.494±0.008	0.52±0.006	0.556±0.012	0.462±0.007	0.362±0.005	0.234±0.007	78.26

The data are expressed in mean ± SEM) (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test .ns-non-significant; UFD- Umbelliferone diglucoside

* $P < 0.05$ is considered as significant

** $P < 0.01$ is considered as very significant

*** $P < 0.001$ is considered as extremely significant

Effect of UFD on the body weight

CFA induced arthritic control group rats continuously decreasing the body weight as compared to the initial body weight till day 28. Normal control group rat and CFA induced arthritic rats group treated with different doses of the UFD and indomethacin continuously increasing the body weight as compared with the initial body weight has been given in the Table 4.

Effect of UFD on clinical signs of arthritis

No sign and symptom of arthritic index was observed in the normal control group rat and normal control group rat treated with UFD dose 40 mg/kg. CFA induced arthritic control group

rats was showing the evidence of clinical inflammation in one or more hind paw from the day 10. CFA induced arthritic control group rats rapidly increased from the day 10 which indicated that the polyarthritic symptoms had certainly developed. CFA induced arthritic rat treated with different doses of the UFD and indomethacin showed little change of the arthritic index till day 15. However, after this CFA induced arthritic development phase, the arthritic indexes started to decrease and finally reached less than 60 % in all group as compared to the CFA induced arthritic control group rats (Table 5).

Effect of UFD on hematological alterations in CFA rats

CFA induced arthritic rats showed the hematological milieu, such as decrease level of the RBC, Hb and increase level of

Table 2 Effect of UFD on formaldehyde induced rat paw edema

S. No	Treatment	Increase in joint diameter					% inhibition
		Day 2	Day 4	Day 6	Day 8	Day 10	
1	Normal Control	0.492±0.013	0.492±0.013	0.492±0.013	0.492±0.013	0.492±0.013	–
2	Normal Control + UFD (40 mg/kg)	0.498±0.015	0.498±0.015	0.496±0.012	0.496±0.012	0.496±0.012	–
3	Arthritic Control	0.6±0.017	0.698±0.017	0.784±0.019	0.898±0.017	1.03±0.024	–
4	UFD I (10 mg/kg)	0.544±0.019 ^{ns}	0.614±0.017 ^{ns}	0.59±0.018*	0.536±0.019**	0.496±0.017***	51.94
5	UFD II (20 mg/kg)	0.55±0.011 ^{ns}	0.612±0.012*	0.574±0.006**	0.464±0.005***	0.358±0.015***	65.31
6	UFD III (40 mg/kg)	0.534±0.011 ^{ns}	0.574±0.012**	0.526±0.006***	0.39±0.005***	0.27±0.015***	73.26
7	Indomethacin (10 mg/kg)	0.518±0.019 ^{ns}	0.568±0.018**	0.522±0.015***	0.426±0.015***	0.314±0.016***	69.57

The data are expressed in mean ± SEM) (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test NS non-significant, UFD umbelliferone diglucoside

* $P < 0.05$ is considered as significant

** $P < 0.01$ is considered as very significant

*** $P < 0.001$ is considered as extremely significant

Table 3 Effect of UFD on CFA induced rat paw edema

S.No	Treatment	Increase in joint diameter					% Inhibition
		Day 10	Day 15	Day 20	Day 25	Day 28	
1	Normal Control	0.51±0.122	0.51±0.122	0.51±0.122	0.512±0.011	0.512±0.011	–
2	Normal Control+UFD (40 mg/kg)	0.528±0.009	0.528±0.009	0.524±0.008	0.524±0.009	0.524±0.009	–
3	Arthritic Control	0.908±0.011	1.09±0.013	1.21±0.011	1.27±0.014	1.34±0.017	–
4	UFD I (10 mg/kg)	0.814±0.015 *	0.87±0.015***	0.688±0.012***	0.57±0.013***	0.446±0.017***	66.61
5	UFD II (20 mg/kg)	0.77±0.008*	0.8±0.007**	0.594±0.017***	0.47±0.014***	0.346±0.014***	74.10
6	UFD III (40 mg/kg)	0.704±0.005**	0.69±0.004***	0.404±0.012***	0.276±0.016***	0.12±0.014***	91.02
7	Indomethacin (10 mg/kg)	0.74±0.013**	0.75±0.013***	0.506±0.011***	0.384±0.014***	0.234±0.012***	82.78

The data are expressed in mean ± SEM) (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. NS non-significant, UFD umbelliferone diglucoside, CFA complete Freund's arthritic

* $P < 0.05$ is considered as significant

** $P < 0.01$ is considered as very significant

*** $P < 0.001$ is considered as extremely significant

WBC, ESR were significantly altered by the UFD treatment. CFA induced hematological perturbations rats favorably altered by UFD but the level of RBC, WBC, Hb and ESR were recorded unchanged in the normal control group rats and normal control group rats treated with the UFD 40 mg/kg. The effect of the UFD were dose dependent (Table 6).

Effect of UFD on antioxidant enzyme in CFA induced arthritic rats

Antioxidant enzyme alterations in CFA induced arthritic rats were normalized after treatment with different doses of the UFD. The CFA-induced antioxidant perturbations, such as an increase in the MDA and decreased level of SOD, GPx, GSH

were also significantly ($P < 0.001$) altered by different doses of the UFD treatment (Table 7).

Discussion

Acute toxicity study of the bioactive compound UFD revealed the non-toxic nature in the lower dose. There was no lethality or any toxic reactions found with the selected doses of the UFD until end of the study (Kumar et al. 2013b).

TO induced paw edema method is characterized by increase vascular permeability and vasodilatation. TO induce paw edema is triphasic in nature; initial phase releasing the histamine and serotonin, intermediate phase kinin and later

Table 4 Effect of UFD on body weight of CFA induced rat paw edema

S.No	Treatment	Increase in joint diameter						Weight Variance
		Day 0	Day 10	Day 15	Day 20	Day 25	Day 28	
1	Normal Control	162.2±1.985	176.4±2.041	185±2.168	198.6±2.966	206.6±2.437	213.8±2.223	51.6
2	Normal Control + UFD (40 mg/kg)	164.6±2.249	175.4±2.168	186±1.594	200±1.806	207.8±1.503	217.6±1.939	53
3	Arthritic Control	168±1.483	157.6±1.435	148.6±1.077	133.2±1.068	125.8±2.059	119±1.871	–49
4	UFD I (10 mg/kg)	163.6±3.932	166±3.808**	167.4±4.057***	176.8±3.056	184.2±2.354	193±1.924	29.4
5	UFD II (20 mg/kg)	167.4±1.661	171±1.14**	172.8±0.735***	186±0.707***	194.8±0.812***	203±0.948***	35.6
6	UFD III (40 mg/kg)	158.8±1.393	163.4±1.288***	166.2±1.356***	182.8±1.497***	193.2±1.281***	204.6±1.143***	45.8
7	Indomethacin (10 mg/kg)	166±2.001	170±2.102***	172.4±1.861***	187.8±1.685***	197.6±1.142***	206.8±1.158***	40.8

The data are expressed in mean ± SEM) (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. NS non-significant, UFD umbelliferone diglucoside, CFA complete Freund's arthritic

* $P < 0.05$ is considered as significant

** $P < 0.01$ is considered as very significant

*** $P < 0.001$ is considered as extremely significant

Table 5 Effect of UFD on the development of CFA arthritic index

S.No	Treatment	Arthritic Index				
		Day 10	Day 15	Day 20	Day 25	Day 28
1	Arthritic Control	7.6±0.401	8.6±0.245	10±0.316	10.6±0.124	11.2±0.254
2	UFD I (10 mg/kg)	6.8±0.209 ^{ns}	7.2±0.211 [*]	6±0.283 ^{***}	5.2±0.374 ^{***}	4.2±0.241 ^{***}
3	UFD II (20 mg/kg)	6.6±0.316 [*]	7±0.312 ^{**}	5.2±0.213 ^{***}	4.2±0.312 ^{***}	3.2±0.187 ^{***}
4	UFD III (40 mg/kg)	6±0.325 ^{**}	6±0.205 ^{***}	3.6±0.376 ^{***}	2.6±0.236 ^{***}	1.2±0.202 ^{***}
5	Indomethacin (10 mg/kg)	6.4±0.509 ^{**}	6.6±0.401 ^{***}	4.6±0.678 ^{***}	3.4±0.512 ^{***}	2.2±0.583 ^{***}

The data are expressed in mean ± SEM (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. NS non-significant, UFD umbelliferone diglucoside, CFA complete freund's arthritic

* $P < 0.05$ is considered as significant

** $P < 0.01$ is considered as very significant

*** $P < 0.001$ is considered as extremely significant

phase releasing the cyclooxygenase and lipoxygenase inflammatory mediators (Di Rosa et al. 1971; Hanson et al. 1974; Singh et al. 2011). In the present investigation, different doses of UFD showed the inhibition in the TO induced paw edema throughout the complete observation period. The result suggests that different doses of UFD influenced all phases of the TO induced inflammatory mediators in the rat paw. However, maximum inhibitory effects of the UFD was seen during the late phase of inflammation, thus result suggesting that the UFD is a considerable cyclooxygenase/lipoxygenase inhibitory effect. The possible mechanism of action of the UFD on the TO induced paw edema was inhibition of the all inflammatory mediator during the all phase of the inflammation.

Formaldehyde is an organic compound, which is widely used for the induction of the acute inflammation. Formaldehyde induced the proliferative and acute inflammation

in the rats (Nair et al. 2011). Formaldehyde induced inflammation is biphasic in nature; firstly affect the neurogenic component and laterly tissue mediators (Owoyele et al. 2011). The different doses of the UFD were able to significantly reduced the proliferatively global edematous in the formaldehyde induced acute arthritic rats. The data showed that the reduction in the joint swelling in the UFD dose 40 mg/kg was higher than the other tested doses of UFD and indomethacin treated group rats. The possible mechanism of action of the UFD to neutralizing the proliferative global edematous.

Most of the researcher claims that inhibition of adjuvant-induced arthritis in the rats is the one of the most suitable method to evaluate the antiarthritic activity. CFA has been shown the number of clinical, chronic and immunological features similar to the human arthritis (Salahuddin and Jalalpure 2010; Jalalpure et al. 2011). Therefore, this model

Table 6 Effect of UFD on hematological parameters in adjuvant-induced arthritis in rats

S. No	Treatment	Antioxidant marker			
		Total WBC count (cells/cu.mm)	RBC count (million/cu.mm)	Hb (cells/cu.mm)	ESR (million/cu.mm) (gm%)
1	Normal Control	6.6±0.245	6.2±0.212	13.2±0.374	9±0.316
2	Normal Control + UFD (40 mg/kg)	6.6±0.245	6±0.316	13±0.316	9±0.214
3	Arthritic Control	8.6±0.234	3.6±0.245	8.4±0.244	13±0.114
4	UFD I (10 mg/kg)	8.4±0.316 [*]	3.8±0.234 [*]	9.8±0.371 [*]	12.6±0.245 [*]
5	UFD II (20 mg/kg)	8±0.234 [*]	4.4±0.244 ^{**}	11.2±0.201 ^{**}	11.6±0.231 ^{**}
6	UFD III (40 mg/kg)	7.2±0.212 ^{***}	6.2±0.212 ^{***}	12.8±0.134 ^{***}	10.4±0.509 ^{***}
7	Indomethacin (10 mg/kg)	7.4±0.249 ^{***}	5.6±0.265 ^{***}	12.4±0.243 ^{***}	10.8±0.374 ^{***}

The data are expressed in mean ± SEM (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. NS non-significant, UFD umbelliferone diglucoside, CFA complete freund's arthritic

* $P < 0.05$ is considered as significant

** $P < 0.01$ is considered as very significant

*** $P < 0.001$ is considered as extremely significant

Table 7 Effect of UFD on the antioxidant marker

S. No	Treatment	Antioxidant marker			
		Malonaldehyde (nmol/mg of protein)	Glutathione (μ mol/g of protein)	Glutathione peroxidase (nmol/mg of protein)	Super oxide dismutase (U/mg of protein)
1	Normal Control	5.2 $\pm$ 0.374	7 $\pm$ 0.316	45.4 $\pm$ 1.077	8.4 $\pm$ 0.509
2	Normal Control + UFD (40 mg/kg)	5.2 $\pm$ 0.374	6.6 $\pm$ 0.245	43.6 $\pm$ 0.927	8 $\pm$ 0.265
3	Arthritic Control	12.8 $\pm$ 0.321	1.6 $\pm$ 0.253	15.8 $\pm$ 0.735	1.6 $\pm$ 0.316
4	UFD I (10 mg/kg)	10.2 $\pm$ 0.243*	2.6 $\pm$ 0.113 ^{ns}	25.8 $\pm$ 0.663*	3.2 $\pm$ 0.215*
5	UFD II (20 mg/kg)	8.8 $\pm$ 0.212**	3.8 $\pm$ 0.373*	31.4 $\pm$ 0.927**	4.8 $\pm$ 0.373**
6	UFD III (40 mg/kg)	7 $\pm$ 0.316***	5.8 $\pm$ 0.342***	41.2 $\pm$ 0.862***	7.4 $\pm$ 0.245***
7	Indomethacin (10 mg/kg)	7.2 $\pm$ 0.234***	5.6 $\pm$ 0.245***	39.4 $\pm$ 0.509***	7 $\pm$ 0.316***

The data are expressed in mean $\pm$ SEM (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. NS non-significant, UFD umbelliferone diglucoside, CFA complete freund's arthritic

* $P < 0.05$ is considered as significant

** $P < 0.01$ is considered as very significant

*** $P < 0.001$ is considered as extremely significant

is used with a relatively high degree of validity for evaluating agents with potential antiarthritic activity. Swelling in the hind paw is the primary reaction; characterized feature of the CFA induced arthritis which persisted for weeks and primary reaction followed by the development of the arthritic nodules in ear and tails and swelling in the contra lateral, this is called delayed response (secondary reaction) (Kaithwas et al. 2012). CFA induced arthritic control group rats showed an increase the paw joint diameter till the day 28, which can be attributed to the delayed immunological flare in the disease (Pearson and Wood 1959). All group rats showed significantly increase in the joint diameter after the day 10 except normal control and normal control group rat treated with UFD 40 mg/kg. The result suggests that different doses of the UFD significantly decrease the humoral immune response (primary reaction). The possible mechanism for inhibiting the acute inflammation is by inhibiting the vascular permeability and other inflammatory mediators. The secondary and delayed reaction was occurred due to the delayed hypersensitivity reaction (Kaithwas and Majumdar 2010). The different doses of the UFD significantly decrease the joint diameter at end of the study. The possible mechanism of the UFD on the second lesion or delayed reaction is due to inhibiting the hypersensitivity reaction. The observation suggests that UFD showed the effective therapy of clinical condition in the treatment of the rheumatoid arthritis.

CFA induced arthritic control group showed the decline in body weight. The decline in body weight of the CFA induced arthritic rats is due to the improper absorption of nutrients through the intestine (Patil and Suryavanshi 2007). CFA induced arthritic rats treated with the different doses of the UFD significantly improving the body weight as compared to the CFA induced arthritic control group rats. The possible

mechanism of action of the UFD on the improvement of the body weight may be due to decreasing the absorption of the nutrient through the intestine and normalize the process of absorption.

Anemia is the most commonly appear with the chronic arthritis patients. The major reason for the anemia is firstly gastrointestinal blood loss due to the arthritic medication and another bone marrow changes with inflammatory arthritis, which inhibit the incorporation of the iron with the RBC and showed the marked decreased level of the RBC count (Paval et al. 2009). Another manifestation in the arthritic condition; marked increased in the level of the WBC was observed due to increase in the level of the IL-1B (Interleukin-1 beta) colony stimulating factor. Due to increase in the level of the IL-1B, the production of the inflammatory GM-CSF (granulocyte and macrophages colony stimulating factors) got increased (Eric and Lawrence 1996; William 1996; Kumar et al. 2013c). Thus, the CFA induced arthritic rats treated with the UFD significantly increased the level of the RBC and decline the level of the WBC further support its anti-arthritic effects. CFA induced arthritic condition increases the level of the ESR and decreased the level of the Hb. CFA induced arthritic rats decreased the level of Hb count due to reduced erythropoietin levels, decreasing the level of the Hb count in arthritic conditions. CFA induced arthritis decreased Hb and response of the bone marrow erythropoietin and premature destruction of red blood cells. Similarly, increase level of ESR is an estimation of the suspension stability of RBC in plasma. ESR is attributed to the accelerated formation of endogenous protein such as especially fibrinogen, alpha and beta globulins. Increase level of the ESR indicates the elevation in the concentration to stress or inflammations like injection, injury, tissue necrosis and surgery (Patil et al. 2011; Parthiban et al. 2013). Thus, the decline

level of the ESR and increase in the Hb count brought about by UFD treatment further support its anti-arthritis effect.

Free radical, reactive oxygen species (ROS), oxidative stress and reactive oxygen play an important role in the tissue damage. The oxidative stress start the degradation of the synovial fluid and induced depolymerization of hyaluronic acid which is turn lead to a loss of viscosity in the joints (Arnett et al. 1988). The oxidative stress or reactive oxygen species produced the cartilage destruction in CFA induced arthritic rats which is turn to decrease when treated with the different doses of the UFD through the increase the activity of the endogenous antioxidant such as SOD, GPx, GSH and MDA. Cancerously, the possible mechanism of action of the UFD neutralization of various free radicals generated at the site of the inflammatory by increasing the activity of the antioxidant enzymes.

Conclusion

In Conclusion UFD shows to beneficial effects on multiple pathological manifestations of turpentine oil, formaldehyde and CFA induced acute and chronic arthritis in rats. Therefore, further study is needed to prove clinical investigation of the isolated compound in addition; the present investigation validates the use of the UFD in the treatment of the pain and arthritic inflammation condition.

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