Natural Product Sciences
7(3) : 76~82 (2001)

Pharmacological Studies of Various Extracts and the Major Constituent, Lupeol, obtained from Hexane Extract of *Teclea nobilis* in Rodents

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Abstract – The pharmacological activities of the acetonitrile (MeCN), hexane extracts and isolated pure terpenoidal compound Lupeol from the leaves of *Teclea nobilis*, Delile (TN), on inflammation induced by carrageenan and implantation of cotton pellets in rats; the nociceptive response using writhing and tail flick tests and the antipyretic activity in yeast-induced fever were examined in mice. Oral administration of TN extracts at doses of 150 and 300 mg/kg and lupeol 5 and 10 mg/kg showed a significant anti-inflammatory activity in rats. The extracts of TN and lupeol significantly decreased the number of contractions and stretchings induced by acetic acid and heat-induced pain in mice. The antipyretic effect of extracts and lupeol was also found to be significant. The behavioral observation of animals showed that the hexane extract and lupeol caused CNS depressant activity and did not produce any toxic or lethal effects in animals at various dose levels. The results suggest that the *Teclea nobilis* extracts and lupeol possesses anti-inflammatory, analgesic and antipyretic activities.
Key words – *Teclea nobilis*, lupeol, inflammation, pain, fever, traditional medicine.

Introduction

Teclea nobilis Delile (Rutaceae) (TN), commonly known as Al-dhureim, is a shrub used in traditional medicine of many African countries and in Saudi Arabia. The various parts of the plant including leaves and stem bark are said to be a remedy for gonorrhea, and pain (Watt & Breyer-Brandwijk, 1962). In this regard, (Mascolo *et al.*, 1988) have reported antipyretic and analgesic activities of the ethanol extract of TN and suggested further pharmacological studies to determine which of the chemical constituents are responsible for the observed analgesic and antipyretic activities of this plant. There is a dearth of literature on TN phytoconstituents and their pharmacological activities. In a previous study the presence of quinoline alkaloids in TN were reported (Yenesew & Dagne, 1988).

Therefore, the present investigation was undertaken to evaluate anti-inflammatory, analgesic and antipyretic activities of acetonitrile (MeCN) and hexane extracts of the leaves of TN and of the isolated triterpenoidal compound named lupeol. Furthermore, the behavioral and acute toxicity studies were also conducted to rationale its use in traditional medicine.

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Experimental

Plant material – Aerial parts of *Teclea nobilis* were collected from Tanumah in Southern Province of Saudi Arabia in March, 1999 and identified by a Taxonomist, Pharmacognosy Department, College of Pharmacy, King Saud University. A voucher specimen is deposited at the Herbarium of the College of Pharmacy.

Phytochemical Studies

Preparation of plant extract – The dried powdered leaves of *Teclea nobilis* were extracted with hexane in a Soxhlet extractor for 72 h. The obtained hexane extract was evaporated under reduced pressure to give 50.0 g. The hexane extract was then partitioned between hexane and acetonitrile pre-saturated with each other to yield pre-saturated hexane fraction 26.0 g and pre-saturated acetonitrile fraction 22.0 g. The pre-saturated hexane fraction (26.0 g) was roughly separated using SiO_2 column chromatography. The column was eluted with 10% ethyl acetate (EtOAc)-hexane (6,000 ml), 30% EtOAc-hexane (3,000 ml), 50% EtOAc-hexane (2,000 ml) and 100% EtOAc (500 ml) to give 46 fractions (each fraction was 250 ml) as follows:

10% EtOAc-hexane 24 fractions

30% EtOAc-hexane 12 fractions
50% EtOAc-hexane 8 fractions
100% EtOAc 2 fractions

Fractions 12-24 from the 10% EtOAc-hexane were combined together due to similarity on TLC behavior. These fractions were further purified by SiO_2 column chromatography using 6% EtOAc-hexane as eluting system to give lupeol as a colorless needles from acetone (3.0 g). The ^1H NMR, ^{13}C NMR and MS and the physical constant were in full agreement with those reported (Reynolds *et al.*, 1986; Sholichin *et al.*, 1980).

Anti-inflammatory activity

Carrageenan-induced paw edema in rats – Pedal inflammation in albino rats (8 to 10 weeks old, six animals in each group) of either sex weighing 180-200 g was induced according to the method described by (Winter *et al.*, 1962). An injection was made of 0.05 ml of 1% carrageenan sodium salt (BDH) into the right hind foot of each rat under the plantar aponeurosis. The test groups of rats were treated orally with various dose levels (150 and 300 mg/kg for MeCN and hexane extracts; 5 mg and 10 mg/kg body weight for lupeol) (extracts were dissolved in saline; lupeol was suspended in CMC) 1 h before the carrageenan injection. At the same time, the control group was given 5 ml/kg of normal saline and the reference for each group was given 100 mg/kg of an aqueous solution of oxyphenbutazone orally. The measurements of foot volume were done by the displacement technique using a plethysmometer (Apelex, France) immediately and +3 h after the injection of carrageenan. The inhibitory activity was calculated according to the following formula (Winter *et al.*, 1962):

$$\text{Percent inhibition} = 100\ [1 - (a - x)/(b - y)]$$

where 'b' is the mean paw volume of control rats at the specified time after carrageenan injection and 'y' (is the mean paw volume of control rats) immediately after the carrageenan injection; where 'x' is the mean paw volume of treated rats immediately after carrageenan before injection and 'a' is the mean paw volume of treated rats at the specified time after carrageenan injection.

Cotton pellet granuloma in rats – The method of (Goldstein *et al.*, 1972) was used with a few modifications. Sterilized cotton pellets weighing 30 mg were introduced s.c. in the groin region of groups of rats (six rats were used in each group). The test groups were treated orally with MeCN and hexane extracts or lupeol once daily for four consecutive days (doses 150 and 300 mg/kg for extracts; 5 and 10 mg/kg for lupeol). Animals in the control groups received normal saline. Oxyphenbutazone 100 mg/kg (used as standard drug) was given in another test group. On the fifth day, the animals were sacrificed with ether, the pellets were removed, freed from extraneous tissue and dried overnight at 60°C and weighed. The percentage decrease in the pellets weights in treated groups was calculated compared with the control group.

Antipyretic activity

Yeast-induced Hyperpyrexia in mice – Hyperpyrexia was induced in mice by s.c. injection of (20% aqueous suspension of brewer's yeast) of 20 ml/kg body weight (6 animals in each group) in the back below the nape of the neck (Loux *et al.*, 1972). The animals were then fasted for the duration of experiment (approximately 26 h); water was made available *ad lib.* Control temperatures were taken 24 h after the yeast injection to determine the pyretic response to yeast. Rectal temperatures taken 1 h prior to drug administration in fevered animals served as a pre-drug control. The extracts (150 and 300 mg/kg) and lupeol (5 and 10 mg/kg) was given orally 24 h after the yeast injection and the temperatures were recorded at 60, 90 and 120 min after their administration.

Analgesic activity

Acetic acid-induced writhing in mice – The test was carried out using the technique of (Siegmund *et al.*, 1957), as modified by (Koster *et al.*, 1959). The MeCN and hexane extracts (150 and 300 mg/kg body weight) and lupeol (5 and 10 mg/kg body weight) were administered orally, to 16 h fasted mice, and divided into groups of six animals each. One hour after treatment, the mice were injected intraperitoneally with 0.2 ml of 3% acetic acid solution to induce the characteristic writhings. The number of writhings occurring between 5 and 15 min after the acetic acid injection in control and treated animals was recorded. The responses of extract-treated groups were compared with those of animals receiving indomethacin (as standard drug), 4 mg/kg, as well as with the control group.

Tail flick test in mice – Acute nociception was induced using a tail flick apparatus (Tail Flick model DS 20 Sorrel Apelex, France) following the method of D'Amour and Smith (1941). Briefly, each mouse

was placed in a restrainer (six animals in each group), 2 min before treatment, and baseline reaction time was measured by focusing on intensity controlled beam of light on the distal one-third portion of the animals tail. The extracts and lupeol were orally administered immediately after this step and 15, 30, 60, 90 and 120 min later; the post drug reaction time was measured. A 10 seconds cut off time was used in order to prevent tissue damage.

Behavioral studies

Behavioral studies were carried out in mice, weighing 25-30 g, according to the scheme of (Irwin 1964). The MeCN and hexane extracts and lupeol were administered intraperitoneally, the animals were observed for excitation, tremors, twitches, motor activity, pinna, corneal reflexes, and respiratory changes for four hours.

Acute toxicity studies

The MeCN and hexane extracts and lupeol were administered by gavage to six (one group served as control) groups of 10 mice each 5 male and 5 female, after an overnight fast. The doses studies (for extracts) were 0.25, 0.5, 1, 2 and 3 g/kg body weight and for lupeol 0.5-40 mg/kg body weight. The animals were observed for seven consecutive days to register mortality or other toxic signs (Al-Yahya *et al.*, 1994).

Statistical analysis

All results are expressed as mean ± S.D. Statistical analysis was performed using one-way ANOVA and statistical significance was checked with Duncan's test.

Results

Effect on carrageenan-induced paw edema – Carrageenan-induced paw edema was tested on different groups of randomly divided rats. The rats were orally treated either with control vehicle (saline) or extracts (MeCN and hexane) and lupeol 150, 300 mg/kg; 5 and 10 mg/kg body weight, respectively or oxyphenbutazone (100 mg/kg). The extracts (MeCN and hexane) and lupeol in the doses used, significantly reduced the carrageenan-induced paw edema.

The effect of the extracts (MeCN and hexane), lupeol and oxyphenbutazone on granuloma pouch in rats is shown in Table 1. Both the extracts and lupeol significantly inhibited granuloma formation in rats except the lower dose of hexane extract used (150 mg/kg) (Table 1).

Hyperthermia induced by brewer's yeast – The extracts (150 and 300 mg/kg), lupeol (5 and 10 mg/kg)

Table 1. Effect of the various extracts and lupeol from *Teclea nobilis* on carrageenan induced paw edema on cotton pellets granuloma formulation in albino rats

Group (n = 6)	Dose mg/kg orally	Paw volume after carrageenan administration		Cotton pellets granuloma formation	
		Increase in paw volume (ml) + 3 h	% Inhibition	Increase in pellets wt. (mg) Mean ± S.E	% Inhibition
Vehicle	Saline	0.60 ± 0.04	–	62.12 ± 4.43	–
MeCN ext.	150	0.36 ± 0.02*	40	44.00 ± 2.42**	29
MeCN ext.	300	0.31 ± 0.01*	48	34.33 ± 2.87***	45
Oxyphenbutazone	100	0.28 ± 0.02*	53	28.33 ± 1.74***	54
Vehicle	Saline	0.84 ± 0.05	–	63.16 ± 2.63*	–
Hexane ext.	150	0.55 ± 0.02*	34	53.83 ± 3.41	15
Hexane ext.	300	0.44 ± 0.02*	48	39.83 ± 2.48***	37
Oxyphenbutazone	100	0.25 ± 0.02*	70	28.66 ± 3.12***	54
Vehicle	CMC	0.79 ± 0.05	–	63.50 ± 0.25	–
Lupeol	5	0.43 ± 0.05*	46	44.83 ± 6.34**	29
Lupeol	10	0.34 ± 0.03*	60	32.16 ± 2.19**	49
Oxyphenbutazone	100	0.28 ± 0.04*	65	28.33 ± 1.99***	55

Statistically significance was determined by one-way ANOVA and Duncan's test.
In carrageenan administration - *refers to significant at P<0.01 level each test group was significantly compared with their correspondent control group.
In cotton pellets formation - the significant level shows as *P<0.05; **P<0.01; and ***P<0.001. Each test group was significantly compared with their correspondent control group.
CMC: Carboxy Methyl Cellulose.

and indomethacin (4 mg/kg) produced a decrease in yeast-induced hyperthermia in mice (Table 2).

Effect on acetic acid-induced writhing in mice – Treatment of mice with MeCN, hexane extracts and lupeol produced a significant reduction in writhes induced by acetic acid, as indicated in Table 3.

Effect on the tail flick response in mice – Pretreatment of mice with MeCN and hexane extracts (150 and 300 mg/kg); lupeol (5 and 10 mg/kg) significantly increased the tail flick latency of mice to the noceceptive stimuli (Table 4).

Effect of behavior – Behavioral studies in mice showed that the extracts (300 mg/kg) and lupeol (10 mg/kg) intraperitoneally produced a CNS depressant effect. Concerning acute toxicity, the extracts up to 3 g/kg and lupeol up to 40 mg/kg did not show mortality.

Discussion

The present study clearly demonstrates that acetonitrile, hexane extracts of leaves of *Teclea nobilis*, and lupeol possesses a potent anti-inflammatory activity against carrageenan-induced rat paw edema and granuloma induced by cotton pellet implantation.

Table 2. Effect of various extracts and lupeol of *Teclea nobilis* on yeast-induced hyperpyrexia in mice

Group n = 6	Dose mg/kg orally	Rectal Temperature °C			
		Pre Drug	Post Drug		
			60 min	90 min	120 min
Vehicle	Saline	37.31 ± 0.21	37.26 ± 0.19	37.26 ± 0.12	37.03 ± 0.17
MeCN ext.	150	37.41 ± 0.17	36.81 ± 0.17*	35.91 ± 0.13***	36.10 ± 0.16***
MeCN ext.	300	37.46 ± 46.020	36.58 ± 0.18**	35.85 ± 0.12***	35.68 ± 0.26***
Indomethacin	4	37.31 ± 0.18	36.33 ± 0.22**	35.56 ± 0.30***	35.5 ± 0.34***
Hexane ext.	150	37.88 ± 0.23	37.11 ± 0.18*	36.9 ± 0.21**	37.20 ± 0.27
Hexane ext.	300	37.35 ± 0.23	36.10 ± 0.31**	36.2 ± 0.11***	36.2 ± 0.20**
Indomethacin	4	37.26 ± 0.20	36.15 ± 0.21**	35.75 ± 0.17***	35.78 ± 0.19***
Vehicle	CMC	37.73 ± 0.29	37.5 ± 0.26	37.35 ± 0.22	36.88 ± 0.08
Lupeol	5	38.10 ± 0.38	37.03 ± 0.40	37.00 ± 0.25*	36.83 ± 0.25**
Lupeol	10	37.66 ± 0.19	36.81 ± 0.09**	36.20 ± 0.08***	36.23 ± 1.4***
Indomethacin	4	37.9 ± 0.27	36.66 ± 0.12**	35.94 ± 0.12***	35.68 ± 0.32***

Statistically significance was determined by one-way ANOVA and Duncan's test.
The significance level showed as *P<0.05; **P<0.01; and ***P<0.001.
Post drug group were statistically compared with pre drug group.
CMC : Carboxy Methyl Cellulose.

Table 3. Effect of various extracts and lupeol of *Teclea nobilis* on acetic acid-induced writhings in mice

Group (n = 6)	Dose mg/kg orally	Number of writhings Mean ± S.E.	% Inhibition
Vehicle	Saline	39.83 ± 2.24	–
MeCN ext.	150	25.50 ± 2.09*	36
MeCN ext.	300	20.83 ± 1.74*	48
Indomethacin	4	15.66 ± 1.64*	61
Vehicle	Saline	38.66 ± 2.03	–
Hexane ext.	150	25.00 ± 1.29*	35
Hexane ext.	300	21.16 ± 1.74*	45
Indomethacin	4	15.5 ± 1.89*	60
Vehicle	CMC	39.33 ± 1.45	–
Lupeol	5	32.66 ± 2.18*	24
Lupeol	10	32.00 ± 2.02*	26
Indomethacin	4	16.16 ± 1.40*	63

Statistically significance was determined by one-way ANOVA and Duncan's test.
*Refers to significant at P < 0.01 level.
Each test group was significantly compared with their correspondent control group.
CMC : Carboxy Methyl Cellulose.

Table 4. Effect of various extracts and lupeol of *Teclea nobilis* on tail flick test in mice

Group n = 6	Dose mg/kg orally	Pre Drug	Reaction Time				
			Post Drug				
			15 min	30 min	60 min	90 min	120 min
Vehicle	Saline	3.03±0.12	3.16±0.09	3.06±0.09	3.01±0.13	3.06±0.13	3.05±0.10
MeCN ext.	150	3.3±0.24	3.8±0.17	4.06±0.24	4.51±0.23**	4.93±0.26**	4.11±0.14**
MeCN ext.	300	3.55±0.24	4.95±0.08***	5.01±0.24**	5.01±0.27**	4.73±0.17**	4.51±0.14**
Indomethacin	4	3.63±0.11	5.30±0.28***	5.63±0.18	5.66±0.13***	4.83±0.18***	4.35±0.17***
Hexane ext.	150	3.08±0.19	4.55±0.26***	4.83±0.19***	5.06±0.18***	4.53±0.18***	4.20±0.14***
Hexane ext.	300	3.55±0.36	4.71±0.26*	4.83±0.44*	5.28±0.32**	5.31±0.31**	4.66±0.25**
Indomethacin	4	3.21±0.11	4.08±0.10***	4.38±0.24*	5.85±0.17***	5.45±0.28**	4.76±0.16**
Vehicle	CMC	3.15±0.13	3.03±0.10	3.48±0.12	3.33±0.12	3.15±0.16	3.3±0.23
Lupeol	5	3.26±0.32	4.11±0.23*	5.2±0.52**	5.06±0.50*	4.5±0.19**	3.70±0.09*
Lupeol	10	3.20±0.16	3.63±0.10*	4.23±0.09***	4.40±0.10***	4.10±0.03***	3.73±0.24
Indomethacin	4	3.58±0.18	5.73±0.23***	6.11±0.20***	6.00±0.25***	4.58±0.17***	4.41±0.11**

Statistically significance was determined by one-way ANOVA and Duncan's test.
The significance level shows * $P<0.05$; ** $P<0.01$; and *** $P<0.001$.
Post drug group were statistically compared with pre drug.
CMC : Carboxy Methyl Cellulose.

Carrageenan-induced paw edema is a test used largely to study anti-inflammatory drugs both steroidal and non-steroidal since it involves several mediators. This kind of test induces an inflammatory reaction in two different phases. The initial phase has been attributed to the action of histamine, serotonin and bradykinin on vascular permeability (Vinger *et al.*, 1987) while the late phase is a result of over production of prostaglandins in tissue (Vinger *et al.*, 1987; Di Rosa, 1974). The extracts and lupeol inhibit carrageenan induced edema, which indicates that the extracts and lupeol could inhibit different aspects of chemical mediators of inflammation (histamine, serotonin, bradykinin and prostaglandins). Moreover, the extracts and lupeol seems to have a protective effect, which may contribute to its anti-inflammatory effect. On the experimental model of chronic inflammation, both the extracts and lupeol were found active. The granuloma formation is caused by leukotriene B_4 (Lee & Katayama, 1992). Taken together, these results produced by the extracts of TN and luepol are presumably related to the presence of terpenoidal principles (Calixto *et al.*, 1990). A large number of reports indicated that the lupeol isolated from various plants has been shown to possess anti-inflammatory activity (Akihisa *et al.*, 1996; Geetha *et al.*, 1999; Hasmeda *et al.*, 1999; Geetha *et al.*, 1999b).

On the other hand, the extracts and lupeol showed antipyretic and analgesic activities in mice. The present finding is in agreement with an earlier report in which an ethanolic extract of TN has shown anti-inflammatory and analgesic activities (Mascolo *et al.*, 1988). Therefore, the findings of this study support the use of the TN in traditional medicine for the treatment of rheumatism, fever, and various types of pains. These properties of the plant resemble those of the non-steroidal anti-inflammatory drugs (NSAIDs), which are known to possess anti-inflammatory, antipyretic, and analgesic activities (Vane & Botting, 1987). One of the major mechanisms involved in the anti-inflammatory activity of NSAIDs is inhibition of prostaglandins biosynthesis (Vane, 1971). Furthermore, fever is known to be promoted by prostaglandin, mainly PGE_2, synthesized upon induction of cytokines released from activated macrophages (Dinarello, 1989). Indomethacin (a NSAID) for example, is known to block the synthesis of prostaglandings (Ferreira & Vane, 1974). The results indicate that the extracts and lupeol exerted both antipyretic and analgesic activities probably by interfering with the synthesis of prostaglandins (Dirosa, 1974; Di Rosa *et al.* 1971). One cannot exclude a probable contribution of terpenoidal compounds to the anti-inflammatory activity of the plant extracts. These compounds are known to possess anti-inflammatory activity (de Farias Freire *et al.*, 1973; Mossa *et al.*, 1995); as the lupeol is a terpenoidal compound. The acute toxicity and behavioral study showed that a high single dose of TN extracts and lupeol did not cause mortality or any severe effect in animals except sedation.

In conclusion, *Teclea nobilis* extracts and lupeol

presented an anti-inflammatory, analgesic and antipyretic effects without causing apparent deleterious effects. These results are encouraging for further investigation of extracts from species of this genus. Further studies are deemed necessary to elucidate exact mechanism(s) of action.

Acknowledgements

This work was supported by a grant from the Research Center (Grant #. C.P.R.C.72), College of Pharmacy, King Saud University. The authors thank Mr. Malik Sawood for his assistance.

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(Accepted July 12, 2001)