

Antidiabetic and Antiinflammatory activities of Ursolic acid (3 - beta-3hydroxy-urs-12-ene -8-oic-acid), a triterpenoid from Leaves of *Stachytarphetajamaicensis*(L) Vahl (Verbenaceae)

ABSTRACT

Background: Ursolic acid, a pentacycliterpenoid carboxylic acid widely found in medicinal plants has been reported to have multifarious biological activities such as antiinflammatory, anticancer, antidiabetic, and antioxidant activities.

Aim: To evaluate the antidiabetic and antiinflammatory activities of Ursolic acid isolated from dichloromethane:methanol extract of leaves of *Stachytarphetajamaicensis*(L) Vahl (Verbenaceae) in alloxan-induced diabetic rats.

Method: A dichloromethane:methanol (1:1) leaves extract was prepared by cold maceration. Dichloromethane fraction obtained from the dichloromethane: methanol extract was subjected to column chromatography and eluted with different solvents of increasing polarity to isolate the bioactive compounds. The structure of the isolated compound was elucidated using spectroscopic techniques (IR, ¹HNMR, ¹³CNMR, MS). Phytochemical analysis of the isolate and acute toxicity study was done following standard procedures. The anti-diabetic potential was assessed by determining fasting blood glucose level on hyperglycemic rats at the dose of 25 and 50 mg/kg for a period of 14 days. The isolated compounds' antiinflammatory activity was investigated using egg albumin-induced rat paw edema in mice at dose of 10 and 20 mg/kg body weight.

Results: Ursolic acid (3-beta-3hydroxy-urs-12-ene -8- oic-acid) isolated from dichloromethane:methanol extract of *Stachytarphetajamaicensis* showed significant ($p < 0.05$) reduction (72.79 and 79.77%) in fasting blood glucose levels at the dose of 25 and 50 mg/kg respectively in treated diabetic animals when compared with the diabetic control. A 77.77 % reduction was observed for standard drug (glibenclamide). Ursolic acid at 20 mg/kg exhibited a maximum inhibition (75.00%, $p < 0.05$) of carrageenan-induced ear edema in mice. It showed positive for terpenoids. There was no death recorded in the acute toxicity test at doses of up to 5000mg/kg.

Conclusion: The results demonstrate that ursolic acid isolated from dichloromethane:methanol extract of *Stachytarphetajamaicensis* (L) Vahl possesses significant anti-diabetic and anti-inflammatory activity.

Keywords: *Stachytarphetajamaicensis*; ursolic acid; antidiabetic; egg albumin; anti-inflammatory

INTRODUCTION

A condition of carbohydrate metabolism known as diabetes mellitus is characterized by a decreased capacity of the body to produce or respond to insulin and, as a result, maintain normal levels of glucose in the blood. As a result, there are many serious side effects and repercussions, including arteriosclerosis, hyperthyroidism, nephropathy, retinopathy, neuropathy, and countless other serious disorders [1, 2, 3]. In fact, other problems are connected to the use of antidiabetic drugs because frequent delivery may have a number of

unfavorable effects [4]. This makes it difficult to achieve adequate control of hyperglycemia when utilizing commercially available anti-diabetic medications, which can have a number of serious effects [5]. A fantastic chance to find new organic therapeutic compounds is provided by medicinal plants. Some of these compounds might improve diabetic patients' glucose homeostasis without having any negative side effects that are currently seen in antidiabetic medications [5, 6].

Inflammation is a pathophysiological response to injury or infection that causes redness, warmth, swelling, pain, and a loss of function. Inflammation, a crucial component of the body's defense system, chases away viruses or foreign objects while protecting the host from further damage. However, if the harmful inflammation is allowed to continue, it will lay the groundwork for the emergence of a number of illnesses, including sepsis, atherosclerosis, and cancer. From mild headaches to rheumatoid arthritis, nonsteroidal anti-inflammatory medications (NSAIDs) are frequently used to treat a variety of inflammation-related diseases. NSAIDs are readily available currently. NSAIDs have unfavorable side effects, including allergic reactions, indigestion, and stomach ulcers [7]. A higher risk of heart attack, stroke, or heart failure has also been associated with long-term NSAID use [8]. Numerous herbal medicines could be anti-inflammatory or analgesic. Furthermore, its side effects are far less severe than those of NSAIDs. As a result, when searching for novel anti-inflammatory drugs, scientists from all around the world are now interested in ethnomedicine.

Stachytarpheta jamaicensis (L.) Vahl belongs to the Verbenaceae family and is also known as Gervao, Brazilian tea, verbena cimarrona, rooster comb, and blue porter weed [9, 10]. This plant is most usually found in the subtropical woodlands of Africa, Asia, and Oceania, as well as the tropical and subtropical sections of America. *S. jamaicensis* has long been regarded as a powerful healer in traditional and folk medicine. Antacid, analgesic [11], anti-inflammatory [12], hypotensive [13], antihelminthic [14], diuretic, laxative, lactagogue, purgative, sedative, spasmogenic, vasodilator, vulnerary, and vermifuge characteristics have been demonstrated [9, 15, 16]. *S. jamaicensis* is rich in bioactive compounds. These bioactive compounds are now known to be the source of their therapeutic actions. Some of the key classes of secondary metabolites found in plants include alkaloids, flavonoids, phenols, steroids, and terpenoids. These bioactive compounds are found throughout the plant. The purpose of this study is to learn more about the anti-diabetic and anti-inflammatory characteristics of ursolic acid, which was extracted from the leaves of *Stachytarpheta jamaicensis*.

MATERIALS & METHODS

Collection, Identification, and Preparation of Plant Material

Fresh leaves of *Stachytarphetajamaicensis* (L.) Vahl was collected in June from Orba, Udenu LGA, Enugu State, Nigeria, and identified by Mr. A.O Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The voucher specimen (UNN/PCG/14/022) was deposited in the Herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. The leaves were air-dried under shade for three weeks. The dried leaves were then ground into powder using a grinding machine.

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Animals

The animals were housed in cages under laboratory conditions at $22 \pm 2^\circ\text{C}$ and 60–65% relative humidity with a normal 12 hr light and dark cycle.

Extraction and isolation of ursolic acid

The powdered leaves (1 kg) were thoroughly macerated for 72 hours with 5 l of a 1:1 mixture of dichloromethane and methanol. It was filtered, and a rotary evaporator was used to concentrate the filtrate in vacuo. The resulting dichloromethane methanol extract was suspended in a mixture of MeOH and water (1:1) before being progressively partitioned into n-hexane, dichloromethane, and aqueous methanol fractions. Hexane:EthylAcetate gradient column chromatography is used to separate the dichloromethane phase with increasing polarity. The fractions were gathered, put through column chromatography, and then TLC-monitored. Similar fractions were combined and the solvent was removed under vacuum. Pure compound isolated was well characterized by melting point, IR, ^1H NMR (400MHz), ^{13}C -NMR (101MHz), and MS. The infrared spectrum was recorded on FTIR Perkin Elmer, ^1H - NMR and ^{13}C - NMR spectra were recorded using CDCl_3 as a solvent on Bruker Advance II 400 NMR spectrometer at the India Institute of Integrative Medicine (iiim) Jammu, India.

Test for terpenoids

Compound 1 was mixed with 2 ml of chloroform and 3 ml of conc.sulphuric acid carefully added to form a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids.

Acute toxicity study

Using the procedure outlined by Lorke[17], the median lethal dosage (LD₅₀) was calculated. The twelve mice chosen for this investigation ranged in weight from 18 to 40g. Before the study, the animals were fasted for 18 hours while receiving just water. Six treatment groups, designated as groups "A to F," were created for the animals. Oral administration of all medicines was used. Three animals were present in each of groups A through C. Group A was given 10 mg/kg of extract, whilst groups B and C were given 100 and 500 mg/kg of extract, respectively. With only one mouse in each group, 1600, 2900, and 5000 mg/kg of extract were given to groups D, E, and F, respectively. For 24 hours following treatment, the animals were monitored for toxicity-related signs and symptoms, including mortality. The final LD₅₀ was determined by taking the geometric mean of successive doses for which survival rates of 0 and 100% were noted and multiplying it by the square root of the product of the lowest lethal dose and the greatest non-fatal dose.

Evaluation of Antidiabetic Activity

Induction of diabetes

Prior to induction, twenty-four (24) rats were fasted for 12 hours at a time. Alloxan monohydrate, 150 mg/kg, was administered intraperitoneally (i.p.) to fasted rats to cause diabetes. In order to counteract the first hypoglycaemic spike brought on by Alloxan (due to a quick surge in the production of insulin), the rats were administered glucose solution (5% w/v) for 24 hours. Each rat was tail-snipped after three days (72 hours) to obtain blood, and each rat's fasting blood glucose level was measured using a glucometer to determine whether it had diabetes. Animals with fasting blood glucose levels of 200 mg/dl and above after about 3 days of induction were considered diabetic[18].

Antidiabetic study

The diabetic rats were randomly divided into four groups of six mice each: Group A (normal control), Group B (diabetes control), Group C (standard drug: glibenclamide), Groups D and E are for diabetes-treated animals (25 and 50 mg/kg body weight of ursolic acid respectively). Each mouse in group D of the diabetes treatment groups received 25 mg/kg of ursolic acid, while each mouse in group E received 50 mg/kg of ursolic acid, both of which were dissolved in sterile saline and observed for 14 days. For 14 days, the rats received one

treatment every day. A 5ml/kg of normal saline was given to the control mice (normal control and diabetic control groups) exclusively. Rats' blood glucose levels were checked using trace blood samples taken from their tail veins at days 0, 7, and 14.

Evaluation of Antiinflammatory Activity

Induction of inflammation

On Albino Wistar rats, 0.1 ml of 1% carrageenan was subcutaneously injected, with sterile saline solution serving as the control, to cause edema. Paw volume was measured prior to the injection as well as at 1, 2, 3, and 4 hr later.

Carrageenan-Induced rat paw oedema

This concept is based on the idea that carrageenan, a substance utilized in investigations of acute inflammation, releases a variety of inflammatory mediators. The release of histamine and serotonin occurs in the initial phase of the biphasic edema development caused by carrageenan in the rat paw. Prostaglandins, protease, and lysosome release signal the start of the second phase. Inflammation is brought on by plasma extravasation, increased tissue water and plasma protein exudation, neutrophil extravasation, and arachidonic acid metabolism in the rat paw after subcutaneous injection of carrageenan. The first phase starts right away after the carrageenan injection and fades away in two hours, whereas the second phase starts at the conclusion of the first phase and lasts for three to five hours [19].

The animals were placed into four groups with three animals in each group, and they were given unlimited access to water the night before the experiment. The test group is given the normal medication, whilst the control group is given the vehicle orally. The level of the lateral malleolus on the left paw is marked with ink, and the volume of the basal paw is measured plethysmographically by volume displacement method using a plethysmometer by submerging the paw until it reaches the level of the lateral malleolus. Drug therapy is administered to the animals. The rats are challenged an hour after dosing with a subcutaneous injection of 0.1 mL of 1% carrageenan solution into the subplantar side of the left hind paw. After the challenge, the paw volume is measured once more at 1, 2, 3, and 4 hours. The difference between the paw volume and the basal volume is expressed as a percentage. For each time interval, the difference in average values between the treated animals and the control group is calculated and statistically assessed. Then the % inhibition was determined.

Inhibition of edema (%) = $100 \times [1 - (a-x)/(b-y)]$, Where; a = mean paw volume of treated rats at various time after carrageenan injection, x = mean paw volume of treated rats before formation of rat paw edema induced by carrageenan during the first, second and the third hour of inflammation carrageenan injection, b = mean paw volume of control rats at various time after carrageenan injection, y = mean paw volume of control rats before carrageenan injection.

Statistical analysis

Statistical analyses were performed using Student's t-test. All values were expressed as mean \pm standard error of means. Values with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

A white, crystalline powder (48 mg) containing ursolic acid (SJ1) was extracted. Its melting point ranged from 285 to 288 OC, and its Rf value was 0.52 (10% EtoAC/Hexane). It passed the terpenoids test, proving by its spectrum data that it is a terpenoid. Based on spectroscopic research and comparison with published data in the literature, the molecule was described. Based on spectroscopic research and comparison with published data in the literature, the molecule was described. The molecule was identified as a triterpenoid by ¹H NMR analysis and other spectral data (Tables 1; images 2 and 3). The melting point is in line with the values for ursolic acid published in the literature. Through the use of high-resolution mass spectrometry, which revealed an M⁺ peak at m/z 456, the chemical formulae were determined to be C₃₀H₄₈O₃. The compound's fragmentation pattern is consistent with terpenoids.

Spectroscopic data of isolated compound

The EI-MS spectrum showed molecular ion peak at m/z: 456.7g/mol (Molecular Formular C₃₀H₄₈O₃).

¹H NMR (400 MHz, CDCl₃) has given signals at δ 12.01 (d, $J = 6.6$ Hz, 1H), 5.25 (s, 1H), 4.12 (s, 1H), 2.19 (s, 1H), 1.99 (s, 1H), 1.70(s, 1H), 1.66 (d, $J = 12.8$ Hz, 1H), 1.58 (s, 1H), 1.53 (s, 1H), 1.46(s, 1H), 1.43(s, 1H), 1.35 (s, 1H), 1.29 (d, $J = 11.8$ Hz, 3H), 1.16 (s, 1H), 1.11 (s, 1H), 1.04(s, 1H), 0.96 (t, $J = 15.3, 6.8$ Hz, 3H), 0.78 (t, $J = 10.0$ Hz, 3H).

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^{13}C NMR (101 MHz, CDCl_3) δ 178.40(C-28) 137.50(C-13), 122.50 (C-12), 78.20(C-3) 56.88(C-5), 53.10(C-18), 48.70 (C-17), 47.01(C-9), 42.53(C-14), 40.75(C-8), 39.20(C-19), 38.90(C-4), 38.40(C-20), 37.70(C-10), 37.48(C-1), 36.98(C-22), 33.38(C-7), 30.52(C-21), 29.40(C-15), 26.38(C-2), 26.10(C-26), 24.30(C-16), 24.57(C-11), 22.17(C-23,24), 20.59(C-30), 18.40(C-6), 17.41(C-27), 16.41(25), 16.20(C-29). The ^{13}C NMR data showed that the compound contain 30 carbon. Comparison of the spectral data with those published before allowed us to establish the structure of compound 3 as Ursolic acid or 3 - beta-3hydroxy-urs-12-ene -8- oic-acid.

IR spectra showed absorption peaks at 3744.53 and 3421.36 cm^{-1} (O-H stretching), 2090.10 cm^{-1} (aromatic ring system), 1642.36(C=C stretching), 1554.92 and 1453.65 cm^{-1} (C-C stretching), 1274.04 cm^{-1} (C-O stretching), 995.53 and 764.90 cm^{-1} (=C-H bending).

LD₅₀ Test

During the experiment, the mice that were being treated appeared to be acting normally. Up to 5000 mg/kg, no harmful effects were noticed, and none of these groups suffered deaths.

Antidiabetic effect of ursolic acid

Table 3 presents the findings of the antidiabetic impact. Alloxan elevated the blood glucose levels in rats, which led to hyperglycemia. Alloxan was used to produce diabetes because it is known to destroy pancreatic beta cells [20]. It is now understood that beta-cell mass and function may gradually decrease in people who have a high risk of acquiring type 2 diabetes. To stop the loss of beta-cell bulk and function, beta-cell stabilization and regeneration are necessary [21]. Ursolic acid-treated rats displayed a significant (p0.05) decrease in blood glucose levels 3 to 14 days after treatment. Despite ursolic acid at 50 mg/kg being a little bit higher than the standard drug glibenclamide (77.77%) after 14 days of treatment, blood glucose levels in the treated groups (25 and 50 mg/kg) were equivalent to those obtained with the reference drug (glibenclamide). The diabetic group's blood glucose levels remained elevated throughout the experiment. On day 14, the blood glucose levels decreased in the 25 and 50 mg/kg groups by 72.79 and 79.77%, respectively. Glibenclamide only directly improves insulin action when insulin is present [22]. As was the case with glibenclamide, it is unknown whether the anti-diabetic benefits of ursolic acid are brought on by increased insulin secretion. This study's antidiabetic activity is in line with other studies that shown ursolic acid can lower blood sugar [23, 24]. These results demonstrate that ursolic acid dramatically reduces blood sugar levels in diabetic rats produced by alloxan. Ursolic acid showed a significant 75.00% reduction in inflammation in the anti-inflammatory research at the two doses (10 and 20 mg/kg), albeit it was less effective than the gold standard medicine, piroxicam (20 mg/kg).

Conclusion

In rats with diabetes induced by alloxan, ursolic acid extracted from *Stachytarpheta jamaicensis* dramatically lowered blood glucose levels. Ursolic acid treatment for just 14 days had a substantial hypoglycemic impact when compared to diabetic control mice ($p < 0.05$), which is similarly equivalent to the positive control, glibenclamide. A sizable anti-inflammatory impact was also noted. It is advised that more research be done on the processes that cause inflammation and diabetes. The discovery could soon result in the creation of secure and efficient hypoglycemic and anti-inflammatory medications.

Figure 1: Ursolic acid (Compound SJ 1), $C_{30}H_{48}O_3$, Mol. Wt: 456.71g/mol

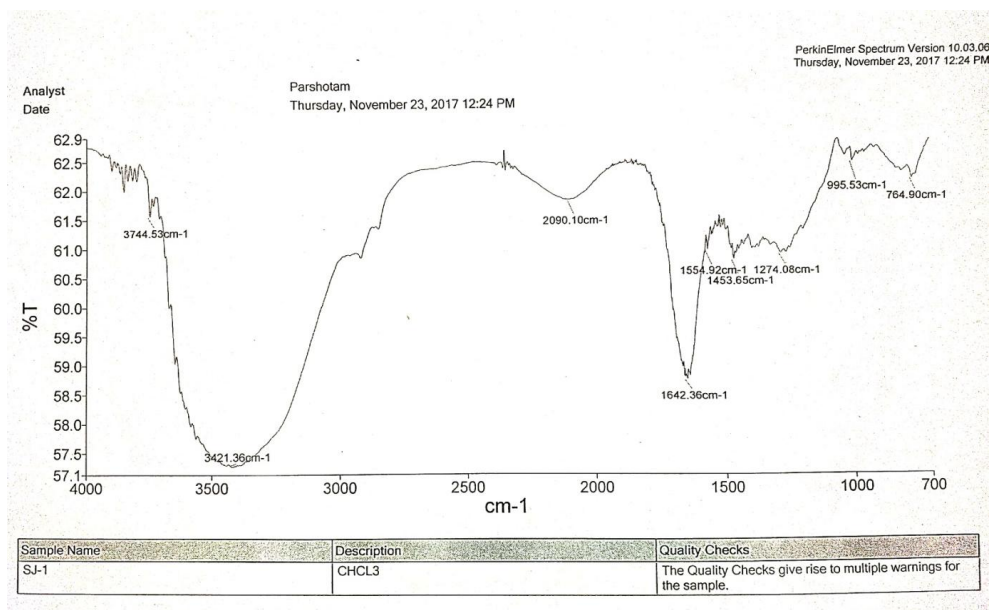


Figure 2: Infra red spectrum of Ursolic acid (Compound SJ 1)

Table 1: ^1H NMR (δ_{H} in ppm, 400MHz) and ^{13}C NMR (δ_{C} in ppm, 101MHz) chemical shift values for Compound SJ 1 (Ursolic acid)

Carbon atom	^{13}C NMR Experimental	^1H NMR Experimental
C-1	37.48	1.66 (d, $J = 12.8$ Hz, 1H)
C-2	26.38	1.70(s, 1H)
C-3	78.20	4.12 (s, 1H)
C-4	38.90	-
C-5	56.88	1.04(s, 1H),
C-6	18.40	1.66 (d, $J = 12.8$ Hz, 1H)
C-7	33.38	1.58 (s, 1H)
C-8	40.75	-
C-9	47.01	1.16 (s, 1H)
C-10	37.70	1.11 (s, 1H)
C-11	24.57	2.19 (s, 1H)
C-12	122.50	5.25 (s, 1H)
C-13	137.5	-
C-14	42.53	1.66 (d, $J = 12.8$ Hz, 1H)
C-15	29.40	1.35 (s, 1H)
C-16	24.30	1.53 (s, 1H)
C-17	48.70	-
C-18	53.10	-
C-19	39.20	1.46(s, 1H)

C-20	38.40	1.43(s, 1H)
C-21	30.52	1.63(s, 1H)
C-22	36.98	1.99 (s, 1H)
C-23	22.17	0.96 (t, $J = 15.3, 6.8$ Hz, 3H)
C-24	22.17	0.96 (t, $J = 15.3, 6.8$ Hz, 3H)
C-25	16.41	1.38(t, 3H)
C-26	26.10	1.11 (t, 3H)
C-27	17.41	1.16 (t, 3H)
C-28	178.40	1.29 (t, $J = 11.8$ Hz, 3H)
C-29	16.20	0.78 (t, $J = 10.0$ Hz, 3H)
C-30	20.59	0.78 (t, $J = 10.0$ Hz, 3H)

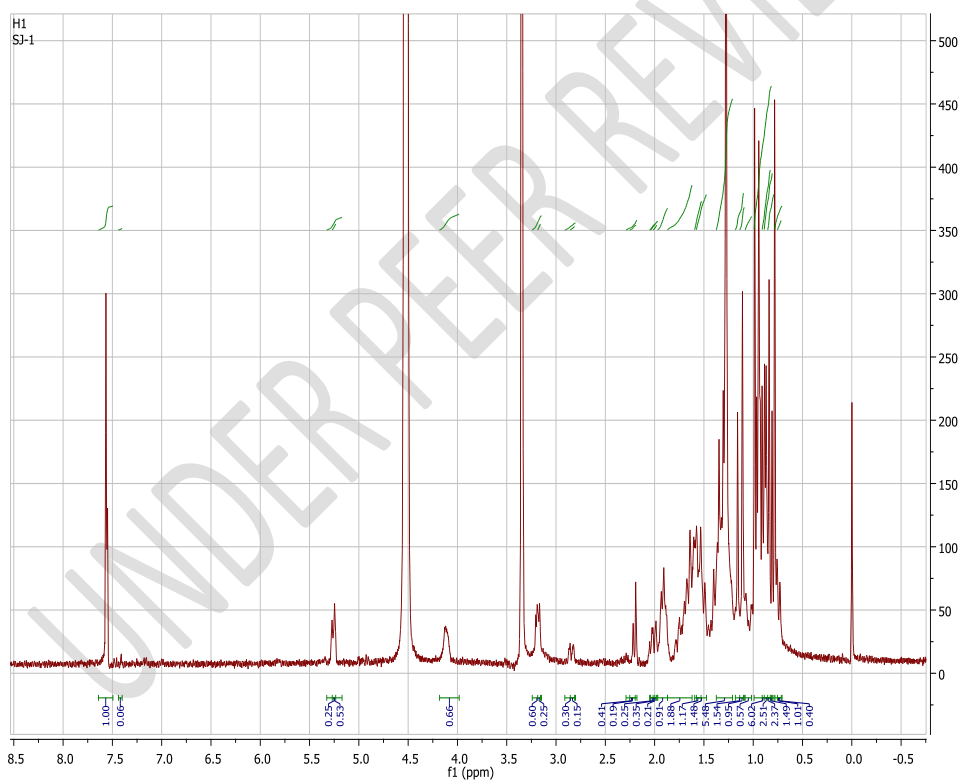


Figure 3: The ¹H-NMR Spectrum of Compound 1- Ursolic acid ((3 - beta-3hydroxy-urs-12-ene -8-oic-acid).

Table 2: Results of antidiabetic effect of Ursolic acid on the fasting blood glucose concentration and percentage reduction in blood glucose concentration of alloxan-induced diabetic rats

Treatment	Dose (mg/kg)	0 day	3 rd day	7 th day	10 th day	14 th day
Ursolic acid	25	346.67±36.45	217.67±49.08	171.67±65.45	96.00±11.85	94.33±9.60
		(37.21)	(47.97)	(68.18)	(72.79)	
Ursolic acid	50	384.33±78.24	134.33±31.94	88.00±3.06	84.67±3.53	78.00±3.46
		(65.00)	(77.10)	(77.96)	(79.70)	
Glibenclamide	5	325.33±62.35	135.00±33.96	96.00±5.51	87.00±4.58	72.33±2.60
		(58.50)	(70.49)	(73.26)	(77.77)	
Negative	-	412.67±15.38	459.33±30.78	554.67±36.70	570.67±20.99	600.00±0.00
		(5ml/kg)	(-10.00)	(-34.41)	(-38.29)	(-45.39)

Values are the mean ± SEM (n=6), * p< 0.05 Vs negative control. Values in parenthesis are percentage reductions

Table 3: Results of antiinflammatory effect of ursolic acid on rat paw edema

Treatment	Dose (mg/kg)	0h½ h	1h	2h	3h	4h
Ursolic acid	100	73.±0.03	1.33±0.06*	1.13 ±0.03*	1.10±0.03*	0.97±0.03* 0.83±0.03*
Ursolic acid	20	0.60±0.06	1.3 ±0.03*	1.07 ±0.03*	0.90 ±0.00*	0.80±0.00*
		0.70±0.00*				
Piroxicam	20	0.73±0.03	1.66±0.03*	1.00±0.00*	0.97±0.03*	0.83±0.03*
		0.77±0.03*				
D.H2O (5ml/kg) -	-	0.70±0.1	1.43±0.03	1.40±0.00	1.30±0.00	
		1.20±0.00	1.10±0.00			

Values are ± SEM, n=5, *. The mean difference is significant at p< 0.05

Table 4: Results of percentage inhibition of the antiinflammatory effect of ursolic acid on rat paw edema

Treatment	Dose (mg/kg)	½ h	1h	2h	3h	4h
Ursolic acid	10	18.15	42.86	38.83	61.05	75.00

Ursolic acid	20	27,29	33.33	50,00	60.00	75.00
Piroxicam	10	40.83	61.86	76.63	89.97	91.48
Negative control (5 ml/kg)	-	0.73	0.70	0.60	0.50	0.40

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