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ORIGINAL RESEARCH

Phytochemical study, cytotoxic, analgesic, antipyretic and anti-inflammatory activities of *Strychnos nux-vomica*

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Abstract The strychnine tree (*Strychnos nux-vomica* L.) (*S. nux-vomica)* belonging to family Loganiaceae has been a very promising medication for certain disorders. Different chromatographic methods were used to isolate the phenolic compounds from the aqueous methanolic extract of the *S. nux-vomica* leaves. Their identification was achieved through spectroscopic techniques. Cytotoxicity, analgesic, antipyretic and anti-inflammatory activities of *S. nux-vomica* leaves extract were evaluated. Five phenolic compounds were isolated and identified; Kaempferol-7 glucoside **1**, 7-Hydroxy coumarin **2**, Quercetin-3-rhamnoside **3**, Kaempferol 3-rutinoside **4**, and Rutin **5**. Furthermore, the cytotoxic activity of the extract was evaluated against different cancer cell lines. The extract showed potential cytotoxic activity against human epidermoid larynx carcinoma cells (Hep-2) and against breast carcinoma cell line (MCF-7). Colon carcinoma cells (HCT) were the least one affected by the extract. In addition, the extract exhibited promising analgesic, antipyretic as well as anti-inflammatory activities. It is concluded that, leaves extract of *S. nux vomica* possess potent cytotoxic, analgesic, antipyretic and anti-inflammatory activities. These activities could be due to the presence of phenolic compounds revealed by our phytochemical investigations.

Keywords *Strychnos nux-vomica* · Phenolics · Cytotoxic · Analgesic · Antipyretic · Anti-inflammatory

Introduction

Herbal medicine is still the mainstay of about 75–80 % of the world population, mainly in the developing countries, with the aim of promoting primary health care with better cultural acceptability, human compatibility and fewer side effects. The introduction of medicinal plants in treatment of various diseases is increasing and becomes now a new trend for natural drug discovery (Abdel-Daim 2012; Abdel-Daim and Halawa 2014). The strychnine tree (*Strychnos nux-vomica* L.) (S. *nux-vomica)* is a very promising drug for certain diseases since long time. Its seed (Kupeelu seed), a known poisonous medication, is used extensively in various Ayurvedic formulations with great therapeutic significance. In Ayurvedic medicine, *S. nux-vomica* L. has been cited for the treatment of

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paralysis, diabetes, gonorrhea, anemia and bronchitis. It has been shown to possess antioxidant and anti-snake venom activity (Tripathi 1998; Tripathi and Chaurasia 2000; Philippe et al. 2004). *S. nux-vomica* L. and its alkaloids have been reported for its analgesic and anti-inflammatory (Yin et al. 2003), anti-tumor (Deng et al. 2006), and anti-diarrhoeal (Shoba and Thomas 2001) activities in different modern literatures. The seeds extract also prevent lipid peroxidation (Tripathi and Chaurasia 2000) and might be a good potential candidate exhibiting antioxidant activity (Vijayakumar et al. 2009). Seeds extract has a suppressive activity on allergen-specific IgE antibody response and suggests its possible application in allergic conditions (Duddukuri et al. 2008).

The root extract was screened using the human MM-cell line, RPMI 8226. The anti-proliferative and cytotoxic activity could be due to the alkaloids; strychnine and brucine, which have been identified by LC–mass (Rao et al. 2009). Concerning the published data on the leaves extract; the petroleum ether extract contained alkaloids, strychnine and vomicine (Quirin et al. 1965; Sefcovic et al. 1968). It was reported that *S. nux-vomica* contained a significant quantity of non-enzymatic and enzymatic antioxidants in the leaves (Vijayakumar et al. 2009).

Since nothing could be traced in literature concerning the phenolic content of leaves of *S. nux-vomica* and as part of an ongoing study to discover potential bioactive phenolics from terrestrial plant sources (Ayoub et al. 2009; Ayoub 2010), the present study was directed to investigate the phenolics present in the aqueous methanol extract obtained from the leaves of *S. nux-vomica* and to investigate their cytotoxic, analgesic, antipyretic and anti-inflammatory activity.

Materials and methods

Strychnos nux-vomica leaves were collected from Giza Animal Zoo (Giza, Egypt) during April, 2011. Experimental design and animal handling were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (approval no. 20151). All efforts were made to minimize animal suffering. Blood sampling was done under light ether anaesthesia. Moreover, after each animal experiment, rats or mice were sacrificed by deep ether anaesthesia.

Phytochemical investigation

Plant source

Strychnos nux-vomica leaves were authenticated by Taxonomy specialist, Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. Voucher specimen was deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. The leaves were dried in shade and reduced to a fine powder.

Extraction and isolation

The intact air dried plant material (1 kg) was comminuted to powder then boiled in distilled water for 2 h then filtered while hot. The filtrate was completely evaporated *in vacuo* at $\approx$55 °C until dryness. The solid residue was then extracted with methanol until exhaustion at 40 °C. The combined methanol extracts were evaporated *in vacuo* until dryness.

Thirty five grams of the extract were applied on a cellulose column. Elution started with water then water and methanol of decreasing polarity. Six grams of water fraction were chromatographed on a polyamide column. Water and water methanol were the solvent system. Fractions 4, 5, 6 and 7 (water eluted) was gathered and applied on paper chromatography using BAW as a solvent system. Two compounds were isolated 1 and 2. Fractions 24–32 (80 %) were pooled and then applied on Sephadex column to isolate two compounds 3 and 4. Fraction 34 (80 %) was applied on a silica gel column to isolate compound no. 5, from solvent system chloroform and methanol.

Chemicals

Kaempferol, quercetin, apigenin and luteolin were obtained from the NMR department, NRC (Cairo, Egypt). Glucose, rhamnose and glucuronic acid were purchased from E. Merck (Darmstadt Germany), Sephadex LH-20 from GE Healthcare (Uppsala, Sweden), Paper chromatography was carried out on sheets of unwashed Whatman No. 1 paper (Whatman Ltd. Maidstone, Kent, England). Silica gel 60 for column chromatography was purchased from E. Merck, particle size (70–230 mesh), precoated silica gel 60 F 254 sheets for TLC was obtained from Riedel-De Haen AG (Seelze, Germany).

Ultraviolet spectrophotometric analysis

Chromatographically pure materials 1 mg each were dissolved in analytically pure methanol then subjected to UV spectroscopic investigation in 4 ml capacity quartz cells 1 cm thick using a Carl Zeiss spectrophotometer PMQ II. $AlCl_3$, $AlCl_3$/HCl, fused NaOAc/H_3BO_3 and NaOMe reagents were separately added to the methanolic solution of investigated material and UV measurements were then carried out.

Nuclear magnetic resonance spectroscopic analysis

The NMR spectra were recorded on a Varian Mercury VX-500 NMR spectrometer (Palo Alto, CA, USA). ^{1}H-spectra run at 500 MHz. Chemical shifts are quoted in δ and were related to that of the solvents.

Cytotoxic activity

Chemical

Dimethyl sulfoxide (DMSO), crystal violet (1 %) and trypan blue dye were purchased from Sigma (St. Louis, Mo, USA), DMEM, RPMI-1640, FBS, HEPES buffer solution, L-glutamine, gentamycin and 0.25 % Trypsin–EDTA were obtained from Bio Whittaker ® Lonza (Verviers, Belgium).

Cytotoxicity evaluation using viability assay

All cell lines of well-differentiated carcinomas; Hep 2 (Larynex), MCF-7 (Breast cancer), HCT (Colon cancer) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cell toxicity was monitored by determining the effect of the test sample on cell morphology and cell viability through viability assay (Mosmann 1983; Vijayan et al. 2004).

In-vivo animal study

Chemicals

Analytical-grade glacial acetic acid (Sigma-Aldrich) was diluted with pyrogen-free saline to provide a 0.6 % solution for i.p. injection. Diclofenac sodium (declophen®) was obtained from Pharco Pharmaceutical (Alexandria, Egypt).

Experimental animals

Healthy male Wistar albino rats weighing between 150 and 200 g and male Swiss mice weighing between 20 and 25 g, were selected for the study and obtained from the animal house of the National Central Institute (Dokki, Cairo, Egypt) and caged in uniform hygienic conditions (eight animals per cage) in the well-ventilated animal house of the Department of Pharmacology, Faculty of Veterinary Medicine, Suez Canal University. Animals were fed on standard pellet and given water ad libitum. Rats and mice were acclimatized to laboratory conditions for 7 days before the beginning of any experiment. Experimental design and animal handling were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Suez Canal University (Ismailia, Egypt) (approval no. 20151). All efforts were made to minimize animal suffering. After each animal experiment, rats or mice were sacrificed by deep ether anaesthesia.

Acute toxicity study

Oral acute toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD). Rats and mice were used and kept fasting providing only water, after which the aqueous methanolic extract was administered orally by stomach tube in different gradual doses (up to 2,000 mg/kg bw), and observed for any toxic symptoms and mortality for 72 h.

Analgesic activities

The antinociceptive activities of the aqueous methanolic extract of the *S. nux vomica* were evaluated using mice acetic acid-induced writhing test, rat tail immersion test as well as mice hot plate test.

Acetic acid-induced writhing test

A writhing was defined as contraction of the abdominal muscles accompanied by an extension of the hind limbs. Abdominal writhing was induced following intraperitoneal injection of acetic acid (0.6 % solution in distilled water, 10 ml/kg bw) (Atta and Abo 2004). Forty Swiss albino mice (20–25 g) were divided into five groups each of eight. The 1st group served as

negative control and received saline, and the 2nd, 3rd and 4th groups received *S. nux-vomica* aqueous methanolic leaves extract orally at doses of 100, 200 and 400 mg/kg bw, respectively, 30 min before acetic acid administration. Diclofenac sodium used as a standard drug, and given orally for the 5th group at a dose of 100 mg/kg bw. The number of writhing and stretching was counted over a 15 min period starting 5 min after injection of acetic acid. The inhibition of nociceptive response by the tested extract was determined for each experimental group as follows:

$$\% \text{ Inhibition} = 100 \times (1 - \text{experimental}/\text{control}).$$

Tail immersion test

Tail immersion was conducted as described by (Toma et al. 2003). This technique involved immersing the extreme 3 cm of the rat's tail in a bath containing water maintained at a temperature of 55 ± 0.5 °C. Within a few seconds, the rats reacted by withdrawing the tail. The reaction time was measured at 0, 60, 120, 180, 240 and 300 min after drug administration. Forty rats were allocated into five groups (each of 8). The 1st group received saline and served as control. The 2nd, 3rd and 4th groups received *S. nux vomica* aqueous methanolic extract orally at doses of 100, 200 and 400 mg/kg bw. The 5th group got orally administered diclofenac sodium (100 mg/kg) and served as a standard group.

Hot plate test

Analgesic activity was tested in mice using the hot plate according to (Abdel-Salam 2005) with slight modifications. Forty Swiss mice were divided into five groups (n = 8). The 1st group was given only distilled water and observed as a control. The 2nd, 3rd and 4th groups received *S. nux vomica* aqueous methanolic extract orally at doses of 100, 200 and 400 mg/kg bw. The last group got orally administered diclofenac sodium (100 mg/kg) and served as a standard group. The mice were placed in a large glass flask as hot plate kept at a temperature of 55 ± 0.5 °C for a maximum time of 15 s. Reaction time was recorded when animals licked their paws or jumped. The responses were taken at a different time interval; 0, 60, 120, 180, 240 and 300 min after the administration of the extract. Cut off time in the absence of a response was 15 s to prevent the animals from being burnt (Laughlin et al. 2002).

Antipyretic activities

The antipyretic activities of the *S. nux vomica* aqueous methanolic extract were evaluated using brewer's yeast-induced pyrexia in rats. For this purpose, forty healthy albino rats weighing 175 ± 15 g were used. The pre-acclimatized rats were then divided into five groups each of eight. Rats were then made hyperthermic by subcutaneous injection of a 15 % w/v suspension of brewer's yeast at a dose of 10 mg/100 g of bw. The prepared test extract were administrated orally—17 hs after the yeast administration—to the experimental rats at doses of 100, 200 and 400 mg/kg bw for the 2nd, 3rd and 4th groups, respectively. The 1st group was left as non-treated feverish control group, which was given normal saline, whereas, the 5th group was given paracetamol orally (150 mg/kg) and acted as a standard group. After the treatment, the body rectal temperature was measured in 1 h intervals for consecutive 6 h by using a digital thermometer.

Anti-inflammatory activities

Paw inflammation and edema were induced by subplanter injection of 0.1 ml of 1 % freshly prepared suspension of carrageenan into the right hind paws of the rats of all groups (Esmat et al. 2012). The thickness of the injected paws and contralateral paws were measured at 0, 60, 120,180, 240 and 300 min intervals using skin caliber. *S. nux vomica* extract (100, 200 and 400 mg/kg) was administered to three groups of animals and the remaining two groups of animals received distilled water (control 10 ml/kg) and diclofenac (100 mg/kg) as a standard drug, respectively. After the last measurement of the paw edema, the rats were lightly anesthetized with ether and the blood was collected from the orbital sinus in heparinized tubes. The blood was centrifuged at 3,000 rpm for 15 min and the plasma was aliquoted and stored at −20 °C until use for estimation of proinflammatory cytokines' activities. After blood collection, the rats were sacrificed by an ether overdose, and then the inflamed hind paws were excised and stored at −80 until use for estimation of lipid peroxidation

and antioxidant activities as well as tissue proinflammatory cytokines.

Plasma and paw tissues, PGE_2 and TNF-α as well as plasma IL-1β and IL-6 were measured using kits from Assay Designs Inc. (Ann Arbor, MI, USA) using The Assay Max Mouse PGE_2, TNF-α, IL-1β and IL-6 ELISA kit according to the manufacturer's instructions through a quantitative sandwich enzyme immunoassay technique. Briefly, The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for each cytokine. The samples were pipetted into the wells of this plate. All unbound material was then washed away. The target cytokine in standards and samples was caught by the immobilized monoclonal antibody in the plate and a secondary biotinylated polyclonal antibody, which is recognized by a streptavidinantibody, which is recognized by a streptavidin-peroxidasubstrate was added. Subsequently, the developed color was stopped, and the plates were read at 490 nm using an ELISA reader. Malonaldhyde (MDA) and superoxide dismutase (SOD) were measured in paw tissue exudates using kits from Biodiagnostics Co. (Cairo, Egypt) according to Mihara and Uchiyama (1978) and Nishikimi et al. (1972), respectively.

Statistical analysis

All data were expressed as mean ± SE and statistically analyzed using SPSS (Statical Pucteage for Social Science) version 16.0 for Windows (SPSS Inc, Chicago, IL). Statistical significance of differences among different study groups was evaluated by one-way analysis of variance (ANOVA) ($P \leq 0.05$). Duncan's multiple range test was used to differentiate between means (to determine differences between means of treatments at significance rates of 0.05).

Results

Phytochemical investigation

Phytochemical investigation of the aqueous methanolic extract of *S. nux vomica* leaves using column fractionation on cellulose, polyamide, Sephadex LH20, silica and paper chromatography resulted in the isolation of 5 compounds (Fig. 1): Kaempferol-7-glucoside **1**, 7 Hydroxy coumarin **2**, Quercetin-3-rhamnoside **3**, Kaempferol 3-rutinoside **4**, and Rutin **5**. The structures of these compounds were unambiguously determined by their chromatographic behaviors as well as spectroscopic analysis via UV and ^{1}H-NMR.

*Compound **1**: Kaempferol-7-O-β-D-glucopyranoside*

UV/Vis (λmax, MeOH, nm): 267, 320sh, 360. ^{1}H-NMR (CD_3OD) δ: 5.19 (d, $J = 6.8$ Hz, H-1″), 6.26 (d, $J = 1.8$ Hz, H-6), 6.46 (d, $J = 1.8$ Hz, H-8), 6.90 (d, $J = 8.4$ Hz, H-3′, 5′), 8.12 (d, $J = 8.4$ Hz, H-2′, 6′) (Xu et al. 2005).

*Compound **2**: Umbelliferone; 7-Hydroxy coumarin*

UV/Vis (λmax, MeOH, nm): 216, 245, 258, 279, 322; ^{1}H-NMR (CD_3COCD_3 and D_2O) δ: 6.06 (d, $J = 9.5$ Hz, H-3), 6.72 (d, $J = 2.3$ Hz, H-8), 6.81 (dd, $J = 8.4$, 2.3 Hz, H-6), 7.63 (d, $J = 8.4$ Hz, H-5), 7.75 (d, $J = 9.5$ Hz, H-4) (Kim et al. 2006).

*Compound **3**: Quercetin 3-O-$^1C_4$-L-rhamnoside; Quercitrin*

UV/Vis (λmax, MeOH, nm) 259, 297sh, 348. ^{1}H-NMR (DMSO-*d*6) 1.01 (d, $J = 6.1$ Hz, CH_3), 5.03 (d, $J = 1.5$ Hz, H-1″), 6.18 (d, $J = 2.2$ Hz, H-6), 6.43 (d, $J = 2.2$ Hz, H-8), 6.88 (d, $J = 8.2$ Hz, H-5′), 7.58 (dd, $J = 2.2$ Hz and $J = 8.2$ Hz, H-6′), 7.67 (d, $J = 2.2$ Hz, H-2′) (Eldahshan 2011).

*Compound **4**: Kaempferol-3-O-α-L-rhamnopyranosyl-(1‴ → 6″)-β-D-glucopyranoside*

UV/Vis (λmax, MeOH, nm):266, 350 nm. ^{1}H NMR (CD_3OD) δ: 6.17 (d, $J = 1.9$ Hz, H-6), 6.36 (1H, d, $J = 1.9$ Hz, H-8), 6.86 (d, $J = 8.5$ Hz, H-3′, H-5′), 8.03 (2H, d, $J = 8.5$ Hz, H-2′, H-6′), 5.08 (d, $J = 7.2$ Hz, H-1″); 1.11 (d, $J = 6.2$ Hz, CH_3 rhamnose), 4.50 (d, $J = 1.5$ Hz, H-1‴) (Kazuma et al. 2003).

*Compound **5**: Quercetin-3-O-α-L-rhamnopyranosyl-(1‴ → 6″)-β-D-glucopyranoside; Quercetin 3-O-rutinoside; Rutin*

UV/Vis (λmax, MeOH, nm): 256, 266, 300 sh, 360. ^{1}H-NMR (DMSO-*d*6) 6.16 (d, $J = 2$ Hz, H-6), 6.35

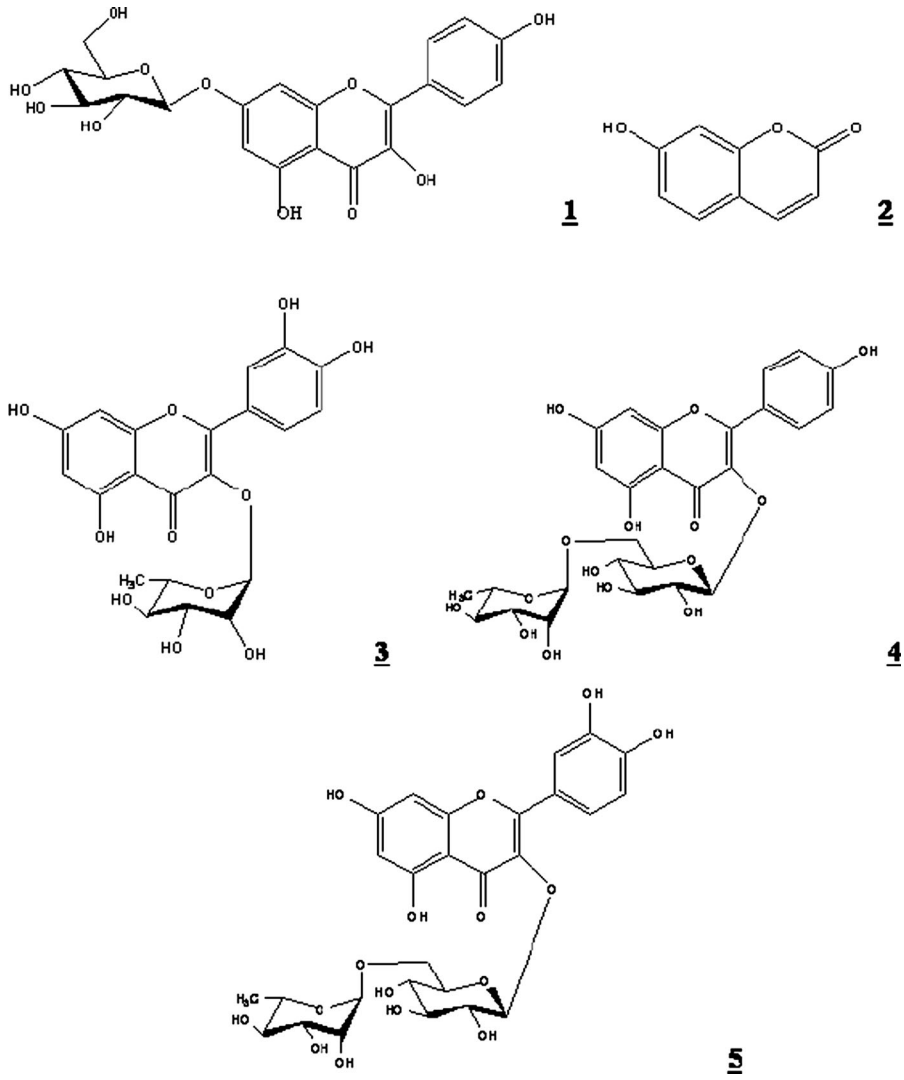

Fig. 1 Chemical compounds isolated from the aqueous methanol leaf extract of *S. nux vomica*: **1** Kaempferol-7-*O*-*β*-D-glucopyranoside; **2** Umbelliferone; **3** Quercitrin; **4** Kaempferol-3-*O*-rutinoside and **5** Rutin

(d, $J = 2$ Hz, H-8), 7.50 (d, $J = 2.1$ Hz, H-2′), 6.81 (d $J = 8$ Hz, H-5′), 7.52 (dd, $J = 8$, 2.1 Hz, H-6′), 4.37 (d, $J = 1.9$ Hz, H-1‴), 5.30 (d, $J = 6.9$ Hz, H-1″), 0.96 (d, $J = 6.3$ Hz, CH_3) (Emam et al. 2010).

Cytotoxic activity

In vitro cytotoxic assay of the aqueous methanol leaves extract of *S. nux vomica* showed potential cytotoxic activity against human epidermoid larynx carcinoma cells, with IC_{50} value 17.8 µg/mL. It also exhibited promising cytotoxic activity against breast carcinoma cell line (MCF-7) with IC_{50} 36.3 µg/mL. Colon carcinoma cells were the least one affected by the extract as its IC_{50} was 41.2 µg/mL (Fig. 2).

In vivo animal study

Acute toxicity studies

The oral acute toxicity test showed no lethality or signs of toxicity for *S. nux vomica* leaves extract up to a dose level of 2,000 mg/kg bw either in rats or mice, and were considered as safe. Based on the results obtained from this study, the doses of 100, 200 and 400 mg/kg bw of the extract were selected for further in vivo

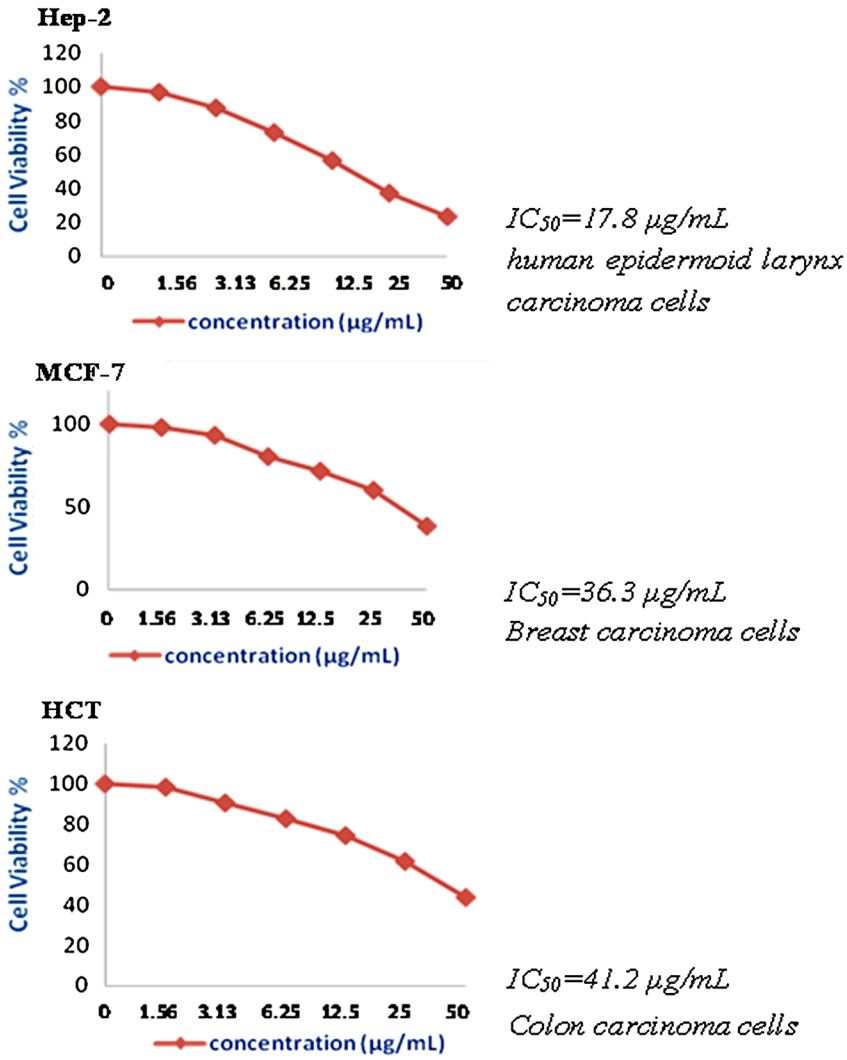

Fig. 2 Cytotoxic activity of the aqueous methanol extract of *S. nux vomica*

Table 1 Effects of the *S. nux vomica* extracts on acetic acid-induced writhing in mice

Treatment	Dose (mg/kg)	No of writhes (15 min)	Inhibition (%)
Control	….	$33.25^{a} \pm 1.82$	…….
S. nux vomica	100	$22.63^{b} \pm 1.29$	31.95
	200	$14.88^{c} \pm 1.49$	55.26
	400	$11.63^{cd} \pm 0.99$	65.04
Diclofenac sodium	100	$10.88^{d} \pm 0.91$	67.29

Results expressed as mean ± SEM

Within the same column, different letters means statistical significance at ($P < 0.05$)

evaluation of analgesic, antipyretic and anti-inflammatory activity.

Analgesic activities

In the mouse writhing assay, the aqueous methanolic extract of *S. nux vomica* caused a significant ($P < 0.05$ %) dose depended inhibition of writhing behavior, and the inhibition percentages were 31.95, 55.26, 65.04 for 100, 200 and 400 mg/kg, respectively (Table 1). The inhibition caused by the highest dose (400 mg/kg bw) was comparable to that of the diclofenac (100 mg/kg bw).

Table 2 Effects of the *S. nux vomica* extracts on rats tail-immersion test

Treatment	Dose (mg/kg)	Post treatment reaction time (s)					
		0	60	120	180	240	300
Control		1.97 ± 0.06	1.86^{a} ± 0.15	2.1^{a} ± 0.19	2.05^{a} ± 0.25	1.74^{a} ± 0.19	1.86^{a} ± 0.15
S. Nux vomica	100	1.85 ± 0.15	3.38^{b} ± 0.30	4.13^{b} ± 0.34	4.50^{b} ± 0.44	3.75^{b} ± 0.29	3.28^{b} ± 0.21
	200	2.11 ± 0.15	5.38^{c} ± 0.32	5.56^{c} ± 0.41	5.10^{bc} ± 0.47	4.94^{c} ± 0.32	3.75^{b} ± 0.40
	400	2.13 ± 0.26	7.10^{d} ± 0.60	6.51^{c} ± 0.62	6.03^{c} ± 0.40	5.70^{cd} ± 0.38	3.88^{b} ± 0.34
Diclofenac sodium	100	1.88 ± 0.26	8.34^{e} ± 0.72	9.04^{d} ± 0.87	7.75^{d} ± 0.94	6.63^{d} ± 0.72	3.99^{b} ± 0.36

Results expressed as mean ± SEM

Within the same column, different letters means statistical significance at ($P < 0.05$)

Table 3 Effects of the *S. nux vomica* extracts on mice hot plate test

Treatment	Dose (mg/kg)	Post treatment reaction time (s)					
		0	60	120	180	240	300
Control		1.61 ± 0.08	1.65^{a} ± 0.08	1.61^{a} ± 0.11	1.60^{a} ± 0.08	1.59^{a} ± 0.11	1.54^{a} ± 0.08
S. Nux vomica	100	1.59 ± 0.09	4.90^{b} ± 0.62	5.88^{b} ± 0.55	4.68^{b} ± 0.53	4.55^{b} ± 0.39	3.41^{b} ± 0.31
	200	1.61 ± 0.11	5.98^{bc} ± 0.38	6.83^{bc} ± 0.43	6.65^{bc} ± 0.32	5.49^{bc} ± 0.35	3.94^{bc} ± 0.23
	400	1.64 ± 0.10	6.64^{c} ± 0.48	7.46^{cd} ± 0.72	6.11^{c} ± 0.63	6.98^{c} ± 0.38	4.21^{bc} ± 0.28
Diclofenac sodium	100	6.63 ± 0.08	9.19^{d} ± 0.55	8.83^{d} ± 0.76	7.7^{c} ± 1.02	7.59^{d} ± 0.81	4.05^{c} ± 0.35

Results expressed as mean ± SEM

Within the same column, different letters means statistical significance at ($P < 0.05$)

Table 4 Effects of the *S. nux vomica* extracts on brewer's yeast-induced pyrexia in rats

Treatment	Dose (mg/kg)	Rectal temperature (°C)					
		0	60	120	180	240	300
Control		38.79 ± 0.22	38.84^{a} ± 0.18	38.74^{a} ± 0.18	38.86^{a} ± 0.18	38.61$_{a}$ ± 0.17	38.56^{a} ± 0.13
S. Nux vomica	100	38.79 ± 0.09	38.12^{b} ± 0.06	37.98^{b} ± 0.07	37.71^{b} ± 0.05	37.67$_{b}$ ± 0.08	37.33^{b} ± 0.11
	200	38.94 ± 0.10	38.06^{b} ± 0.09	37.71^{bc} ± 0.11	37.40^{c} ± 0.10	37.2$_{c}$ ± 0.08	37.10^{bc} ± 0.10
	400	38.95 ± 0.10	37.98^{b} ± 0.11	37.68^{c} ± 0.08	37.39^{c} ± 0.08	37.16$_{c}$ ± 0.07	36.98^{c} ± 0.06
Paracetamol	150	38.83 ± 0.17	37.96^{b} ± 0.19	37.39^{d} ± 0.08	37.21^{c} ± 0.10	37.15$_{c}$ ± 0.08	36.9^{c} ± 0.06

Results expressed as mean ± SEM

Within the same column, different letters means statistical significance at ($P < 0.05$)

The effects the extract on rat tail immersion test are shown in Table 2. The extract induced a dose depended inhibition of pain all over the experimental period of 6 h with maximal tail withdrawal times at 180 min for 100 mg/kg bw, 120 min for 200 mg/kg bw and 60 min for the extract at the dose of 400 mg/kg bw.

As shown in Table 3 using mice hot plate test, the extract induced a dose-dependent increase in reaction time with maximal effect at 120 min.

Antipyretic activities

The effect of the aqueous methanolic extract of *S. nux vomica* on experimental rats using yeast induced pyrexia is given in Table 4. Subcutaneous yeast injection markedly increased rectal temperature 17 h after administration, and the hyperthermia was recorded and continued throughout the experiment. It was observed that the plant extract produced a

Table 5 Effects of the *S. nux vomica* extracts on carrageenan-induced rat paw oedema

Treatment	Dose (mg/kg)	Post treatment paw thickness (cm)					
		0	60	120	180	240	300
Control		0.88 ± 0.04	0.83^{a} ± 0.02	0.89^{a} ± 0.02	0.83^{a} ± 0.03	0.90^{a} ± 0.04	0.89^{a} ± 0.04
S. Nux vomica	100	0.91 ± 0.04	0.77^{b} ± 0.02	0.73^{b} ± 0.03	$0.68^{b,c}$ ± 0.02	0.66^{b} ± 0.02	0.62^{b} ± 0.02
	200	0.94 ± 0.05	0.77^{b} ± 0.02	0.71^{b} ± 0.02	$0.65^{b,c}$ ± 0.02	$0.62^{b,c}$ ± 0.02	0.58^{b} ± 0.01
	400	0.95 ± 0.05	0.76^{b} ± 0.02	0.68^{b} ± 0.02	$0.61^{c,d}$ ± 0.01	0.59^{d} ± 0.01	$0.56^{b,c}$ ± 0.02
Diclofenac sodium	100	0.92 ± 0.06	0.68^{c} ± 0.02	0.61^{c} ± 0.01	0.59^{d} ± 0.01	0.56^{d} ± 0.02	0.51^{c} ± 0.02

Results expressed as mean ± SEM

Within the same column, different letters means statistical significance at ($P < 0.05$)

Table 6 Effect of *S. nux vomica* extracts on paw and/or plasma cytokines, Malonaldhyde and superoxide dismutase in rats with carrageenan-induced paw oedema

Treatment	Carrageenan +ve	Carrageenan inflamed *S. Nux vomica*-treated			Diclofenac sodium
Dose (mg/kg)		100	200	400	100
Paw tissue exudates					
PGE_2 (pg/ml)	814.88^{a} ± 18.27	453.25^{b} ± 21.10	374.00^{c} ± 18.80	316.50^{d} ± 18.71	312.25^{d} ± 10.21
TNF-α (pg/ml)	236.00^{a} ± 5.75	223.13^{a} ± 4.32	178.25^{b} ± 6.46	149.38^{c} ± 4.61	143.25^{c} ± 5.36
MDA (mM/100 mg)	14.25^{a} ± 0.61	10.13^{b} ± 0.63	6.75^{c} ± 0.42	5.63^{c} ± 0.48	5.50^{c} ± 0.49
SOD (mM/100 mg)	5.81^{a} ± 0.22	$6.44^{a,b}$ ± 0.25	$6.61^{a,b,c}$ ± 0.51	$7.01^{b,c}$ ± 0.38	7.53^{c} ± 0.40
Plasma					
PGE2 (pg/ml)	512.75^{a} ± 6.62	278.25^{b} ± 4.95	229.63^{c} ± 3.60	204.00^{d} ± 5.51	201.13^{d} ± 2.55
TNF-α (pg/ml)	370.63^{a} ± 4.35	259.25^{b} ± 9.41	217.88^{c} ± 7.27	163.25^{d} ± 3.91	148.25^{d} ± 3.24
IL-1β (pg/ml)	46.63^{a} ± 1.65	37.50^{b} ± 1.23	31.75^{c} ± 1.04	27.38^{d} ± 0.57	23.25^{e} ± 1.29
IL-6 (pg/ml)	397.38^{a} ± 4.02	269.25^{b} ± 3.01	252.88^{b} ± 3.63	181.38^{c} ± 12.58	145.25^{d} ± 12.10

Results expressed as mean ± SEM

Within the same column, different letters means statistical significance at ($P < 0.05$)

dose-dependent antipyretic effect started as early as 1 h and maintained up to 5 h after the extract administration. The inhibitory effect of 400 mg/kg bw was comparable to that of the reference drug.

Anti-inflammatory activities

The anti-inflammatory activities of *S. nux vomica* leaves extract were evaluated on Carrageenan-induced rat paw edema using experimental rats. The rat paw edema was markedly inhibited by oral pre-treatment with the extract (100, 200 and 400 mg/kg bw) and diclofenac sodium (100 mg/kg bw) with maximal inhibitory effect at 60 min post administration (Table 5). In the acute inflammation model, a dose of 400 mg/kg bw of the extract showed significant inhibition ($P < 0.05$) at 1 h up to the end of the experimental observation, and it was comparable to that of the standard.

In order to deeply understand the mechanism by which the extract exert its anti-inflammatory activities, paw tissue exudates and plasma level of PGE_2 and TNF-α were measured. Carrageenan injection into rat paws induced high PGE_2 and TNF-α level in both paw tissue exudates as well as plasma of rats. *S. nux vomica* extract induced a dose-dependent decrease on these parameters compared to carrageenan induced inflamed non-treated group. Similar results were found for IL-1β and IL-6 measured in rats' plasma as well as MDA in inflamed rat's paw. The results of SOD measured in inflamed rats' paw were recorded. *S nux vomica* extract induced a significant ($P < 0.05$) dose dependent

increase of SOD compared to the inflamed-non treated group (Table 6).

Discussion

Herbal plants and plant-derived drugs have been widely used in traditional cultures everywhere in the world and have gained popularity in modern society as natural alternatives to produce new potential therapeutic agents for combating diseases (Abdel-Daim et al. 2013; Azab et al. 2013; Madkour and Abdel-Daim 2013). For example, 60 % of the current available anticancer agents derived from natural products. Comprehensive data in the literature show that polyphenols present antioxidant, anticancer and anti-inflammatory activities as their major biological characteristics.

Phytochemical investigation of *S. nux vomica* leaves extract led to the isolation and characterization of five compounds; Kaempferol-7-glucoside **1**, 7 Hydroxy coumarin **2**, Quercetin-3-rhamnoside **3**, Kaempferol 3-rutinoside **4**, and Rutin **5**. The current study indicates that these phenolic compounds are promising molecules with useful cytotoxic, analgesic, antipyretic and anti-inflammatory activity profiles. A potent cytotoxic activity was detected against human epidermoid larynx carcinoma cells, with IC_{50} value 17.8 µg/mL, followed by breast carcinoma cell line (MCF-7) and then colon carcinoma cell line, which was the cell line least affected by the extract. The cytotoxics activities could be due to the presence of apoptotic and anti-proliferative flavonoids and other phenolics (Xu et al. 2008; Li et al. 2007).

In the present study, the leaf extract of *S. nux vomica* expressed potent analgesic activities using mice acetic acid induced writhing test and hot plate test as well as rat tail immersion test. In addition, the extract revealed strong antipyretic as well as anti-inflammatory effects. This study has shown that the *S. nux vomica* leaves extract has a potent antinociceptive effect and thus indicates the presence of analgesic components that might influence the prostaglandin pathways. A significant decrease in acetic acid-induced writhes as well as a significant increase in the reaction time for tail immersion and hot plate tests indicated nociception and pain perception inhibition as well as peripheral and central analgesic mechanism of action.

Several flavonoids isolated from medicinal plants have been discovered to possess significant analgesic and anti-inflammatory effects (Ofuegbe et al. 2013). The analgesic and anti-inflammatory activity of the methanol extract of the leaves of *Phyllanthus amarus* may be due to the presence of flavonoids and other phenols. Kaempferol-7 glucoside and rutin are phenolic compounds isolated and characterized from the extract are known for their ability to have antioxidant anti-inflammatory and anticancer activities (Liu and Chou 2007; Karimi et al. 2012).

The carrageenan-induced rat paw edema is a common model to study inflammation and examine anti-inflammatory agents (Nantel et al. 1999). The swelling or edema, one of the cardinal signs of acute inflammation, is an important parameter to be considered when testing compounds with a potential anti-inflammatory activity (Morris 2003). In this model, edema is a biphasic process. The release of serotonin or histamine occurs in the 1st phase, and the 2nd phase is associated with the production of lysosome, protease, bradykinin (BK) and prostaglandin (Ouachrif et al. 2012). The role of PGE_2 in the carrageenan-induced edema has been well documented elsewhere (Nantel et al. 1999; Zanin and Ferreira 1978; Mnich et al. 1995; Portanova et al. 1996). PGE_2 and BK are responsible for the edema formation and also for the pain that accompanies the inflammatory reaction (both PGE_2 and BK and are able to sensitize primary afferent neurons) (Williams and Peck 1977; Calixto et al. 2003). Therefore, the effect of *S nux vomica* leaves extract in inhibiting edema could be attributed to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of PGE_2 synthesis.

The aqueous methanolic extract was found to possess a prominent anti-inflammatory activity, showing inhibition to the paw edema induced by carrageenan all over the experimental period. The effectiveness of these extracts at 1 and 3 h in carrageenan induced paw edema indicates their antagonist effect at the levels of serotonin, histamine, bradykinin and prostaglandin. Because the release of serotonin and histamine occurs 1 h after carrageenan whereas BK and PGE_2 are released 2 and 3 h, respectively, after carrageenan injection (Di Rosa and Willoughby 1971). The extract is found to be comparable to diclofenac sodium specially when used at higher doses. As the carrageenan-induced inflammation model is a significant predictive test for anti-

inflammatory agents acting by inhibiting the mediators of acute inflammation, these results are an indication that *S. nux vomica* leaves extract could be an effective treatment for acute inflammatory disorders. Since inflammation is also associated with pain, the majority of anti-inflammatory drugs have analgesic activity. The peripheral analgesic effect of drugs may be mediated through inhibition of cyclo-oxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action may be mediated through inhibition of central pain receptors.

It was proven that, flavonoids isolated from some medicinal plants have an antinociceptive and/or anti-inflammatory effect (Eldahshan and Azab 2012). Flavonoids inhibit the biosynthesis of prostaglandins, which are involved in various immunologic responses and are the end products of the cyclooxygenase and lipoxygenase pathways (Moroney et al. 1988; Di Rosa and Willoughby 1971). Protein kinases are another class of regulatory enzymes affected by flavonoids. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory processes (Manthey et al. 2001).

Fever is defined as an elevation in body temperature characteristically exhibited by most species in response to an invasion of infectious agents. When a pyrogenic agent, such as LPS or Brewer s yeast, enters the body through a break in its natural barriers, it will interact with immune cells, and promote the synthesis and release of endogenous mediators, such as PGE_2 and cytokines (e.g. TNF-α, IL-1β, IL-6) (Kluger 1991; Romanovsky et al. 2005; Rudaya et al. 2005). In the current study administration of *S. nux vomica* leaves extract induced a significant dose-dependent reduction of body temperature of brewer's yeast-induced fever in rats.

Cytokines are regulatory proteins that are not constitutively produced under normal physiological conditions. However, inflammatory stimuli induce gene expression of cytokines, initiating the inflammatory response (Santos et al. 2003). Tumor necrosis factor alfa (TNF-α) is a major cytokine involved in the initiation of the inflammatory response. Its actions include the induction of other cytokines such as interleukin 1 (IL-1β) and interleukin 6 (IL-6), priming of PMN, up-regulation of adhesion molecules and activation of arachidonic acid metabolism (Santos et al. 2003). Arachidonic acid metabolites include prostaglandins and thromboxanes (via cyclooxygenases, COX) and leukotrienes (via lipoxygenase). PGE_2 derived from COX metabolic pathway, is able to promote changes in vascular tonus and blood flow leading to edema as a cardinal sign of inflammation. Cox_2 and PGE_2 are not only inflammatory mediators, but also they act as biomarkers in different cancer cell types (Abdel-Daim et al. 2010a, b; Funasaka et al. 2012; Elzagheid et al. 2013; Karavitis et al. 2012; Perez-Ruiz et al. 2012). So, it is important to examine the cytotoxic activities of the extract beside its anti-inflammatory activity. Many previous researchers have also revealed that carrageenan injection resulted in lipid peroxidation and oxidative stress in the inflamed paw, which could be inhibited by many tested anti-inflammatory compounds (Liao et al. 2013; Khayyal et al. 2009).

To examine the exact mechanism of action of the extract as anti-inflammatory agent, PGE_2 and TNF-α were further analyzed in the rat plasma and the inflammatory exudates of the paws in the same model. Our results indicate that *S. nux vomica* leaves extract caused statistically significant reduction of plasma and inflammatory exudates PGE_2 and TNF-α content. This finding clearly underlines the anti-inflammatory effect of the extract. TNF-α is a pleiotropic cytokine which plays a critical role in both chronic and acute inflammation (Holtmann et al. 2002). In addition, TNF-α promotes the synthesis of PGE_2 (Arai et al. 1990). The reduction of TNF-α release by the extract was parallel with the PGE_2 decrease observed in the current study. In addition to evaluating PGE_2 and TNF-α level in our experiments, paw MDA and SOD as well as plasma interleukin 1 (IL-1β) and interleukin 6 (IL-6) were analyzed. Our results indicated that the *S nux vomica* leaves extract induced a dose-dependent reduction in paw MDA and SOD as well as plasma IL-1β and IL-6 indicating the antioxidant mechanism of action besides downregulation of PGE_2 and cytokines' production.

Flavonoids are believed to act as health-promoting agents, through their antioxidants, cytotoxic, analgesic, antipyretic and anti-inflammatory properties (Middleton et al. 2000; Havsteen 2002).

Conclusion

The current study confirms that *S nux vomica* leaves extract has a great value as a source of compounds for pharmaceutical applications. Phytochemical

investigation of the extract led to the isolation and characterization of five phenolic compounds. A potent cytotoxic activity was detected against human epidermoid larynx carcinoma, breast carcinoma (MCF-7) and colon carcinoma cell lines. Moreover, the leaves extract presented antinociceptive, antipyretic and anti-inflammatory activities in animal models of writhing test, tail immersion test, hot plate test as well as Brewer's yeast-induced fever and carrageenan-induced inflammation, respectively. These activities may be due to the inhibitory action of the extract on the synthesis and/or release of inflammatory mediators involved in these responses, such as PGE_2, TNF-α. Additional experiments are necessary in order to confirm these hypotheses and to clarify the true target for the anti-inflammatory and analgesic effects of *S nux vomica* extract.

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Conflict of interest Both authors declare that there is no conflict of interest relevant to this study.

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