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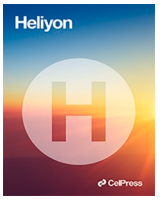

Research article

Muscle relaxant and antipyretic effects of pentacyclic triterpenes isolated from the roots of *Diospyros lotus* L

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A R T I C L E I N F O

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A B S T R A C T

The present article describes the muscle relaxant and antipyretic effects of pentacyclic triterpenes, oleanolic acid (**OA**), ursolic acid (**UA**) and betulinic acid (**BA**) isolated from roots of *Diospyros lotus* in animal models. The muscle relaxant effects of isolated pentacyclic triterpenes were determined by chimney and inclined plane tests. In the chimney test, pretreatment of pentacyclic triterpenes evoked significant dose dependent influence on muscle coordination. When administered intraperitoneally (i.p.) to mice at 10 mg/kg for 90 min, **OA**, **UA**, and **BA** exhibited muscle relaxant effects of 66.72 %, 60.21 %, and 50.77 %, respectively. Similarly, **OA**, **UA,** and **BA** (at 10 mg/kg) illustrated 65.74 %, 59.84 % and 51.40 % muscle relaxant effects in the inclined plane test. In the antipyretic test, significant amelioration was caused by pretreatment of all compounds in dose dependent manner. **OA**, **UA,** and **BA** (at 5 mg/kg) showed 39.32 %, 34.32 % and 29.99 % anti-hyperthermic effects, respectively 4 h post-treatment, while at 10 mg/kg, **OA**, **UA,** and **BA** exhibited 71.59 %, 60.99 % and 52.44 % impact, respectively. The muscle relaxant effect of benzodiazepines is well known for enhancement of GABA receptors. There may exist a similar mechanism for muscle relaxant effect of pentacyclic triterpenes. The *in-silico* predicted binding pattern of all the compounds reflects good affinity of compounds with $GABA_A$ receptor and COX-2. These results indicate that the muscle relaxant and antipyretic activities of these molecules can be further improved by structural optimization.

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1. Introduction

Diospyros (Ebenaceae) is a genus, which consists of trees and woody shrubs. Several studies reported the presence of lupine triterpenes, naphthoquinones, dimeric naphthoquinones, and naphthalene derivatives in various species of *Diospyros*. Around 500 species are known worldwide for this genus, out of which twenty-four exists within India [1]. Among these species, *Diospyros lotus* L. is found in China and Asia. *D. Lotus* is a deciduous tree, it is cultivated for its fruit which is used as febrifuge, laxative, nutritive, sedative, antiseptic, and astringent. Moreover, it has antidiabetic and antitumor properties. *D. Lotus* fruit is also effective against constipation, diarrhea, coughs and hypertension [2] and it contains some non-volatile acids, phenolic compounds, fatty acids, and sugar molecules [3,4].

Pentacyclic triterpenes are pharmacologically active phytochemicals, widely distributed in many plants used in folk medicine as anti-inflammatory remedies [5]. During last three decades, triterpenes has demonstrated anti-inflammatory, anti-tumor, anti-viral, hepatoprotective, anti-bacterial, and wound healing with low toxicity [6–11].

Pyrexia (fever) is increased body temperature above normal range, caused by physiological stress, amplified thyroid secretion, lesions in central nervous system (CNS), viral or bacterial infection and leukemia [12–15]. In human physiological system, natural defense mechanism is activated during any infection to initiate an inhospitable environment for the infectious agent [14,16]. The damaged tissues and infectious agent in the body then stimulate the production of proinflammatory cytokines, including interleukin 1α, interleukin 1β, and tumor necrosis factor alpha (TNF-α). These cytokines, in turn, promote the production of prostaglandin E2 (PGE2) near the hypothalamus, leading to an increase in body temperature [17]. The body temperature is controlled by the nervous system's feedback mechanism. When the body temperature rises, blood vessels dilate, and sweating increases to cool the body down. Conversely, vasoconstriction occurs when the body temperature drops to help maintain the internal temperature [18].

Increased body temperature further progress the disease status by increasing tissue catabolism, and dehydration [12,19]. Antipyretic drugs inhibit the expression of cyclooxygenase 2 (COX-2) enzyme, thus the synthesis of prostaglandin is stopped, as a result the elevated body temperature is lowered [20–23]. Although synthetic antipyretic drugs selectively inhibit COX-2, however, they have also shown toxic effects on several organs (glomeruli, brain cortex, heart, and hepatic cells). On the other hand, COX-2 inhibitors from natural sources have fewer side effects but they also have lower selectivity [24,25]. The objective of this study was to examine the muscle relaxant properties and fever-reducing potential of three pentacyclic triterpenes (**OA**, **UA**, and **BA**) isolated from *D. lotus* using animal models. The chemical structures of **OA**, **UA**, and **BA** are shown in Fig. 1. Additionally, their interaction with potential targets was analyzed using *in-silico* docking methods.

2. Materials and methods

2.1. Plant collection

The plant *D. lotus* was gathered from Toormang Razagram, Dir, KPK, Pakistan, in May 2009, and authenticated by Dr. Abdur Rashid, a Taxonomist in the Department of Botany at the University of Peshawar. The roots of *D. lotus* were utilized for isolating compounds. The voucher (Bot.20036 (PUP)) was placed in the Herbarium of the Department of Botany, University of Peshawar.

2.2. Extraction and isolation of pentacyclic triterpenes

The pentacyclic triterpenes were extracted and isolated as follows: *D. lotus* roots (14 kg) were dried in the shade and ground into a powder. The powder was then soaked in methanol at room temperature for 150 h with continuous stirring. The methanolic extract was concentrated using a rotary evaporator, resulting in a dark-red residue (202 g). This residue was suspended in water and sequentially partitioned with *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), and n-butanol (*n*-BuOH). The chloroform fraction (30 g) underwent column chromatography on silica gel, eluted with a gradient of *n*-hexane/EtOAc (100:0 to 0:100). Thin-layer chromatography (TLC) was used, and 105 sub-fractions were collected. Elution with *n*-hexane/EtOAc (100:0 to 10:90) resulted in the separation of fatty acid oil (reddish in color) followed by colorless needles (1.25 g), identified as **OA** after further purification [26,27]. The fraction eluted at 20 % EtOAc/*n*-hexane was subjected to silica gel column chromatography, yielding white crystalline solid of **UA** (100:0 to 10:90) [26,28]. **BA** was isolated from the chloroform fraction using the solvent system *n*-hexane/EtOAc (100:0 to 16:84) as white crystals, with its physical and spectral data matching previously reported data for **BA** [28].

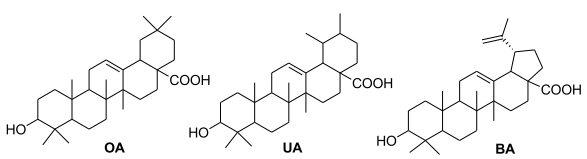

Fig. 1. Chemical structure of compounds isolated from *D. Lotus*.

2.3. Chemicals and instruments

Melting points were determined using a Bicote (Biby Scientific Limited) apparatus. UV–visible spectra were recorded on a Hitachi-U-3200 spectrometer, and FT-IR spectra were obtained using a Nicolet 380 instrument from Thermo Scientific. UV–visible spectra were also recorded on a Shimadzu spectrometer. NMR spectra (1H at 600 MHz, 13C at 150 MHz) and HMBC spectra were recorded on an Avance AV-600 Cryoprob NMR instrument in CDCl3. EI-MS measurements were conducted on a JEOL JMS 600H mass spectrometer with an EI source set at 70 eV. Diclofenac sodium was purchased from Suzhou Ausun Chemical Co., Ltd., China. Acetic acid and silica gel 60 (0.063–0.200 mm, 5 × 60 cm) were purchased from Merck (Germany). Normal saline was used as a control in all experiments.

2.4. Animals

BALB/c mice of both sexes were obtained from the Department of Pharmacy, University of Peshawar, and housed under standard conditions (25 °C and 12-h light/dark cycles). Animals were provided with standard food and water *ad libitum*. All experimental procedures were conducted following the approved protocols of the ethical committee of the Department of Pharmacy, University of Peshawar.

2.5. Chimney test for motor coordination

A chimney test was performed to evaluate the effects of compounds on motor coordination. A Pyrex glass tube (30 cm in length, 3.0 cm in diameter), marked at 20 cm from the bottom, was used for the test. Animals were divided into separate groups (n = 6) and administered normal saline (10 ml/kg), diazepam (0.5 mg/kg), and the isolated compounds (5 mg/kg and 10 mg/kg) via intraperitoneal injections (i.p.). They were then observed for 30, 60, and 90 min. Each animal was placed into the tube from one end and allowed to climb. When an animal reached the 20 cm mark, the tube was tilted vertically, and the animal attempted to ascend. Failure to reach the marked point within the initial 30 s of the tube being vertical was considered indicative of muscle relaxation [29].

2.6. Inclined plane test for motor coordination

In this study, an inclined plane consisting of two plywood boards joined together at a 65° angle was employed. Animals were segregated into different groups (n = 6) and treated with either normal saline (10 ml/kg), diazepam (0.5 mg/kg), or isolated pentacyclic triterpenes (5 mg/kg and 10 mg/kg i.p.). Following treatment, all animals were positioned at inclined plane's top for 30 s, and their ability to either cling on or fall off was evaluated at 30, 60, and 90-min intervals [29,30].

2.7. Yeast induced hyperthermia test

Antipyretic impact of **OA**, **UA** and **BA** was examined in yeast induced hyper thermic mice as reported previously [31,32]. The hyperthermia was induced by administration (18 h before experiment) of bravery's yeast. After 18 h the rectal temperature was checked for induction of hyperthermia. Only the animals with confirmed hyperthermia were included in the test study. The samples to be tested were administered to each animal of classified groups and then after 30 min of administration the rectal temperature was noted periodically. After completion of study the percent effect was calculated [33].

2.8. Statistics

The results are expressed as mean ± S.E.M. Statistical analysis involved one-way ANOVA to compare significant differences among groups, followed by Dunnett's multiple comparison posttest. A significance level of $P < 0.05$ was considered statistically significant for all tests.

2.9. Molecular docking

Docking of **OA**, **UA** and **BA** was carried out with COX-2 and $GABA_A$ receptor by MOE (Molecular Operating Environment version 2022.02). Compound's structures were drawn in MOE, and MM94x forcefield was applied to minimize their energies until gradient of 0.05 kcal/mol/Å was obtained. COX-2 complexed with inhibitor SC-58 (PDB code 1CX2) and $GABA_A$ receptor complexed with diazepam, GABA, and antibody Mb38 (PDB code 6HUP) was used in this study. COX-2 active site was defined around His90, Gln192 and Tyr355, while diazepam binding site was selected for docking in $GABA_A$ receptor. After removal of water molecules in protein with the help of protonate-3D, the structures of both proteins were optimized. MOE-Dock in combination of Triangle Matcher and London dG was used. The best conformations of the compounds were examined for their binding interactions.

Before docking our compounds, the docking method (docking algorithm/scoring function) was validated by re-docking of known inhibitors in their respective targets (SC-58 in 1CX2 and diazepam in 6HUP). The known ligands were re-docked with excellent fitting in their cognate binding sites with RMSD ≤0.2 Å in each target, which reflects good docking capability of the selected docking algorithm. The re-docked conformations of known ligands are given in Figs. S1–S2 in supplementary file along with their exact RMSD and docking scores.

2.10. ADMET Prediction of compounds

The physicochemical and ADMET profile compounds were estimated by admetSAR server (http://lmmd.ecust.edu.cn/admetsar2/) by uploading the SMILE format of these molecules. admetSAR predicts Absorption, Distribution, Metabolism, Excretion, and Toxicity of molecules using ~50 ADMET endpoints with QSAR models.

3. Results and discussion

3.1. Isolation of pentacyclic triterpenes (**OA**, **UA**, and **BA**)

OA, **UA**, and **BA** were obtained as white crystals, with respective melting points of 306–310 °C, 282–284 °C, and 295–298 °C, isolated from the chloroform fraction of the plant. The EI MS spectrum of each compound exhibited a molecular ion peak at *m/z* 456 a.

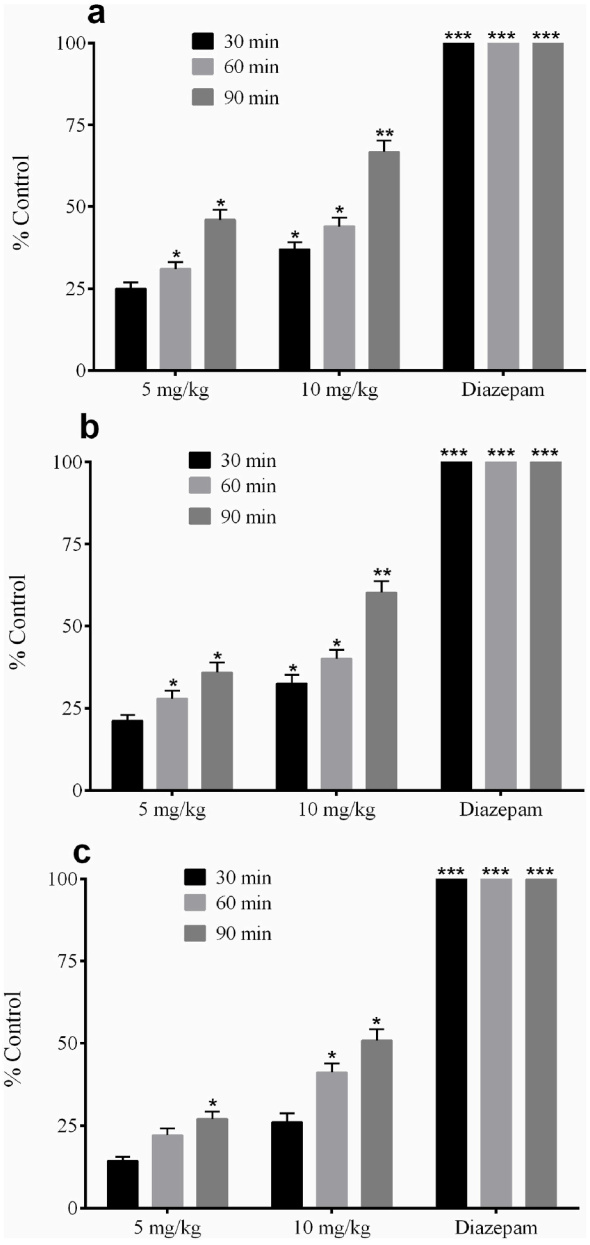

Fig. 2. Percent muscle relaxant effects of (a) **OA**, (b) **UA** (c) **BA** are shown in chimney test after 30, 60 and 90 min. Data represented as mean ± S.E.M (n = 6). *$P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, the results are compared with control.

m.u., consistent with the molecular formula $C_{30}H_{48}O_3$. The IR spectra of **OA** showed absorption bands at 3550 (OH), 2932, 2990 (CH stretching), and 1670 (CO stretching), while UV spectroscopy revealed absorption peaks at 254, 264, and 441 nm. Similarly, the IR spectra of **UA** displayed absorption bands at 3540 (OH), 2930, 2995 (CH stretching), and 1660 (CO stretching), with UV absorption peaks at 244, 264, and 441 nm. The spectral data for **OA** and **UA** closely matched those of oleanolic acid [26,27], as confirmed by HMBC correlations (Figs. S3–S4).

The IR spectrum of **BA** exhibited absorption bands at 3500 (OH), 2933, 2991 (CH stretching), and 1675 (CO stretching), while UV spectroscopy showed absorption peaks at 247 and 261 nm. The spectral data for BA corresponded to the reported structure [26], further supported by HMBC correlations (Fig. S5).

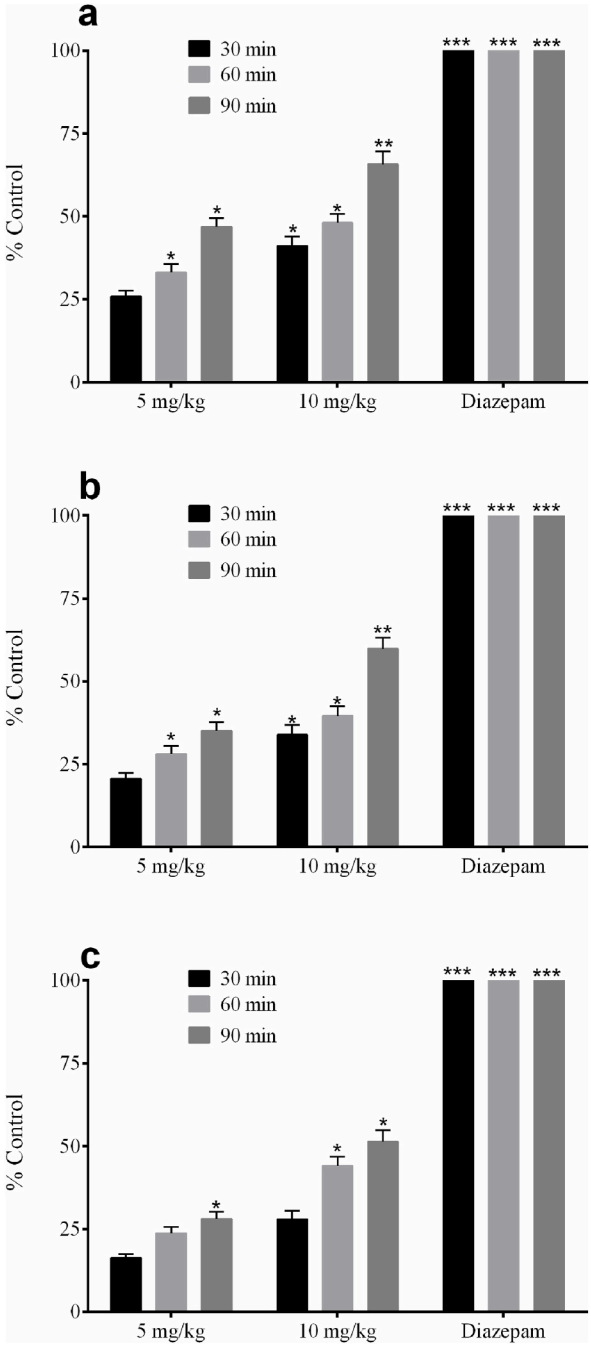

Fig. 3. Percent muscle relaxant effects of (a) **OA** (b) **UA** (c) **BA** are shown in inclined plane test after 30, 60 and 90 min. Data represented as mean ± S.E.M(n = 6)*$P < 0.05$,** $P < 0.01$,*** $P < 0.001$, all compared with control.

3.2. Effect of compounds in chimney test

In-vivo chimney test has been widely used [30,34]. The effect of isolated pentacyclic triterpenes in chimney test is illustrated in Fig. 2. **OA** induced a significant muscle relaxant effect at all assessment times (30, 60, and 90 min). The maximum effect was observed 90 min post-treatment, with values of 46.04 % (at 5 mg/kg) and 66.72 % (10 mg/kg) (Fig. 2a). Post-treatment with **UA** demonstrated a significant dose-dependent muscle relaxant effect. After 90 min, **UA** exhibited 35.88 % (5 mg/kg) and 60.21 % (10 mg/kg) muscle relaxant effects (Fig. 2b). **BA** displayed muscle relaxant effects of 27.00 % (5 mg/kg) and 50.77 % (10 mg/kg) 90 min post-treatment (Fig. 2c). The order of increasing muscle relaxant effect among the isolated pentacyclic triterpenes was **BA** < **UA** < **OA**.

3.3. Effect of isolated pentacyclic triterpenes in inclined plane test

Inclined plane test is another sensitive parameter usually employed to assess the spontaneous loco motor and muscle-coordination activity of compounds [35]. The effects of isolated pentacyclic triterpenes in inclined plane test are shown in Fig. 3. These compounds demonstrated significant muscle relaxant effects at 30, 60, and 90 min in a dose-dependent manner. **OA** (Fig. 3a), **UA** (Fig. 3b), and **BA** (Fig. 3c) showed a maximum muscle relaxant effect of 46.90 %, 35.05 %, 27.90 %, respectively at 5 mg/kg, and 65.74 %, 59.84 %, and 51.40 %, respectively at 10 mg/kg 90 min post-treatment (Fig. 3a–c). The inclined plane test results follow same pattern as observed in chimney test, with the effects decreasing in the order: **BA** < **UA** < **OA**.

3.4. Effect of ***OA, UA,*** and ***BA*** in yeast-induced pyrexia test

In this test, pretreatment with OA, UA, and BA resulted in a dose-dependent improvement in induced pyrexia in hyperthermic mice, as observed at various time intervals (Fig. 4a–c). **OA** (at 5 mg/kg) demonstrated a noticeable impact 3 h post-treatment and a substantial effect at 10 mg/kg (i.p.) first hour post-treatment. The maximum anti-hyperthermic impact of **OA** was 39.32 % (5 mg/kg) and 71.59 % (10 mg/kg) 4 h post-treatment (Fig. 4a). **UA** also reduced induced pyrexia from 1 to 5 h post-treatment. At 5 mg/kg, **UA** showed a noteworthy impact 3 h post-treatment and a significant effect after the first hour of treatment at 10 mg/kg, displaying the highest antipyretic impact of 34.32 % (5 mg/kg) and 60.99 % (10 mg/kg) 4 h post-treatment (Fig. 4b). **BA** at 5 mg/kg (Fig. 4c) exhibited a good effect 4 h post-treatment and a significant effect at 10 mg/kg 3 h post-treatment. The maximum antipyretic effect was 29.99 % [5 mg/kg (i.p.)] and 52.44 % [10 mg/kg (i.p.)] 4 h post-treatment. However, paracetamol (positive control) produced dominant results, as shown in Fig. 4d.

From a clinical perspective, fever and pain are among the most common symptoms of ill health. The hyperthermic effect is orchestrated by CNS through endocrine, neurological, immunological, and behavioral mechanisms [36,37]. Various exogenous and endogenous substances can induce fever. The degree of initiation, expression, and regulation of fever depends on the pyrogenic and anti-pyrogenic effects of these agents. Fever is characterized by a sustained increase in body temperature, surpassing normal fluctuations, and is associated with an elevated thermoregulatory set point [36]. Thermoregulatory neurons monitor changes in blood temperature as well as signals from skin and muscle receptors (cold and warm), maintaining a delicate balance between heat production and loss [32,38]. In the present study, the compounds **OA**, **UA**, and **BA** exhibited significant attenuation of induced pyrexia in hyperthermic mice, with effects that were dose-dependent. Among the compounds tested, **OA** and UA demonstrated pronounced

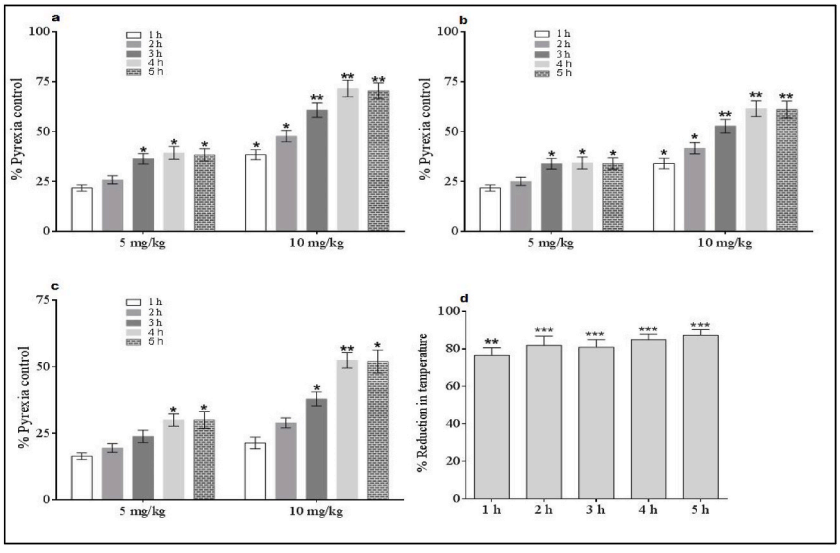

Fig. 4. Antipyretic activity of (a) **OA**, (b) **UA** (c) **BA** and (d) paracetamol is shown. Data represented as mean ± S.E.M(n = 6)*$P < 0.05$,** $P < 0.01$,*** $P < 0.001$, all compared with control.

effects over several hours, with notable effects persisting up to approximately the fifth hour post-administration.

3.5. Molecular docking

The muscle relaxant effect of benzodiazepines (BDZs) like diazepam is primarily attributed to their enhancement of gamma-aminobutyric acid (GABA) action at the GABAA receptor ($GABA_AR$) [39,40]. BDZs bind to the γ subunit of the $GABA_AR$, causing structural changes in the receptor that increase its activity. Unlike GABA, which binds to the α-subunit of $GABA_AR$, BDZs do not directly substitute for GABA. Instead, they increase the frequency of channel opening events, leading to increased chloride ion conductance and inhibition of the activation potential [41]. This mechanism may explain the muscle relaxant effects of **OA**, **UA,** and **BA**. Through docking experiments, the binding modes of **OA**, **UA**, and **BA** were predicted in the $GABA_AR$. **OA** (−6.00 kcal/mol), **UA** (−5.73 kcal/mol), and **BA** (−5.61 kcal/mol) exhibited highly negative docking scores in the GABA receptor.

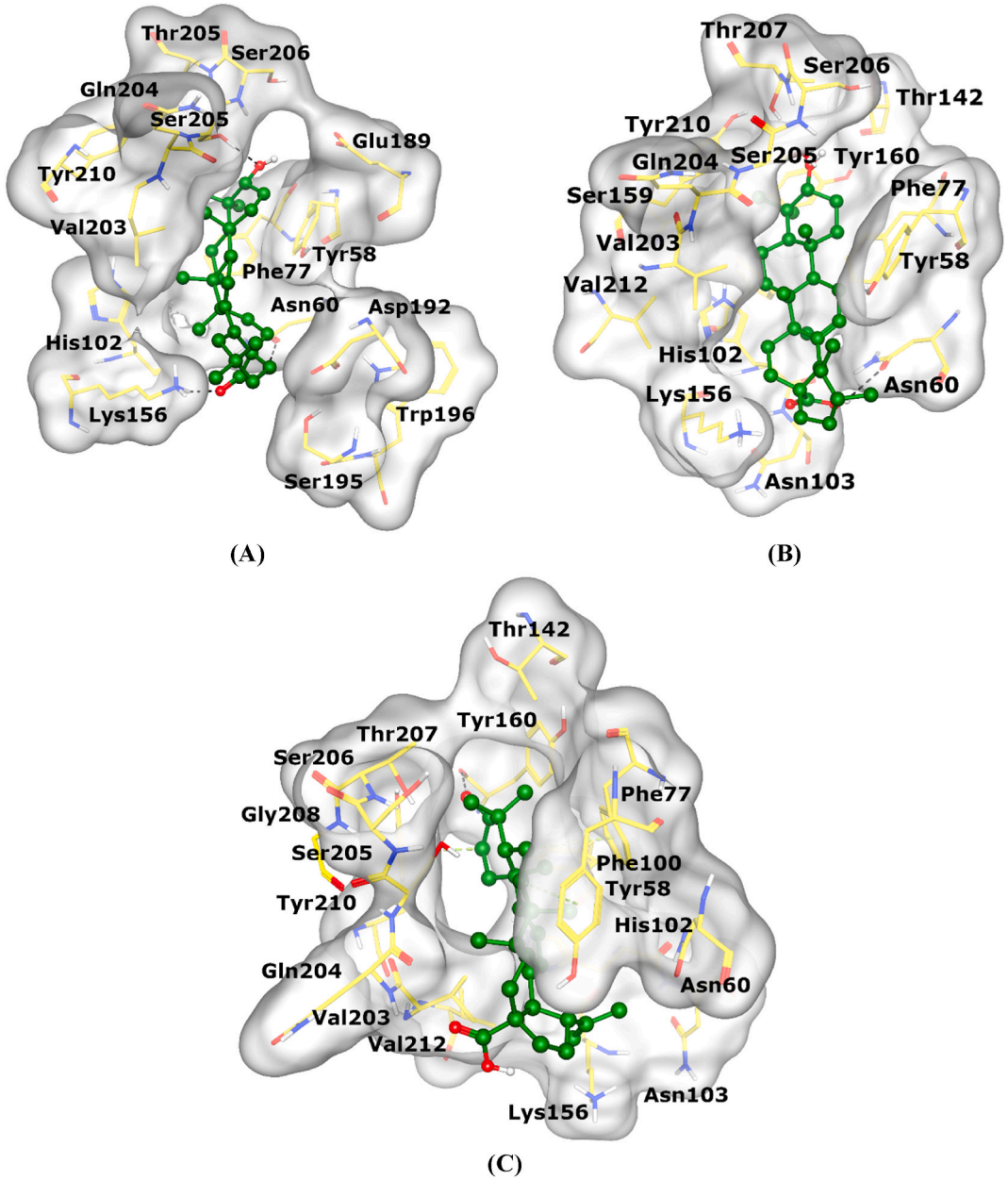

Fig. 5. The docked view of (A) **OA**, (B) **UA**, and (C) **BA** is shown in the ligand binding site of $GABA_A$ receptor. Ligands are presented in green ball and stick model, binding site residues are shown in yellow sticks with surface presentation, and H-bonds are depicted in black dotted lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

OA mediated strong hydrogen bonds (H-bonds) with Asn60, Lys156 and Ser205. **OA**'s hydroxyl group formed H-bond with Ser205 (2.74 Å), and its acetate moiety with Asn60 (3.18 Å) and Lys156 (2.68 Å) (Fig. 5A). While **UA** formed H-bond with Asn60 (2.36 Å) through its acetate moiety (Fig. 5B). In **BA,** the –OH moiety mediates a strong H-bond with Tyr160 (2.72 Å) (Fig. 5C). Acetate moieties of **UA** and **BA** do not interact with the surrounding residues, this may be the reason of their decreased bioactivity, compared to **OA**, which is also indicated by their docking scores. Fig. 5A-C illustrates the docked orientations of compounds in $GABA_AR$.

Insights into COX-2 active site have provided valuable guidance for designing selective inhibitors of this enzyme [38,42]. The COX-2 active site contains an additional pocket of 2 Å which is absent in COX-1, a structural difference crucial for designing selective COX-2 inhibitors. Valine residue at position 523 in COX-2 is replaced by isoleucine in COX-1, therefore, the smaller side chain of Val523 (in COX-2) creates a conformational change in Tyr355, thereby creating this 2 Å pocket in COX-2. Similarly, His513 in COX-1 is replaced with Arg513 in COX-2 significantly impacts network of hydrogen bonds present in the binding site of COX-2. His90, Gln192, and Tyr355 are the residues which regulate the entry of ligands to this 2 Å pocket of COX-2. The time-dependent inhibition of COX-2 requires particular interaction of drug with Arg513 [42–44].

Upon docking of **OA, UA,** and **BA** into COX-2 active site, **OA** (docking score = −14.11 kcal/mol) showed the most significant impact, as evident by pyrexia test. The acetate moiety of **OA** formed H-bonds with Arg120 (2.2 Å) and Tyr355 (2.7 Å, 1.7 Å) (Fig. 6**A**) [42]. **UA** exhibited a lesser effect in yeast-induced pyrexia test compared to **OA**, as indicated by its docking score (−13.97 kcal/mol). **UA** also formed H-bonds with Arg120 (2.1 Å) and Tyr355 (1.6 Å) through its acetate group (Fig. 6**B**). The carbonyl moiety of **BA** (−13.86 kcal/mol) interacted with Tyr355 (1.9 Å) through a H-bond (Fig. 6**C**). The docking results are summarized in Table 1, and

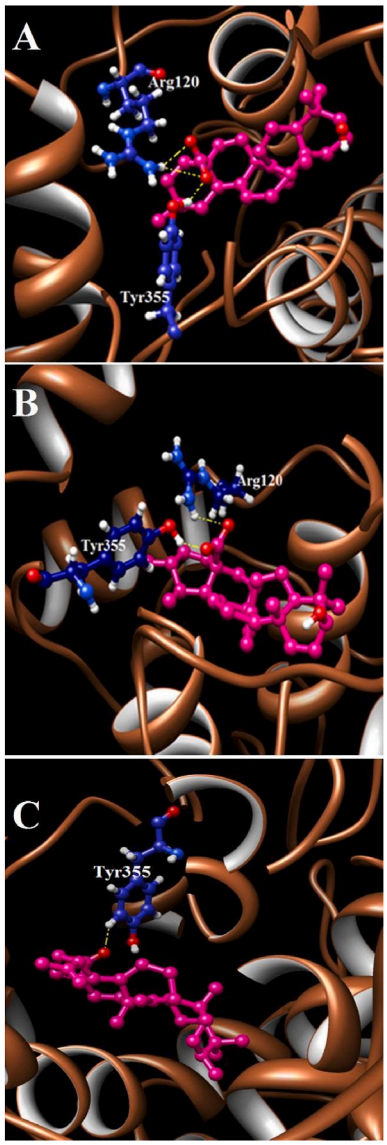

Fig. 6. The binding modes of **OA** (**A**), **UA** (**B**) and **BA** (**C**) are shown in the binding pocket of COX-2 enzyme.

Table 1
Docking results of **OA**, **UA** and **BA** are summarized in both the targets.

Compounds	Score (Kcal/mol)	Ligand Atoms	Residues	Hydrogen bond Distance (Å)
GABA receptor				
OA	−6.00	Acetate	Asn60	3.18
		Acetate	Lys156	2.68
		OH	Ser205	2.74
UA	−5.73	Acetate	Asn60	2.36
BA	−5.61	-OH	Tyr160	2.72
COX-2				
OA	−14.11	Acetate	Arg120	2.2
		Acetate	Tyr355	2.7 and 1.7
UA	−13.97	Acetate	Arg120	2.1
		Acetate	Tyr355	1.6
BA	−13.86	carbonyl	Tyr355	1.9

2D-interactions are given in Fig. S6 (supplementary file).

This study investigated the pharmacological properties of isolated pentacyclic triterpenes from roots of *D. lotus*, particularly focusing on their effects on motor coordination, and antipyretic activities. Comparing our findings with previous studies revealed consistent trends in the muscle relaxant and antipyretic effects of **OA**, **UA**, and **BA**. Notably, **OA** demonstrated the most pronounced muscle relaxant effect, followed by **UA** and **BA**, consistent with their respective molecular docking scores in the ligand binding site of $GABA_AR$. This suggests a potential mechanism of action involving modulation of GABAergic neurotransmission, akin to benzodiazepines, though further mechanistic studies are warranted. Additionally, our docking experiments indicated potential interactions of **OA** with key residues of COX-2, supporting their observed antipyretic effects. However, it is important to acknowledge several limitations of this study. Firstly, the extrapolation of our findings to human physiology requires caution due to the use of animal models.

Table 2
Physicochemical and of OA, UA, and BA.

Physicochemical properties	OA	UA	BA
Molecular Weight	456.71	456.71	453.69
LogP	7.23	7.09	6.97
H-Bond Acceptor	2	2	2
H-Bond Donor	2	2	2
Water solubility (logS)	−4.388	−4.388	−4.416
Plasma protein binding (100 %)	0.767	1.039	1.08
Acute Oral Toxicity [log(1/(mol/kg))]	2.466	1.455	1.93
	OA	**UA**	**BA**
Human Intestinal Absorption	+	+	+
Caco-2	+	+	+
Blood Brain Barrier	–	–	–
Human oral bioavailability	+	+	+
P-glycoprotein inhibitor	–	–	–
P-glycoprotein substrate	–	–	–
CYP3A4 substrate	+	+	+
CYP2C9 substrate	–	–	–
CYP2D6 substrate	–	–	–
CYP3A4 inhibition	–	–	–
CYP2C9 inhibition	–	–	–
CYP2C19 inhibition	–	–	–
CYP2D6 inhibition	–	–	–
CYP1A2 inhibition	–	–	–
CYP2C8 inhibition	–	–	+
CYP inhibitory promiscuity	–	–	–
Carcinogenicity	–	–	–
Eye corrosion	–	–	–
Eye irritation	–	–	–
Skin irritation	+	+	+
Skin corrosion	–	–	–
AMES mutagenesis	–	–	–
Human Ether-a-go-go-Related Gene inhibition	–	–	–
Hepatotoxicity	–	+	–
skin sensitization	+	+	–
Respiratory toxicity	+	–	+
Reproductive toxicity	+	+	+
Nephrotoxicity	–	–	–
Acute Oral Toxicity (c)	III	III	III

Secondly, while molecular docking provides valuable insights, it is a computational tool and may not fully capture the complexities of ligand-protein interactions *in vivo*. Moreover, scope of this study was limited to the evaluation of motor coordination and antipyretic activity; future investigations should be carries out to explore additional pharmacological properties and potential therapeutic applications of these pentacyclic triterpenes. Despite these limitations, this study contributes valuable insights into the pharmacological profile of *D. lotus* derived triterpenes and underscores their potential as therapeutic agents for conditions involving motor dysfunction and fever.

*3.6. Prediction of ADMET profile of **OA, UA**, and **BA***

The physicochemical and AMDET properties of compounds were estimated through admetSAR server which reflect that these molecules have molecular weight less than 500, high LogP (partition coefficient) values, 2 number of hydrogen bond acceptor atoms, and 2 hydrogen bond donor atoms, less water solubility, good binding with plasma protein, and acute oral toxicity dose from 1.4 to 2.5 mol/kg (Table 2).

Moreover, these molecules have high intestinal absorption, oral bioavailability and Caco-2 permeability, and no ability to cross blood brain barrier. These molecules do not act like inhibitors or substrates for P-glycoprotein. They are non-carcinogenic and non-mutagenic according to AMES mutagenicity test. They do not exhibit eye corrosion or eye irritation and skin corrosion, however, may cause Skin irritation, while **OA** and **UA** have skin sensitization ability. They do not have the ability to inhibit human ether-a-go-go-related genes. Furthermore, only **UA** may cause hepatotoxicity, whereas **OA** and **BA** can cause respiratory toxicity, and all these molecules may cause reproductive toxicity. Whereas none of these compounds can cause nephrotoxicity. These molecules fall into the category III of acute oral toxicity which means their oral toxicity dose would be 300 mg/kg, and dermal would be 1000 mg/kg, indicating their safety level.

The metabolic profile of these molecules indicates that they may serve as substrate for Cytochrome P_{450}3A4, whereas they don't have substrate-like properties for CYP2C9 and CYP2D6, and can't inhibit the function of CYP3A4, CYP2C9, CYP2C19, CYP2D6, CYP1A2. However, only **BA** can have inhibitory potential for CYP2C8. The results are given in Table 2, indicating their safety profile.

4. Conclusion

The current investigation revealed significant muscle relaxant potential of isolated pentacyclic triterpenes from the roots of *D. lotus* in animal models. **OA** exhibited the most promising muscle relaxant effect and antipyretic activities, followed by **UA** and **BA**. Those pentacyclic triterpenes (**OA, UA,** and **BA**) have profound antipyretic effect in animal model of yeast induced pyrexia. Furthermore, it is predicted by *in-silico* docking that these molecules may target $GABA_A$ receptor and COX-2 to exhibit muscle relaxant and antipyretic activities. However, further studies are required to explore their mechanism of action and clinical potential.

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Ethics approval

The study was conducted according to the guidelines of the Animals' Scientific Procedures Act (UK), 1986, and approved by the ethical committee, Department of Pharmacy, University of Peshawar (reference number 12/EC-17/Pharm).

CRediT authorship contribution statement

Ajmal Khan: Writing – review & editing, Conceptualization. **Hamdy Kashtoh:** Formal analysis, Data curation. **Abdur Rauf:** Writing – original draft, Methodology, Conceptualization. **Sobia Ahsan Halim:** Software, Methodology. **Awan A. Aleem:** Investigation, Formal analysis, Data curation. **Haji Bahadar** Validation, Methodology. **Huma Shareef:** Investigation, Formal analysis, Data curation. **Fazal Mabood:** Software, Methodology, Investigation. **Asaad Khalid:** Writing – review & editing, Visualization, Validation. **Kwang-Hyun Baek:** Writing – review & editing, Resources, Formal analysis. **Ahmed Al-Harrasi:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Authors Ajmal Khan and Sobia Ahsan Halim are working as associate editor in section "Pharmaceutical Sciences" of this journal.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30547.

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