Original Research Article

Computational and pharmacological evaluation of stevioside derivatives for antinociceptive and anti-inflammatory potential

Sadaf Ahmad[1], Arif-ullah Khan[1], Muhammad Faheem[1]*, Muhammad Shahid Iqbal[2], Mohammad Akbar Hossain[3], Asad Ullah[4], Shabir Ahmad[4]

[1]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan, [2]Department of Clinical Pharmacy, College of Pharmacy, Prince Sattam Bin Abdulaziz University Alkharj, Saudi Arabia, [3]Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al-Qura University, Makkah al Mukarramah, Saudi Arabia, [4]Department of Pharmacology and Toxicology, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia

*For correspondence: **Email:** muhammad.faheem@riphah.edu.pk, **Tel:** +92-3009021523

Abstract

Purpose: To carry out computational and pharmacological evaluation of two stevioside derivatives in order to develop more effective candidates for analgesia and inflammation.
Methods: Primarily, compounds were docked against targets of nociception and inflammation such as cyclooxygenase-1, cyclooxygenase-2, 5-lypooxygenase 12-lypooxygenase, 15-lypooxygenase, prostaglandin synthase, leukotrienes C4 synthase, mu, kappa, and delta receptors to obtain their possible binding modes. Test compounds were then screened in animal model of nociception and inflammation.
Results: The results of docking show that IO possesses good affinity when compared to ID. IO showed two hydrogen bonds against COX-1 and COX-2. IO also demonstrated good binding against 5-LOX, 12-LOX and 15-LOX, exhibited four, one and two hydrogen bonds respectively. Against PG synthase and LTC4, both IO and ID produced moderate binding. IO also showed significant binding against opoid receptors (p < 0.05). IO and ID significantly decrease the number of writhes to 21.20 ± 2.1 and 27.0 ± 2.12 at 10 mg/kg in acetic acid mediated pain test respectively. In hot plate method, IO and ID increase the latency period of mice to 14.14 ± 0.40 and 10.50 ± 0.34 s, respectively. IO and ID significantly reduced the paw edema to 1.69 ± 0.14 and 1.94 ± 0.14 mL, respectively, in acute inflammation (p < 0.05). In chronic inflammatory model, IO and ID decreased paw volume to 3.26 ± 0.38 and 4.20 ± 0.38 mL, respectively.
Conclusion: The results show that IO is a promising candidate for further development as analgesic and anti-inflammatory agents. However, their pharmacokinetic and pharmacodynamic profiles need to be investigated.

Keywords: Computational, Stevioside, Docking, Analgesic, Anti-inflammatory


INTRODUCTION

Stevioside was first isolated from leaves of *Stevia rebaudiana Bertoni,* a perennial herb belongs to family asteraecea [1]. It is composed of steviol, a dieterpene carboxylic alcohol and three molecules of glucose and is used as a sweetener. Steviol is the metabolite, obtained by enzymatic hydrolysis of Stevioside [2]. Pharmacologically, stevioside are effective in controlling obesity, atherosclerosis [3], hypertension, diabetes mellitus [4], cancer, inflammation [5], and free radical scavenging activities [6]. Based on their wide spectrum in both medicine and food, two derivatives of steviol glycosides, namely isosteviol 16-Oxime (IO) and isosteviol 16-2, 4 dinitrophenyl hydrazine (ID) were utilized in this research. The synthesis, antitrypanosomal and antimalarial potential of these compounds have already been published [7]. The structures of IO and ID are shown in Figure 1. Both compounds were screened to check their binding affinity against various targets of pain and inflammation. After *in-silico* screening, IO and ID were also evaluated for *in-vivo* experimentation in determining their analgesic and anti-inflammatory potential in different animal models as acetic acid induced pain, thermal nociception, carrageenan and formalin mediated paw inflammation. The first two models were utilized for evaluating pain perception and thermal latency. The latter two models were used for determining the acute and chronic anti-inflammatory potential of mentioned derivatives.

(IO)
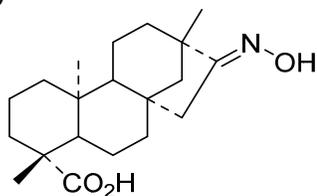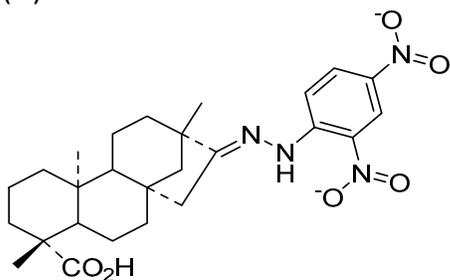

(ID)

Figure 1: Structure of compounds: Isosteviol 16-Oxime (IO) and Isosteviol 16-2, 4 dinitrophenyl hydrazine (ID)

EXPERIMENTAL

Materials

Acetic acid, carrageenan, diclofenac sodium, dimethylsulfoxide (DMSO), formalin, tramadol. (Sigma Chemicals. Dt. Louis, MO, USA). IO and ID were used in 5 and 10 mg/kg in all experimental model. Diclofenac sodium and toradol were used in 20 and 30 mg/kg respectively.

Animals

Swiss albino mice (25 – 30 g) and Sprague Dawley rats (200 – 250 g) were utilized. The animals were kept under control environment (23 - 25 °C) without restriction to water and food. The approval of research was granted by Research and Ethical Committee of Riphah Institute of Pharmaceutical Sciences (ref no. REC/RIPS/2016/007). The research was carried as per guidelines of Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council, 1996 [8].

Computational evaluation

The three-dimensional target proteins were obtained from (http://www.rcsb.org/pdb/) in PDB format with their respective protein data bank entries shown in Table 1. The water molecules and attached ligand were removed through Biovia Discovery Studio Visualizer Client (DSVC) 2016. The 3D structures of ligands were drawn and saved in PDB format through DSVC 2016. The targets and ligands were further converted to PBDqt format through Auto dock vina and saved for further processing. The 3D structure of the standard drugs including diclofenac sodium (PubChem CID: 5018304) and zilueton (PubChem CID: 60490) and morphine (PubChem CID: 249332) for the purpose of analgesic (Central and peripheral analgesic activity) and anti-inflammatory activities were obtained from pubchem (https://pubchem.ncbi.nlm.nih.gov/search/).

Assessment of acetic acid induced writhing in mice

The analgesic effect of IO and ID was determined by using acetic acid induced writhing method in mice [10]. Before 0.7 % injection of acetic acid, the mice were injected with saline, test compound and diclofenac sodium and writhes were counted for 20 min.

Table 1: Targets (COX 1, COX 2, 5 LOX, 12 LOX, 15 LOX, PG Synthase, LTC4 Synthase, Mu, Kappa, Delta) and their PDB IDs, score/E-value (Kcal/mol), no of hydrogen bonds, amino acid residues of isosteviol 16-Oxime (IO), isosteviol 16-2, 4 dinitrophenyl hydrazine (ID) diclofenac sodium, zileuton and morphine interpreted through Biovia Discovery Studio Visualizer client 2016

Target	PDB IDs	IO			ID			Standard drugs			
		Score	No of H-Bond	Bonding residues	Score	No of H-Bond	Bonding residues	Standard	Score	No of H-bond	Bonding residues
COX 1	3KK6	-9.8	02	SER 127 ILE 125	-9.1	00	-	Diclofenac sodium	-7.9	01	GLY 25
COX 2	5IKQ	-10	02	PHE 471 GLU 525	-8.9	01	TYR 131	Diclofenac sodium	-7.8		GLY 25 HIS 26 VAL 28
5 LOX	1YPQ	-7.4	04	ARG 138, 165 ASP 166 TYR 142	-6.5	02	TYR 100, 142	Zileuton	-7.2	01	TYR 81
12 LOX	3D3L	-9.1	01	ASP 646	-6.3	00	-	Zileuton	-7.4	02	LEU 597 CYS 656
15 LOX	4NRE	-8.7	01	TYR 107	-6.4	01	ARG 407	Zileuton	7.0	02	GLN 108 TYR 149
PG Synthase	3DWW	-8.8	01	TYR 55	-6.2	01	SER 217	Zileuton	-6.3	03	ARG 66 ASN 198 SER 200
LTC4 Synthase	3PNO	-7.0	01	GLY 124	-6.6	02	ILE 168 SER 96	Zileuton	-5.2	01	SER 122
Mu	4DKL	-7.8	02	HIS 967 TYR 962	-6.8	00	-	Morphine	-8.9		ASN 990 ASP 1015 LEU 1026
Kappa	4DJH	-8.2	01	TYR 386	-7.4	00		Morphine	-9.3	01	GLY 2
Delta	4EJ4	-7.5	01	THR 260	-6.6	01	SER 143	Morphine	-9.4	01	TYR 308

Aspartic acid = ASP, Cysteine=CYS, Glutamine = GLN, Glutamic acid = GLU, Glycine = GLY, Histadine = HIS, Asparagine = ASN Arginine = ARG, Isoleucine = ILE, Lysine = LYS, Leucine = LEU, Methionine = MET, Phenylalanine = PHE, Serine = SER Tryptophan = TRP, Threonine = THR, Tyrosine = TYR, Valine

Hot plate test

The pain killing effect of IO and ID was determined using hot plate method in mice [11]. The mice were pretreated with saline, test compound and tramadol before placing on hot plate. Paw licking/ jumping from the plate, noted as latency period at 30, 60, 90 and 120 min. The cutoff time was 20 sec to avoid paw injury of mice.

Determination of carrageenan-induced paw edema

The anti-inflammatory effect of IO and ID was determined using carrageenan mediated acute inflammation in rats [12]. After 30 min of treatment, 0.1 mL carrageenan (1%) was injected in sub-aponeurotic region of hind paw and changes in the paw volume were recorded at 1, 2, 3 and 4 h by using plethysmometer (model: UGO Basile S.R.L, 7141).

Evaluation of formalin-induced paw edema

The effect against chronic inflammation was determined by using formalin mediated chronic inflammation rats [13]. After 30 min of treatment with saline, test compound and diclofenac sodium chronic inflammation was induced by sub-aponeurotic injection of formalin (0.1 mL). The changes in paw edema were recorded for ten days and the results were analyzed.

Statistical analysis

Graph Pad Prism (Graph-Pad, San-Diego-CA, USA) was used for statistical analysis. Data are shown as mean ± standard error of mean (SEM). ANOVA with post-hoc Tukey's test was applied to interpret data. $p < 0.05$ was considered as significant.

RESULTS

Docking results

The ligand IO and ID were docked against different targets of pain and inflammation to obtain possible binding modes and binding affinities. The targets and their PDB IDs, binding affinities of ligand (IO and ID), standards (diclofenac sodium, zilueton, and morphine) and number of hydrogen bonds are shown in Table 1.

Effect on acetic acid induced analgesia

Acetic acid-induced pain method was used to determine the peripheral analgesic activity of

both IO and ID and the results are shown in Figure 2. At 5 and 10 mg/kg, IO significantly decrease the writhes to 40 ± 1.94 and 21.2 ± 2.10 ($p < 0.001$) respectively. ID at 5 & 10 mg/kg also significantly decrease the writhes to 57.6 ± 1.50 and 27.0 ± 2.12 ($p < 0.001$) respectively.
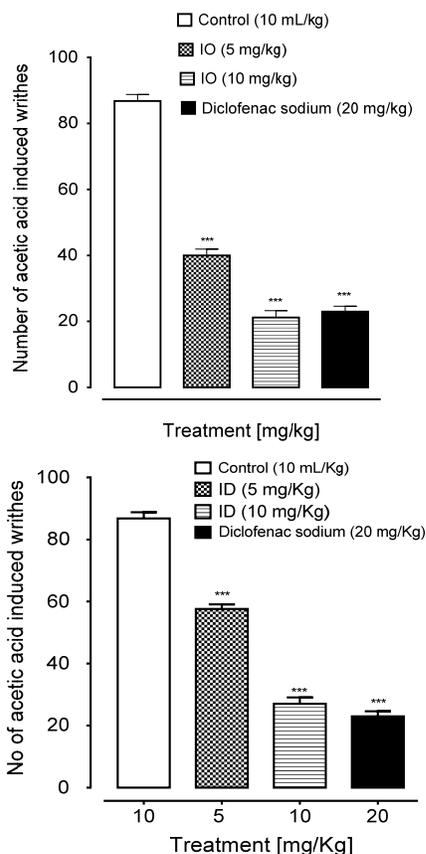

Figure 2: Bar chart showing inhibition of acetic acid-induced writhings by isosteviol 16-Oxime (IO), isosteviol 16-2, 4 dinitrophenyl hydrazine (ID) and diclofenac sodium in mice. Data shown as mean ± SEM, n = 5; ***$p < 0.001$ vs. control, one-way ANOVA with post-hoc Tukey test

Effect of treatment on latency time

Hot plate method was used to determine the central analgesic effect of both IO and ID and the results are shown in Figure 2. IO at 10 mg/kg also significantly increase the latency period ($p < 0.001$) after 60 min. ID at dose 10 mg/kg increase the significantly increases latency period ($p < 0.01$).

Effect on carrageenan-induced paw edema

The anti-inflammatory effect of both IO and ID was determined by carrageenan-induced hind paw edema method and the results are shown in

Figure 4. The mean paw volume recorded for IO at a dose of 5 and 10 mg/kg was significant at ($p < 0.01$) and at ($p < 0.001$). The mean paw volume recorded for ID at a dose of 5 and 10 mg/kg was significant at ($p < 0.01$) and at ($p < 0.001$).

Effect on formalin induced paw edema

The test compounds IO and ID were screened for chronic inflammation through formalin-induced paw edema and the results are shown in Figure 5. The paw volume displacement recorded for IO at dose 5 mg/kg and 10 mg/kg was significant at ($p < 0.001$). The paw volume displacement recorded for ID at dose 5 mg/kg and 10 mg/kg was significant at ($p < 0.001$).
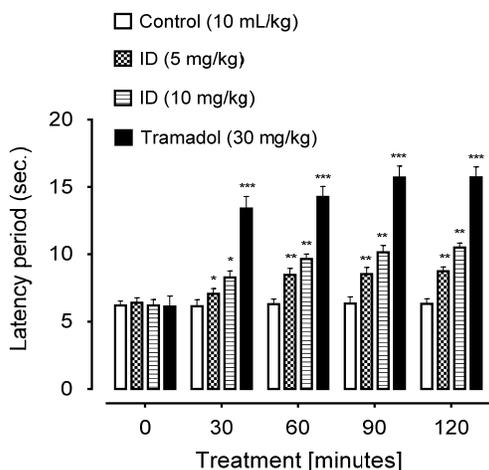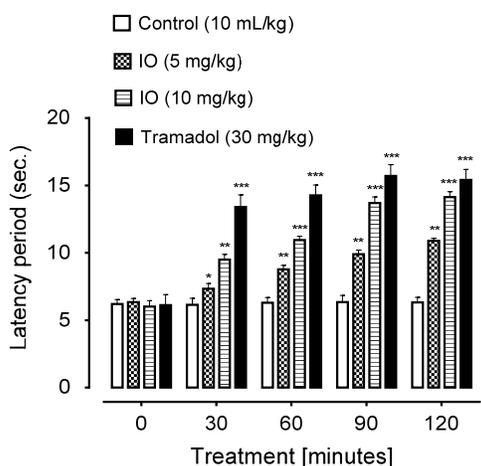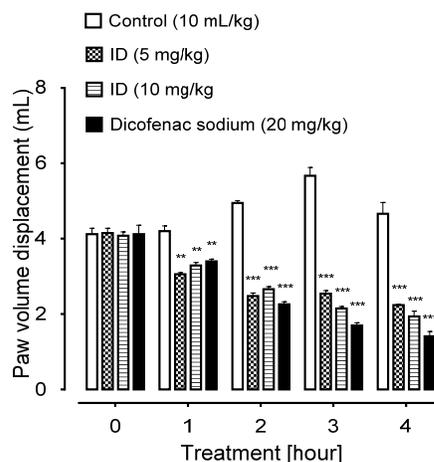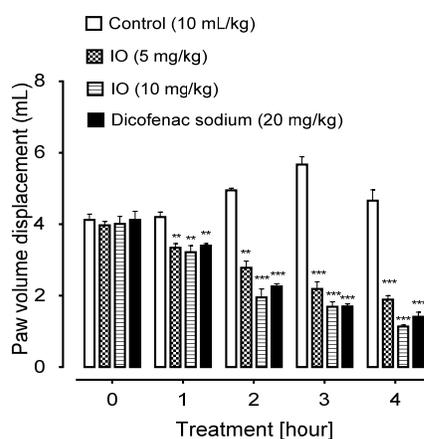

Figure 4: Bar chart showing inhibition of carrageenan induced paw edema by isosteviol 16-Oxime (IO), isosteviol 16-2, 4 dinitrophenyl hydrazine (ID) and diclofenac sodium in mice. Data expressed as mean ± SEM, n=5. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control, one-way ANOVA with post-hoc Tukey test

Figure 3: Bar chart showing the effect of isosteviol 16-Oxime (IO), isosteviol 16-2, 4 dinitrophenyl hydrazine (ID) and tramadol on latency period of mice on hot pla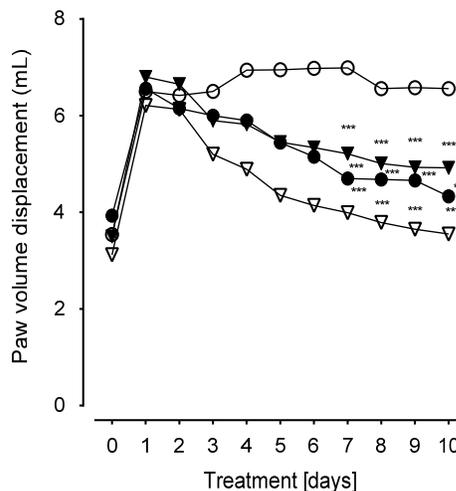te. Data expressed as mean ± SEM, n=5. $^{**}p < 0.01$ and $^{**}p < 0.001$ vs. control, one-way ANOVA with post-hoc Tukey test
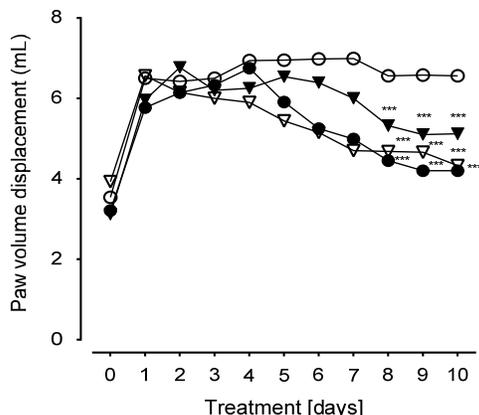
Figure 5. Bar chart showing inhibition of formalin induced paw edema by isosteviol 6-Oxime (IO), isosteviol 16-2, 4 dinitrophenyl hydrazine (ID) and diclofenac sodium in mice. ○ control, ▼ 5 mg/kg, ▽ 10 mg/kg, ● diclofenac sodium. Data expressed as mean ± SEM, n=5. ***$p < 0.001$ vs. control group, one-way ANOVA with post-hoc Tukey test

DISCUSSION

Docking is carried out with auto dock for the purpose to find the best interaction of the ligand (IO and ID) with target protein. This preliminary screening is helpful in order to confirm that weather the ligand molecule have the ability to become a drug candidate or not. These interactions are hydrogen bonds, hydrophobic interaction and wander wall forces [14]. The best and favorable interaction of a ligand with targets is correlated with good drug candidate of the future [15].

According to the Lipinski rule of five a good drug should of small size with molecular weight less than 500 Da [16]. The ligand IO gives best binding affinity against various targets because of its small size. Against COX-1 it gives two hydrogen bonds compare to ID which do not gives good binding with COX-1. The unfavorable bumps shown in red is the reason for the ID which make it less superior against COX-1. IO is also found a good binder of COX-2 because it gives two hydrogen bonds with less bombs as compare to ID which gives one hydrogen and with 3 bumps. The superiority of IO is continued against 5-LOX, 12-LOX and 15-LOX compare to ID in both *in-silico* and *in-vivo* models. Against PG synthase, comparatively IO gives moderate binding better than ID.

Based on the bumps, the ID is again found not a good ligand of the LTC4 as compare to IO. The unfavorable bumps in ID makes are less affective against LTC4. IO was also noticed to possess moderate binding modes against all the opioids receptors. In short IO is comparatively good ligand against targets for analgesia and inflammation. Pain and inflammation move side by side and starts in response to any type of stimulus/injury which provokes immune system. The stimuli may be mechanical or thermal origin [17]. Various inflammatory mediators are released to starts the process of inflammation. The mediators which are released in the early phase of the inflammation are histamine, bradykinin and 5-hydroxy tryptamine.

Some mediators like prostaglandins are released in the late phase of immune response [18]. The release of these mediators from different types of immune cells is termed as immunomodulation [19]. The immunomodulation is either immunostimulation or immunosuppression. The agents which play their role in causing immunomodulation are called as immunomodulator. These agents are gaining importance as they are involved in suppressing immune system and thus optimize response. These agents not only reduce inflammatory process but also lower pain perception associated with various pathological abnormalities [20]. According to literature, the stevioside and steviol glycoside are the key agents in attenuating inflammatory process.

The mechanism involved is the inhibition of various inflammatory cytokines (Nuclear factor-κB (NF-κB), inhibitory kappa B (IκBα) protein, p38, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) or proinflammatory mediators [21]. One another study also focuses the way of how stevioside lowers the immune response by preventing the over expression of certain inflammatory mediators, involved in pain and inflammation. It was also found that stevioside decreases the proinflammatory cytokines both in *in-vivo* and *in-vitro* models [22].

CONCLUSION

The results show that IO is a promising candidate for the management of pain and inflammation. However, further studies, including pharmacokinetics and pharmacodynamics, are required to ascertain their suitability for clinical use.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Sadaf Ahmad design and performed the experimental work. Arif Ullah khan and Muhammad Faheem work in writing of the manuscript. Asad Ullah and Shabir Ahmad synthesis and provide compounds. Muhammad Shahid Iqbal and Muhammad Akbar Hossain helps in improving the English language of the manuscript and financial support.

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