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In Silico EVALUATION OF CHEMICAL TOXICITY OF CERTAIN NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In our study, we collected five commonly used non-steroidal anti-inflammatory drugs, including Ketorolac, Aspirin, Naproxen, and Diclofenac. In our DFT results, Diclofenac has the lowest energy gap (-0.5827 eV), highest ionization potential (5.0983 eV), highest electron affinity (5.6810 eV), highest electronegativity (5.3897 eV), lowest chemical potential (-5.3897), lowest dipole moment (1.1282) and lowest energy (-1657.106). The Pro Tox II web server was used to determine the toxicity of drugs based on their chemical structure. Diclofenac has the lowest LD50 (53 mg/kg) value in comparison to Ketorolac (LD50=189 mg/kg), Naproxen (LD50=248 mg/kg), Aspirin (LD50=250 mg/kg), and Ibuprofen (LD50=189 mg/kg). All these non-steroidal anti-inflammatory drugs target hepatotoxicity, as well as nuclear receptor signalling pathways, including aminooxidase A and prostaglandin G/H synthase 1. Diclofenac was found to be more toxic than other NSAIDs in toxicity studies, and its results matched those found in DFT studies.

Keywords: NSAIDs; DFT studies; Pro tox II; ibuprofen; aspirin and diclofenac.

1. INTRODUCTION

Over 100 million prescriptions for non-steroidal anti-inflammatory drugs (NSAIDs) are written worldwide every year [1,2]. NSAIDs are also used by patients for self-treatment in a large number of non-prescription drugs [3]. Anti-rheumatic drugs most commonly prescribed are non-steroidal anti-inflammatory drugs (NSAIDs). These drugs have adverse effects on the gastrointestinal tract, resulting in gastroduodenal ulcers and their complications [4-5]. NSAIDs are known to cause adverse effects throughout the alimentary tract. For example, those with oesophageal reflux who take NSAIDs are more likely to develop oesophageal stenosis [6]. NSAIDs have significant adverse effects on the stomach, duodenum, and lower gastrointestinal tract. These include nausea, vomiting, dyspepsia, diarrhoea, constipation, ulcerative colitis, and mucosal irritation [7,8].

In evaluating newly designed molecules, non-bonding interactions and toxicity predictions are important factors [9]. Anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), are commonly prescribed for pain relief, fever treatment, and arthritis treatment. NSAIDs reduce prostaglandin

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synthesis by inhibiting cyclooxygenase (COX) [10]. Since the COX enzyme, especially COX-2 enzyme, catalyzes the inflammatory intermediate prostaglandin synthesis, prostacyclin and thromboxane, suppressing COX-2 enzyme activity may prove beneficial for treating inflammation [11,12]. The DFT method has been used in the calculation of free energy, electronic energy, enthalpy, dipole moment, electrostatic potential, homo-lumo gap, chemical potential, and toughness of toxic chemicals [13]. The ProTox II web server is a free tool that offers applications based on chemical similarity and fragment-based toxicity estimation. It has high performance compared to currently available QSAR-based methods. Using Pro-Tox server, toxicity classes can be predicted based on similarity and fragmentation calculations, such that potential toxic targets can be alerted, providing insight into the mechanisms underlying toxicity [14]. Based on the above-mentioned facts and in keeping with our research interests in in-silico toxicity testing of some nonsteroidal anti-inflammatory drugs.

2. MATERIALS AND METHODS

2.1 Collection and Identification Chemical Substances of NSAIDs

The five nonsteroidal anti-inflammatory drugs found in Nagercoil are Aspirin, Diclofenac, Ibuprofen, Ketorolac, and Naproxen, which can be purchased at local medical shops. Each tablet contains a chemical substance containing an acid (-COOH) functional group. This acid functional group dissolved the ice-cold sodium bicarbonate solution and separated it from the solution. To obtain the respective chemical substance, 1:1 HCl was used to neutralise the filtrate. Melting points and Thin Layer Chromatography experiments were performed using a chloroform-ethanol mixture to determine the chemical substance (8:2).

2.2 DFT Study- Quantum Chemical Calculation

The properties of molecular structures were studied using Gaussian 09 B3LYP/6-31G (d,p), program [15]. By using the following equations, the DFT method was analysed using ionization potential (IP), electron affinities (EA), electronegativity (χ), chemical potential (μ), global hardness (η), global softness (σ), and electrophilicity index (ω) using the following equations [16-20]:

$$IP = - EHOMO \quad (1)$$

$$EA = -ELUMO \quad (2)$$

$$\eta = E_{LUMO} - EHOMO \quad (3)$$

$$\sigma = 1 / \eta \quad (4)$$

$$\chi = - (EHOMO - ELUMO) / 2 \quad (5)$$

$$\mu = (EHOMO - ELUMO) / 2 \quad (6)$$

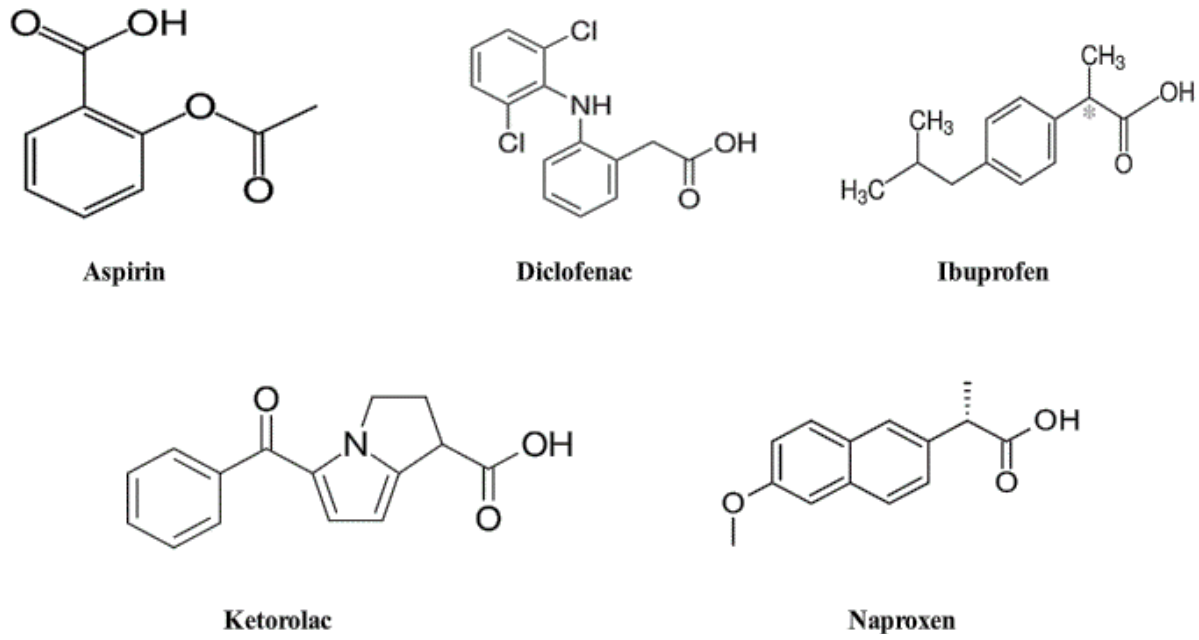

Fig. 1. Molecular formula of isolated NSAIDs

2.3 Toxicity Study-Pro Tox II

By using *in silico* prediction methods, we can reduce the cost, time, and animal experimentation associated with preclinical drug development. ProTox-II covers molecular similarity, pharmacophores, fragment probabilities and machine-learning models that include hepatotoxicity, cytotoxicity, mutagenicity, immunotoxicity, adverse outcomes pathways (Tox21), and toxicity targets [21,22].

2.4 Input Parameter

Users of ProTox II can draw structured diagrams with an embedded chemical editor, allowing them to search for chemical structures by name with an easy-to-use interface. ProTox II requires only the 2D structure of the molecule for which toxicity will be predicted. In addition, users can upload files that contain more than one compound in either mol or SMILE format [23].

2.5 Output Information

Toxicology prediction reports for a compound are generated within seconds, and they can be divided into two parts: the prediction of acute oral toxicity and the indication of potential toxicity targets (Fig. 1). The oral toxicity prediction results are based on the analysis of similarities in 2D and the recognition of toxic fragments, as described below. As well as predicting the LD50 of the input compound, it is classified into a toxicity class ranging from I to VI.. (GHS, United Nations, first revised edition 2005). The second part of the paper discusses possible binding to drug-related toxicity targets. At the present time, 15 of these toxicity targets have been linked with adverse drug reactions. [24,25]. ProTox-II consists of five different classification steps: (i) acute toxicity (oral toxicity model with six different classes); (2) organ toxicity (one model); (3) toxicological endpoints (four models); (4) toxicological pathways (12 models) and (5) toxicity targets (15 models. This webserver provides detailed information about the features that appear in the natural training set (for both active and inactive molecules) along with references, performance scores, and frequency distributions [21].

3. RESULTS AND DISCUSSION

3.1 Isolation of Chemical Constituents from NSAIDs

Sodium bicarbonate solution was used to dissolve acid functional group tablets, and the undissolved filling material was filtered and neutralized with 1: 1 HCl. The chemical constituent precipitates were filtered and dried, and their amounts determined. The amount of each chemical component in each NSAID can be found in Table 1.

According to the notified amount, nearly equal amounts of chemical components of selected non-steroidal anti-inflammatory drugs were obtained.

3.2 DFT Analysis-Quantum Chemical Calculation

3.2.1 Geometry optimization

Geometry optimization of all chemical constituents of selected non-steroidal anti-inflammatory drugs were performed in DFT/B3LYP/6-31G++(d,p) level calculations. The optimized structure is shown in Fig. 2.

Analysis of the wave function indicates that the electron absorption occurs at the transition between ground state and excited state, where the electron donor distribution occurs in the most occupied molecular orbital (HOMO) while the electron acceptor distribution occurs in the least occupied molecular orbital (LUMO. Table 3 and Fig. 3 illustrate the molecular energy, LUMO represents the ability to gain an electron, so the HOMO represents an ability to lose an electron. The energy of the HOMO is directly proportional to the ionization potential and the energy of the LUMO is directly proportional to the electronic affinity. The difference in orbital energy between the HOMO and LUMO is called the HOMO-LUMO gap. The high HOMO energy corresponds to a molecule more reactive with electrophiles in reactions, low energy LUMO is reactive with nucleophiles. According to the theory of molecular orbitals, a high HOMO energy of one reagent molecule and a low LUMO energy of another reagent are advantageous for the reaction between the two molecules, because the electron transfers are easier from the HOMO of a LUMO reagent on the other in the orbital interaction.

The HOMO, LUMO and the energy difference (HOMO-LUMO) of the monomer in the DFT with the 6-31G base (d, p) were calculated. The HOMO-LUMO energy gap reveals that the difference in energy reflects the chemical activity of the molecule. Table 3 and Fig. 2 illustrate the molecular energy, LUMO represents the ability to gain an electron, sothe HOMO represents an ability to lose an electron. The energy of the HOMO is directly proportional to the ionization potential and the energy of the LUMO is directly proportional to the electronic affinity. The difference in orbital energy between the HOMO andLUMO is called the HOMO-LUMO gap. The high

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Table 1. Amount of isolated chemical substance

S. No	Name of the NSAIDs	Amount of chemical substance in per tablet	No. of tablet	Total amount of chemical substance	Isolated amount of chemical substance
1	Ibuprofen	200 mg	20	4.0 g	3.9546 g
2	Aspirin	300 mg	20	6.0 g	5.9862 g
3	Naproxen	250 mg	20	5.0 g	4.9123 g
4	Ketorolac	10 mg	30	0.3 g	0.2942 g
5	Diclofenac	75 mg	20	1.5 g	1.4934 g

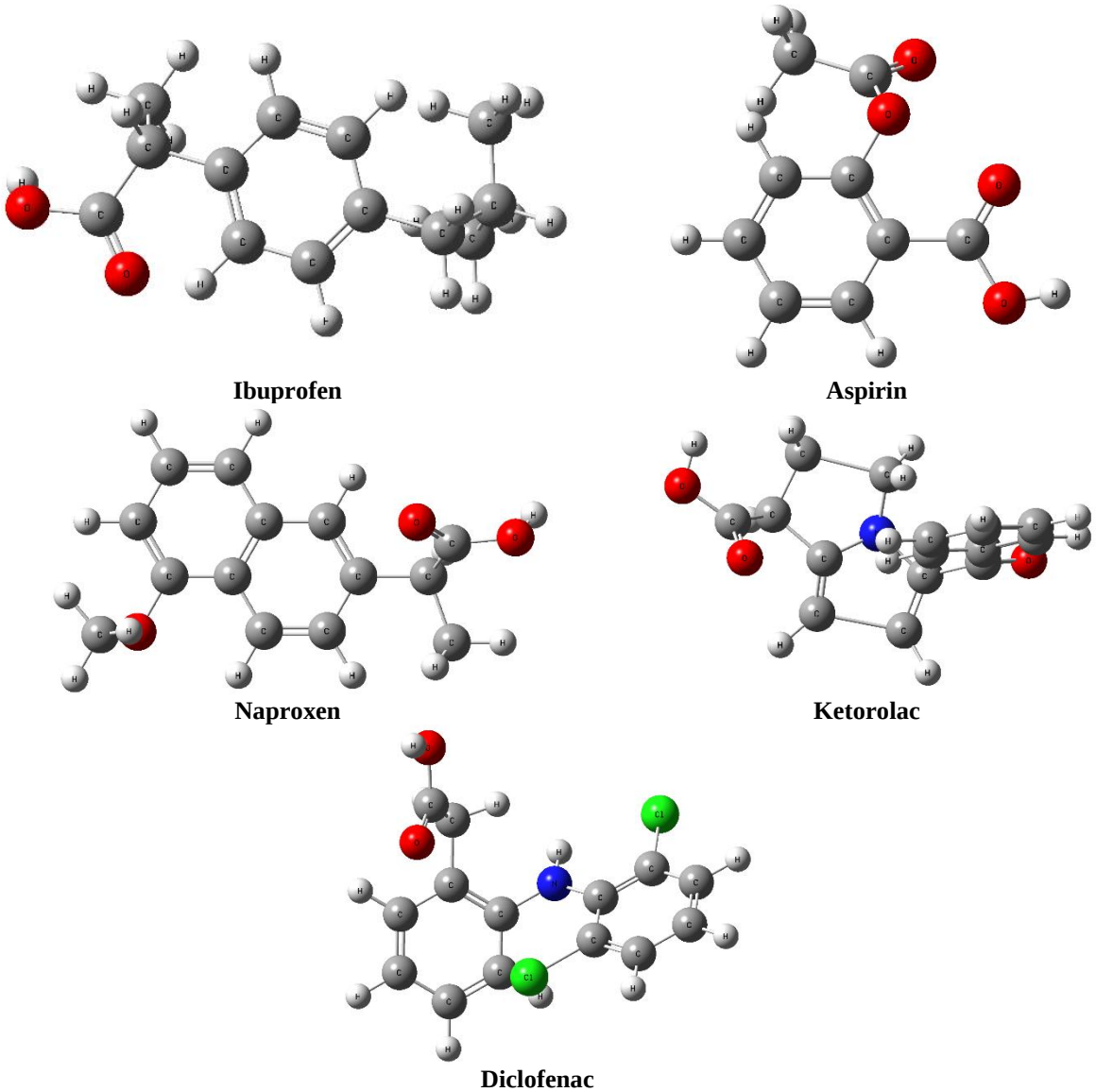
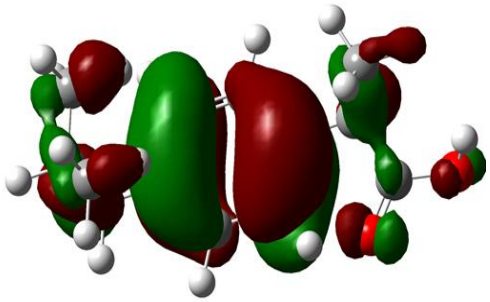
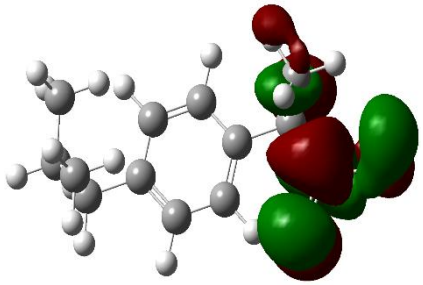
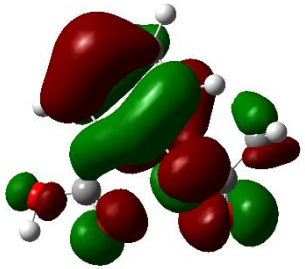


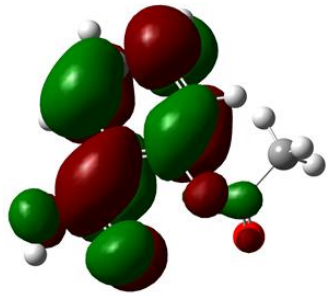
Fig. 2. Optimized structure of chemical constituents of selected non-steroidal anti-inflammatory drugs



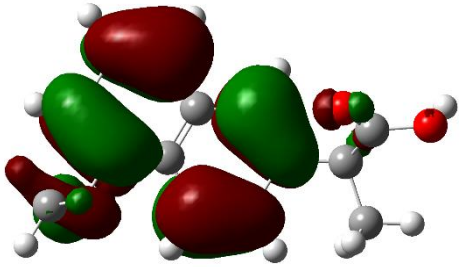
HOMO and LUMO Structure Ibuprofen



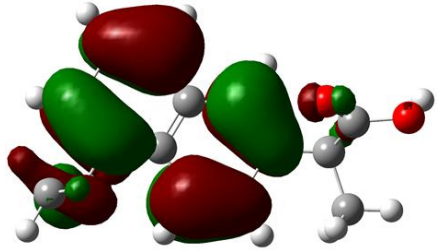


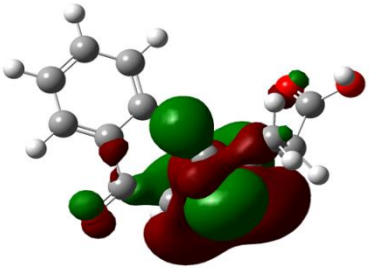


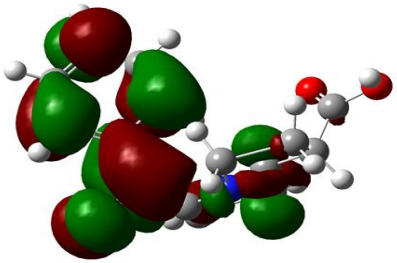
HOMO and LUMO Structure Aspirin



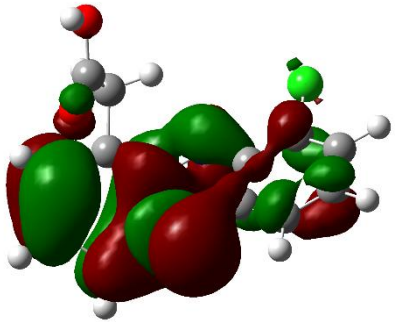
HOMO and LUMO Structure Naproxen

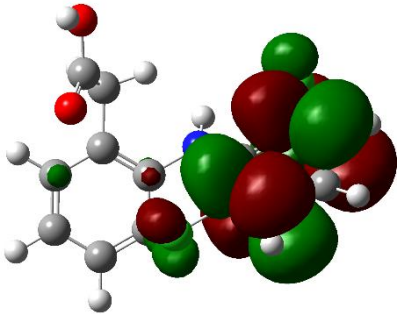






HOMO and LUMO Structure Ketorolac





HOMO and LUMO Structure of Diclofenac

Fig. 3, Frontiers orbital of the of chemical constituents of selected non-steroidal anti-inflammatory drugs

Table 3. Calculated DFT properties of chemical constituents of selected non-steroidal anti-inflammatory drugs

NSAIDs	E_{HOMO}	E_{LUMO}	Energy gap (eV)	Ionization Potential (IP) (eV)	Electron Affinities (EA) (eV)	Electronegativity (χ) (eV)	Chemical potential (μ) (eV)	Hardness (η) (eV)	Dipole moment	Etotal
Ibuprofen	-6.1155	-0.8630	5.2525	6.1155	0.8630	3.4893	-3.4893	2.6263	4.5386	-556.417
Aspirin	-6.0750	-1.5754	4.4996	6.0750	1.5754	3.8252	-3.8252	2.2498	3.9644	-648.429
Naproxen	-5.7102	-2.0149	3.6953	5.7102	2.0149	3.8626	-3.8626	1.8477	2.8338	-767.343
ketorolac	-5.3411	-2.7827	2.5584	5.3411	2.7827	4.0619	-4.0619	1.2792	2.7969	-859.365
Diclofenac	-5.0983	-5.6810	-0.5827	5.0983	5.6810	5.3897	-5.3897	-0.2913	1.1282	-1657.106

The HOMO-LUMO energies, hardness, softness, and chemical potential of all drugs are presented in Table 3 and Fig 4. The electronic absorption relates to the transition from the ground to the first excited state and mainly described by one electron excitation from HOMO to LUMO [26]. The chemical hardness, softness, and chemical potential values depend on the energy of HOMO-LUMO [27,28]. Kinetic stability increases with the increase of the HOMO-LUMO gap. As a result, removal of electrons from ground state HOMO to excited state LUMO requires more energy. In our studies, Diclofenac showed lowest energy gap (-0.5827 eV), highest ionization potential (5.0983 eV), highest electron affinity (5.6810 eV), highest electronegativity (5.3897 eV), lowest chemical potential (-5.3897), lowest dipole moment (1.1282) and lowest energy (-1657.106). As a result, clearly indicate the Diclofenac shows that higher chemical reactivity and highest toxic effect than the other non-steroidal anti-inflammatory drugs and Ibuprofen shows the lowest toxic effect than the other non-steroidal anti-inflammatory drugs.

3.3 Toxicity Study-ProTox-II

3.3.1 Generation of the chemical structures to SMILE

A web-based in-silico toxicity tool was used to estimate the *in-silico* toxicity of each of the NSAIDs by submitting the chemical structure in the form of canonical simplified molecular input line entry (SMILE) Table 4.

Table 4. IUPAC and SMILE file of the NSAIDs

S. No	NSAIDs	IUPAC	SMILE
1	**Aspirin**	2-(acetyloxy)benzoic acid	CC(=O)Oc1ccccc1C(=O)O
2	**Ibuprofen**	2-[4-(2-methylpropyl)phenyl]propanoic acid	CC(Cc1ccc(cc1)C(C(=O)O)C)C
3	**Ketorolac**	6-benzoyl-2,3-dihydro-1H-pyrrolizine-3-carboxylic acid	OC(=O)C1CCc2n1cc(c2)C(=O)c1ccccc1
4	**Naproxen**	2-(6-methoxynaphthalen-2-yl)propanoic acid	COc1ccc2c(c1)ccc(c2)C(C(=O)O)C
5	**Diclofenac**	2-{2-[(2,6-dichlorophenyl)amino]phenyl}acetic acid	OC(=O)Cc1ccccc1Nc1c(Cl)cccc1Cl

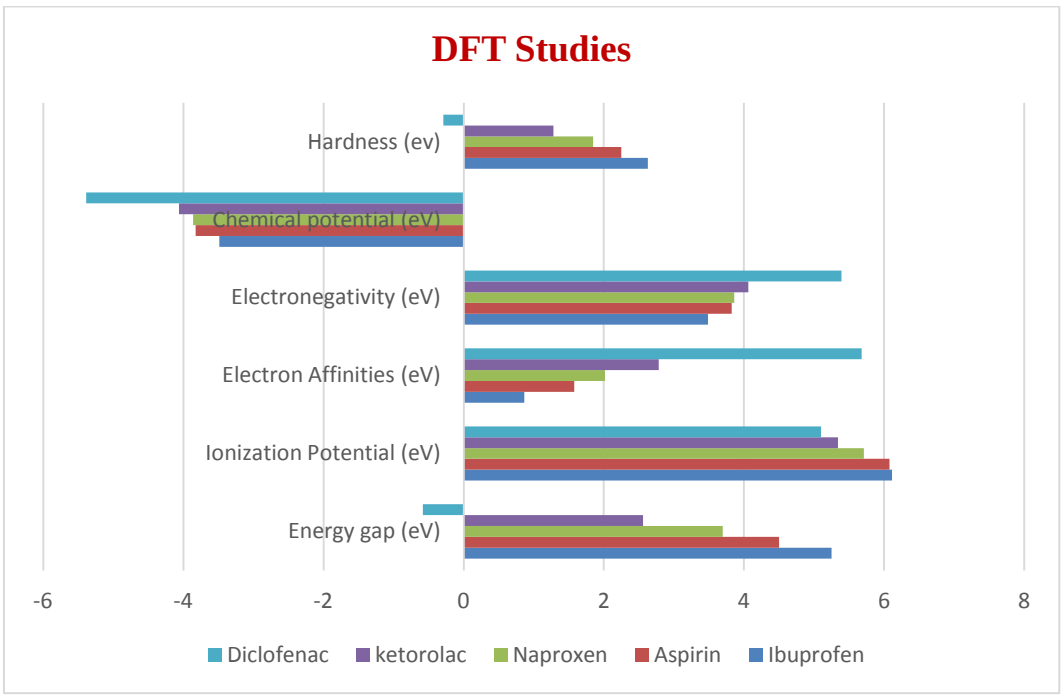


Fig. 4. DFT studies of NSAIDs

Table 5. Toxicity prediction of non-steroidal anti-inflammatory drugs

S. No	Non-steroidal anti-inflammatory drugs	Predicted toxicity class	Predicted LD50	Type of toxicity	Toxicity target	Average pharmacophore fit
1	Ibuprofen	3	299 mg/kg	Hepatotoxicity	Amine Oxidase A	58.13%
					Prostaglandin G/H Synthase 1	68.32 %
2	Aspirin	3	250 mg/kg	Hepatotoxicity	Amine Oxidase A	34.45 %
					Prostaglandin G/H Synthase 1	-
3	Naproxen	3	248 mg/kg	Hepatotoxicity	Amine Oxidase A	63.73 %
					Prostaglandin G/H Synthase 1	68.21 %
4	ketorolac	3	189 mg/kg	Hepatotoxicity	Amine Oxidase A	62.11 %
					Prostaglandin G/H Synthase 1	49.36 %
5	Diclofenac	3	53 mg/kg	Hepatotoxicity and Nuclear receptor signalling path ways	Amine Oxidase A	68.72 %
					Prostaglandin G/H Synthase 1	70.73 %

3.3.2 Toxicity prediction

The chemical structure of Ibuprofen, Aspirin, Naproxen, ketorolac and Diclofenac was input in the Pro Tox II web server (Table 5 and Figs. 5 & 6). ProTox-II which includes molecular similarity for acute toxicity prediction, pharmacophore-based models for 15 toxicity targets, fragment properties and machine learning models for 17 different toxicity end points. In our studies, All the selected drugs showed 3 class toxicity. The Lethal Dose (LD50) value of all the isolated drugs ranged from 299 mg/kg to 53 mg/kg. The LD50 value for Diclofenac is found to the 53 mg/kg predicting it as more toxic. Ibuprofen, Aspirin, Naproxen, and ketorolac exhibited Hepatotoxicity alone, but Diclofenac exhibited Nuclear receptor signalling path way along with Hepatotoxicity. The Ibuprofen, Naproxen, ketorolac and Diclofenac was found to of Amine oxidase A and Prostaglandins G/H synthesis 1. But the toxicity target of Aspirin was found to be Amine oxidase A alone.

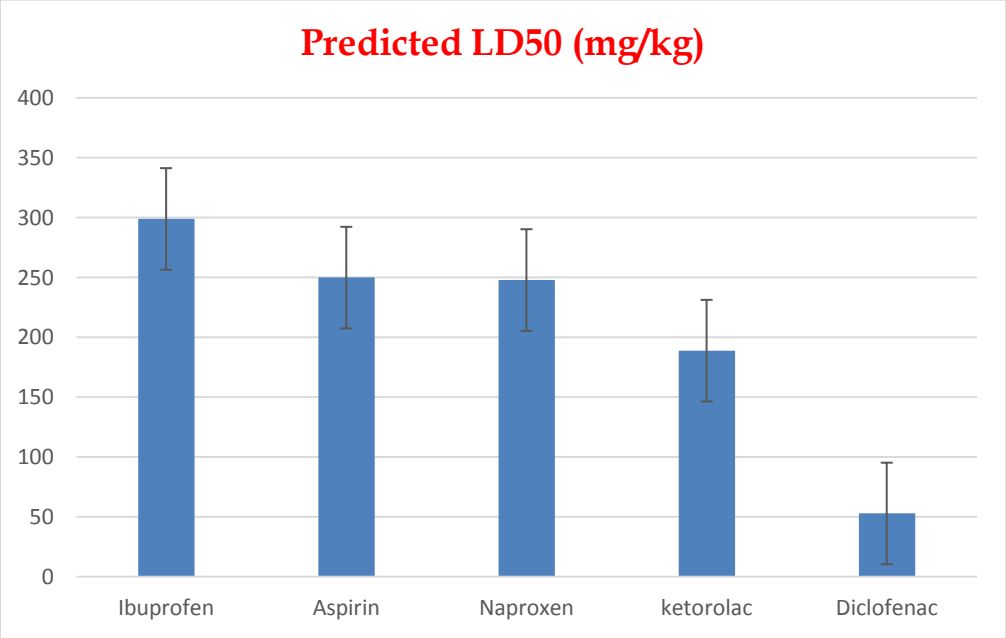


Fig. 5. Toxicity predicted (LD50)

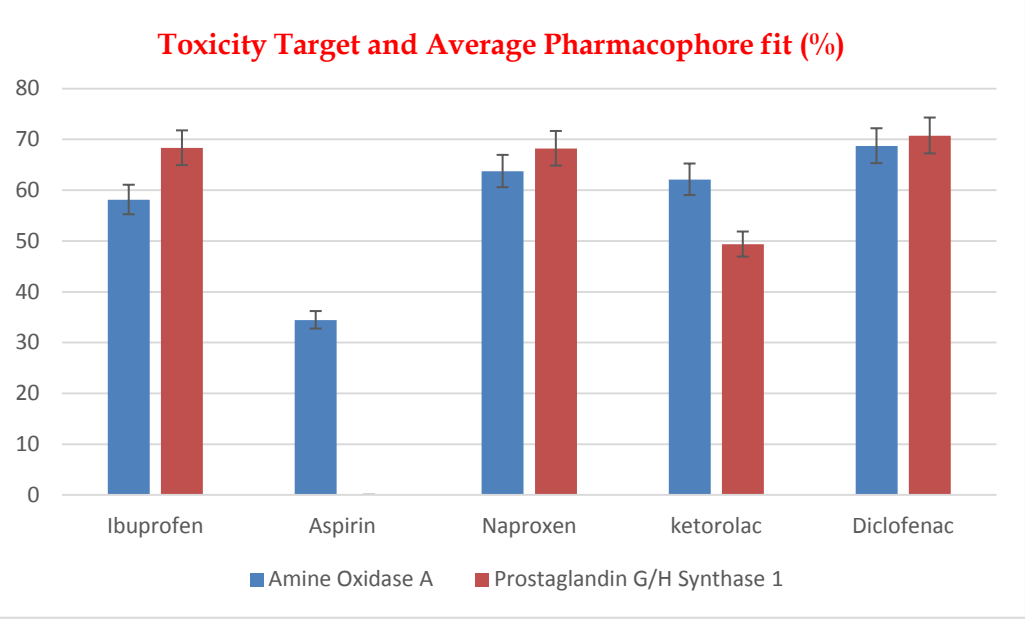


Fig. 6. Toxicity target

4. CONCLUSION

Five commonly used non-steroidal anti-inflammatory drugs were collected, including ketorolac, aspirin, naproxen, and diclofenac. On the basis of the DFT study of Diclofenac, it showed the lowest energy gap (-0.5827 eV), the highest ionization potential (5.0983 eV), the highest electron affinity (5.6810 eV), the highest electronegativity (5.3897 eV), the lowest chemical potential (-5.3897), the lowest dipole moment (1.1282) and the lowest energy (-1657.106). Using the ProTox II web server, the toxicity of drugs was determined based on their chemical structure. Results showed that Diclofenac has the highest chemical reactivity and the highest toxic effect among the non-steroidal anti-inflammatory drugs, while Ibuprofen has the lowest toxic effect. Based on the studies conducted through the ProTox II web server, Diclofenac demonstrated the lowest LD50 (53 mg/kg) value compared to Ketorolac (LD50=189 mg/kg), Naproxen (LD50=248 mg/kg), Aspirin (LD50=250 mg/kg), and Ibuprofen (LD50=189 mg/kg). The Diclofenac showed two types of toxicity targets, including hepatotoxicity and nuclear receptor signalling pathways, while other non-steroidal anti-inflammatory drugs showed only one type of toxicity target. It can be concluded from the toxicology results of Diclofenac that it is a more toxic NSAID than other NSAIDs, and to some extent, these findings are in accordance with those derived from DFT studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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