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Repositioning of 2,4-Dichlorophenoxy acetic acid as a potential anti-inflammatory agent: *In Silico* and Pharmaceutical Formulation study

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ABSTRACT

2,4-Dichlorophenoxy acetic acid (2,4-D) is a well-known plant auxin which is widely used in plant tissue culture experiments as well as a weed killer and a herbicide. In this study, 2,4-D was rediscovered as a new anti-inflammatory agent through an *in silico* molecular modeling and docking studies along with drug formulation and *in vivo* anti-inflammatory inspection. The molecular modeling and docking studies indicated high affinity of 2,4-D toward COX-2 enzyme in a way similar to Ibuprofen, suggesting a higher anti-inflammatory activity. Molecular docking by both MOE 2013.08 and Leadit 2.1.2 revealed excellent binding pattern compared to some of well-known non-steroidal anti-inflammatory drugs. 2,4-D was formulated in different gel bases. *In vitro* drug release experiments were used to examine the best 2,4-D formula for *in vivo* studies. *In vivo* carrageenan-induced hind paw edema inflammatory model in rats was used to test the *in silico* finding. 2,4-D showed potential *in vivo* anti-inflammatory activity and significantly reduced the concentration of prostaglandin E_2 in hind paw tissues in a way similar to Ibuprofen. These results may open the door to introduce a new anti-inflammatory molecule; especially that 2,4-D is a well-investigated regarding its toxicity and side effect.

1. Introduction

Drug discovery is a tedious, time-consuming and expensive process results in the development of a new drug. This was the rationale behind the emergence of drug repositioning concept as a way for rediscovery of already known drug for new uses and applications (Ashburn and Thor, 2004). Drug repositioning, drug re-profiling, drug rediscovery and drug repurposing are all synonyms for recognizing the presence of new medicinal use for old, already known drug or chemical (Da Fonseca and Casamassimo, 2011; Padhy and Gupta, 2011; Tobinick, 2009). This concept has rapidly developed as a cost-lowering alternative that supply new medicinally active drugs to the market. Recently, many old drugs have been rediscovered for their new activity as medicinal agents, examples are aspirin, which is an anti-inflammatory drug, is rediscovered as an anti-platelet and cancer prevention drug. Another example is anti-fungal drug Amphotericin B which is used now in the treatment of Leishmaniasis (Sekhon, 2013). Many old drugs have been repurposed for the treatment of many diseases such as Alzheimer (Corbett et al., 2012) and stroke (Fagan, 2010). The repurposing process should provide new indication for compounds

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and/or chemicals in a new formulations, and route of delivery (Liu et al., 2013). 2,4-Dichlorophenoxy acetic acid (2,4-D) is one of the very well-known plant hormones belonging to the tribe of auxins. 2,4-D has been extensively used in plant tissue culture experiments for induction of callus (Yang et al., 2013)or for organogenesis (Cheruvathur et al., 2013; Kumar et al., 2012; Lü et al., 2013). The drug is also known especially in the USA as a weed killer and herbicide (Garry et al., 1999; Grossmann, 2010; Song, 2014). The use of 2,4-D as a herbicide enabled the study of its effects on human due to close contact. Studies showed that 2,4-D is considered to be a drug of low toxicity to animals with a LD_{50} of 553 mg/kg in rats which is similar to the LD50 of ibuprofen (Gorzinski et al., 1987). There is no evidence that this drug can cause any type of cancer, or to affect any vital system in the human body including the reproductive system in both male and female (Burns and Swaen, 2012; Garabrant and Philbert, 2002; Ibrahim et al., 1991; Saghir et al., 2013). 2,4 D is also cheap and available either from nature or with simple chemical synthesis with high yield and easy purification.

Molecular Modeling tools have recently been discovered and developed to be used in design, modify and discovery of new chemical entities. These tools have been used for the prediction of the biological activities of unknown compounds compared to reference drugs. The recent advances in computer-aided drug design enabled the scientists to interpret the mode of action of many enzyme inhibitors. The use of such tools in the process of drug repurposing may be of great interest, providing a powerful way to find new medical uses either for old drugs or for those with non-medical use in new dosage forms. Drug Discovery tools help in identifying new targets of known drugs in new diseases (Gad, 2005).

Topical drug delivery systems are widely used and many drugs have been successfully delivered via this route for both local and systemic action. Additionally, drug delivery through skin is a promising route due to its easy access, and large surface area exposed to the circulatory and lymphatic networks. In particular, gels have better potential as a vehicle to deliver drugs topically in comparison with ointments, as they are non-sticky and require low energy during formulation (Jasti et al., 2005).

The aim of this study was to investigate the anti-inflammatory activity of 2,4-D as a potential new and cheap medicinal agent through the use of drug repurposing protocols. *In silico* comparative molecular modeling study was performed to predict the anti-inflammatory effect of the compound compared to Ibuprofen. Different pharmaceutical gel preparations were investigated in order to ensure the drug delivery to the site of inflammation locally and systemically.

2. Materials and methods

2.1. Materials

Molecular Operating Environment (MOE) 2013.08 package (Molecular Operating Environment (moe), 2014, 2013.08, Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7) license was purchased from Chemical Computing Group Inc, Sherbooke St, Montreal, QC, Canada (2014). Leadit 2.1.2 software license was purchased from BioSolveIT GmbH, Germany (Rarey et al., 1996).2,4-Dichlorophenoxy acetic acid (M.P = 136–140 °C), Ibuprofen (M.P = 77–78 °C),DMSO, HPMC (K15M), HEC, Carrageenan and Propylene glycol were purchased from Sigma Aldrich. Na CMC was purchased from El- Nasr Pharmaceutical Chemicals Co., Egypt. Cellophane membrane (Spctraper, M.W: Cutt of 12.000-14.000) was purchased from Fisher. Sci. Co., USA. Enzyme-linked immunoassay assay kit for prostaglandin E_2 (PGE_2) kits was purchased from My Biosource (USA).

2.2. Molecular docking studies

The molecular docking studies were done using MOE 2013.08 and Leadit 2.1.2 software.

2.2.1. Molecular docking studies with MOE 2013.08

All compounds were built and saved as moe. Rigid receptor was used as a docking protocol. Both receptor-solvent were kept as a "receptor". Triangle matcher was used as a placement method. Two rescoring were computed, rescoring 1 was selected as London dG. Rescoring 2 was selected as affinity. Force field was used as a refinement.

2.2.2. Molecular docking studies with Leadit 2.1.2

All compounds were built and saved as Mol2. The crystal structure of COX-2 enzyme complexed with Naproxen was downloaded from protein data bank (PDB: 3NT1). The protein was loaded into Leadit 2.1.2 and the receptor components were chosen by selection of chain A as a main chain which in complexed with Naproxen. Binding site was defined by choosing Naproxen as a reference ligand to which all coordinates were computed. Amino acids within radius 6.5 Å were selected in the binding site. All chemical ambiguities of residues left as default. Ligand binding was driven by enthalpy (classic Triangle matching). For scoring, all default settings were restored. Intra-ligand clashes were computed by using clash factor = 0.6. Maximum number of solutions per iteration = 200. Maximum of solution per fragmentation = 200. The base placement method was used as a docking strategy.

2.3. Pharmaceutical formulation

2.3.1. Gels preparation

Formulations FI and FII were prepared, as mentioned in the table below, by sprinkling the gelling agent on the surface of water; the vehicle was stirred until the gel was formed (Paranjyothy, 1994). In case of formulation FIII, the gelling agent was sprinkled in 250 ml beaker containing boiled distilled water and stirred at high speed until thin dispersion was formed. The solution was kept overnight at refrigerator (Tayel and Osman, 1995). 2,4-D was dissolved in a mixture of ethanol, propylene glycol and DMSO then added to previously prepared gel with continuous steering for 30 min.

Ingredients	Formulations		
	FI	FII	FIII
Drug (gm)	5	5	5
Na CMC (gm)	3	–	–
HEC (gm)	–	2.5	–
HPMC K15M (gm)	–	–	10
Ethanol (mL)	10	10	10
Propylene glycol (mL)	15	15	15
DMSO (mL)	10	10	10
Distilled water up to (mL)	100	100	100

2.3.2. Rheological studies

Viscosity determination of the different formulations was done at room temperature using Viscostar® (Fungilab SA., Spain) viscometer. The dynamic viscosity measurement was made using spindle R 6 at 1.5 rpm.

2.3.3. In-vitro release studies

Circular plastic holders of 2.9 cm inner diameter and 1 mm edge height were used as backing membrane reservoirs for the prepared formulae. The prepared formulation was weighed in these holders as 1 g for each holder. The holders provided even spread of