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TOPICAL ANTI-INFLAMMATORY AND HYDROGEN PEROXIDE SCAVENGING EVALUATION OF NICOTINAMIDE: DESCRIPTION IN THE EARLY STAGES OF ANTI-INFLAMATORY DRUG DISCOVERY PROCESS, WITH AND WITHOUT THE USE OF ANIMALS

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ABSTRACT
The aim of our work was to study in silico and the in vivo the challenge of topically applied drugs, using thenew approaches of the early stages of drug discovery process, with and without the use of animals. this work aimed to verify in silico the skin permeability and skin sensitization effect of a natural heterocyclic compound; vitamine B3 (Nicotinamide), and to evaluate in vivo the anti-inflammatory effect and in vitro hydrogen scavenging activity of this heterocyclic bioactive substance, which compared with the anti-inflammatory drug; indomethacin. The early results show the skin permeability of Nicotinamide, with (LogKp) values of -7.31 cm/s.In addition results of HOMO energies indicate that Nicotinamide has a lower skin sensitization effect with P value of 1.3452. Furthermore, the topical anti-inflammatory activity of Nicotinamide was investigated in Xylene-induced ear edema in mice model; our findings revealed that Nicotinamide at the dose of 2mg/mL caused a significant reduction of this edema, with 0.25±0.013 of thickness. Also, Nicotinamide present IC50 value of 333.44µg/ml against hydrogen peroxide. These early results demonstrate the significant activity, and the safety of Nicotinamide for the development of new topical formulation for inflammatory disease treatment.

KEY WORDS: Heterocyclic, Nicotinamide, Inflammation, Skin, Permeability, Sensitization, Animal.

INTRODUCTION
The search of new molecules with therapeutic interest and the development of efficient formulationsfrom bioactive molecules have become one of the major problems of scientists.The chemistry of nitrogen compounds has long been the preferred source for many

study subjects. In addition, heterocyclic chemistry has an interest in the pharmaceutical fields, due to the high importance of heterocyclic compounds, in particular nitrogen heterocycles.Among the different classes of these compounds; pyridine and its derivatives which play an interesting role in the synthesis of many other pharmacologically and biologically active products. They are insecticides, herbicides, antifungals, antibacterials, antivirals, and anticancers[i]. In the inflammatory disease treatment, some drugs induce adverse effects.In addition, the oral anti-inflammatory drugs, pose risks of gastrointestinal and renal toxicity[ii]. The major risks of traditional non steroidal drugs induced gastric damages and ulcers which the responsible of the deaths of 16.000 persons in the US and 10,000 in Canada in a year[iii]. As a result, the search for the development of new safe formulations with anti-inflammatory effects is very practical for protecting human health,withthe objective to treat local indications by topicalmedication. For this reason, the principle ingredient of each formulationis obliged to pass to acceptable safety test, which is the determination of any adverse or undesirable effect. Actually, the computational approaches lead to verify the adverse effect in toxicity assessment, which can lead to select a numbers of molecules in a short moment and with low costs[iv]. Furthermore, these efficient methods have a benefit of being capable to estimate of formulation's toxicity before the experimental development. In this context, and according to the early stages of anti-inflamatory drug discovery process, with and without the use of animals, this work aimed to verify in silico the skin permeability and the skin sensitization effect of a natural heterocyclic compound; vitamine B3 (Nicotinamide), and to evaluate in vivo the anti-inflammatory and in vitro hydrogen scavenging activity of this heterocyclic bioactive substance, which compared with the anti-inflammatory drug; indomethacin.

EXPERIMENTAL

REAGENTS

The chemical reagents used in this study are all of analytical quality and are purchased from Sigma Aldrich; Nicotinamide, *O*-Xylene, Ferrous ammonium sulphate, Hydrogen peroxide, 1,10-Phenanthroline.

SKIN PERMEABILITY COEFFICIENTS PREDICTION

The server SwissADME accessible at (http://www.swissadme.ch)[vi] was used in order to predict skin permeability coefficients of nicotinamide and Xylene.

DENSITY FUNCTIONTHEORY STUDY

The optimized structure and frontier molecular orbitals (HOMOs and LUMOs) were calculated using Software Gaussian 03[vii].

SKIN SENSITIZATION CALCULATION

Skin sensitization prediction was calculated using P value from E_{HOMO} energy (Hartree), with the following formula:(P) = 15.3× E_{HOMO} + 5.08[viii].

IN VITRO HYDROGEN SCAVENGING EFFECT EVALUATION

In this experiment, 125 µL of ferrous ammonium sulfate (1 mM) was mixed with 750 µL of test compounds of different concentrations, and 31.25 µL of hydrogen peroxide (5 mM) was added. The reaction mixture was incubated in the dark for 5 minutes, and then 750 µL was added. 100 µL 1,10-phenanthroline (1 mM). After 10 minutes of incubation in the dark, the absorbance was recorded at 510 nm[ix]. Use the following equation to calculate the hydrogen peroxide scavenging activity:

Hydrogen peroxide scavenging effect (%)=100x(A0-A1)/A0

A0 is the absorbance of the control; A1 is the absorbance of the test compound

IN VIVO TOPICAL ANTI-INFLAMMATORY EFFECT EVALUATION

ANIMALS

In this assessment, adult female albino mice (25-27 g) were used. These animals were purchased from 'Institut Pasteur d'Algérie', Algiers. Mice were housed in cages under standard conditions of 12:12 h light/dark cycle and 24 ± one °C for seven days before the experiments and kept under standard conditions.

XYLENE-INDUCED EAR EDEMA

Mice were randomly divided into three groups (n = 6):

Group 1 (positive control): received topically with indomethacin used as a standards drug (2 mg/ear),

Group 2 (negative control): received topically with Xylene (30 µL/ear)

Group 3: received topically with Nicotinamide (2 mg/ear).

The topical anti-inflammatory activity of Nicotinamide was investigated in xylene-induced ear edema in mice model. The application of Xylene on the ear causesthe accumulation of fluid leading to the formation of edema which is characteristic of acute inflammation[x]. First, 30 µL of Xylene was locally applied at the inner surface of the right ear of each mouse. Simultaneously, 30 µL of sodium chloride (0.9 %) containing 2 mg of Nicotinamide and 0.5 mg of indomethacin respectively, were topically applied at the corresponding place of the ear. Edema in the negative control group was provoked topically by Xylene in each mouse. The thickness of the ear was measured with a digital caliper before and two h after the xylene application.

RESULTS AND DISCUSSION

STRUCTURE OPTIMIZATION

Geometry optimization of Nicotinamide and *O*-Xylene was achieved by minimizing the energy (The most stable structures). The optimized molecular structures are shown in **Fig.1**.

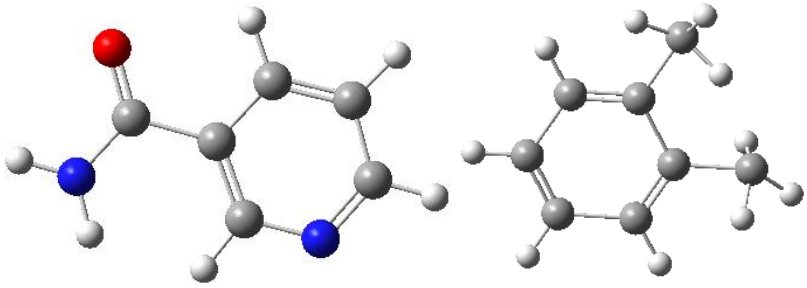

Figure1. Geometry optimisation of Nicotinamide and *O*-Xylene

HOMO AND LUMO ANALYSIS

For Nicotinamide, LUMO and HOMO orbitals are located on the whole molecule, with the values of -0.0857 ev and -0.2441 ev respectively **(Fig.2)**. The HOMO orbitals of Xylene are sitedon the whole molecule with the value of -0.1249 ev, and the LUMO orbitals are limited to a big area with the value of 0.0056 ev**(Fig.3)**.

The HOMO-LUMO energy gap (ΔE_{gap}), is an important factor for the characterization of molecular reactivity and stability.The small value of ΔE_{gap} indicates the high reactivity, and low stability[xi]. Our findings demonstrate the higher chemical reactivity of *O*-Xylene with ΔE_{gap}value of 0.1193 ev. Whereas, Nicotinamide has the value of 0.1584 ev. These results predict the possible toxicity of *O*-Xylene.

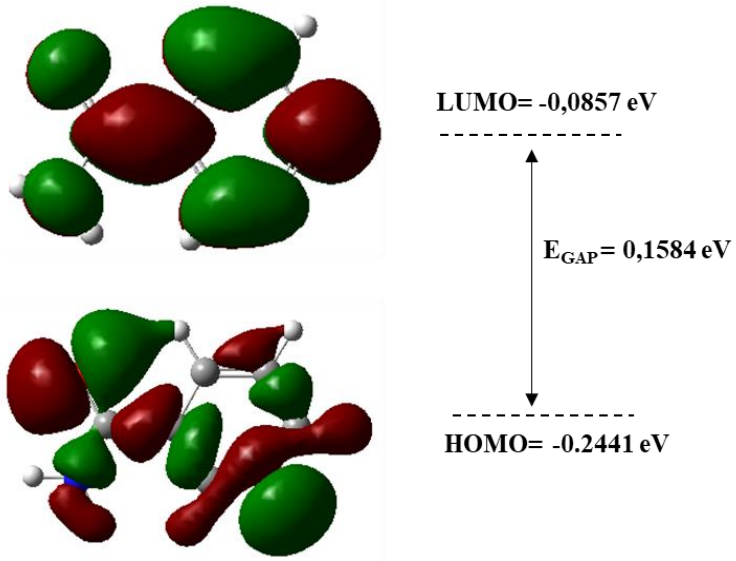

Figure2. The frontier molecular orbitals density distributions Nicotinamide compound

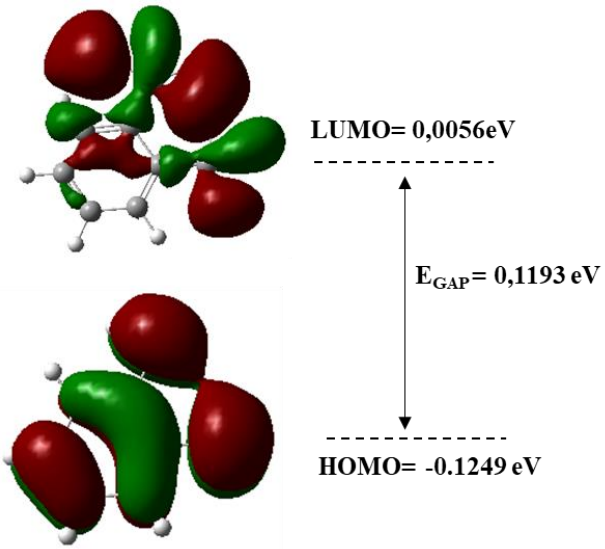

Figure3. The frontier molecular orbitals density distributions *O*-Xylene compound

SKIN PERMEABILITY COEFFICIENTS PREDICTION

The skin permeability constant (Kp) defines the speed of a chemical penetrating through the stratum. This value is widely used to quantitatively describe the transport of molecules in the outermost layer of the skin layer and indicate the importance of skin absorption. In literature, many in silico models for skin permeability prediction have been reported[xii]. Results in the **table1**show the skin permeability of Nicotinamide, followed by Indomethacin and *O*-Xylene with (LogKp) values of -7.31, -5.45 and -4.73 cm/s respectively. These results display the possibility to use Nicotinamide in dermatological application.

Table 1. Predicted values of skin permeability coefficient (LogKp)

Compound	**LogK (cm/s)**	**Prediction**
Nicotinamide	-7.31	Permeable
Indomethacin	-5.45	Permeable
***O*-Xylene**	-4.73	Permeable

SKIN SENSITIZATION CALCULATION

In toxicology, skin allergic reactions are essential questions for scientists[xiii]. The predictions of

the skin sensitization effect of drug arefacile methods, with low charge, and minimize the use of animals before and in the experiments[xiv]. In this instigation, the highest occupied molecular orbital energies (E_{HOMO}) of Nicotinamide and*O*-Xylenewere employed for skin sensitization calculation by using the P values. Results, exhibited in **Table 2**, demonstratehigh sensitizer effect of *O*-Xylene with P value of 4.9176. In addition Nicotinamide has lower sensitization effect with P value of 1.3452.

In the edema model induced by Xylene has been extensively used for assessing the acute anti-inflammatory characteristics of potential drug applicants. Displayed in **Table 2,**are the results of our study of the anti-inflammatory activity of Nicotinamideas well as indomethacin in mice. Our findings revealed that Nicotinamide at a dose of 2 mg caused a significant reduction of edema induced by Xylene in mice, with 0.25 ± 0.013 of thickness. In addition, the non-steroidal anti-inflammatory drug indomethacin reduced the edema inflammation; with 0.26 ± 0.010 of thickness at 0.5 mg. Topical employment of Xylene immediately results in an increase in vascular permeability as a response to the irritation produced by the chemical. The characteristic of acute inflammation was increased vascular permeability allows the infiltration of fluid and protein into the extravascular area, resulting in edema[xv]. This could suggest that the Nicotinamide reduces the vasodilation and plasma oxidation caused by substance P[xvi], which may be due to suppression of phospholipase A2 that is linked with the pathophysiology of inflammation caused by Xylene[xvii]. In literature, studiesreported that the effect of indomethacin on inflammation could be demonstrated by the inhibition of pro-inflammatory prostaglandin synthesis. Along this line, the presence of Nicotinamide may be effective for suppressing the exudative phase of acute anti-inflammatory features and then pain.

Theoretical findingare in good accordance with the achieved experimental results, which reveal that the use of computational method is an essential step before the experiment in the drug discovery process.

Table2. Theoretical and experimental results of skin sensitization effect

Theoretical results			**Experimental results**
Compound	**P values**	**Skin sensitization prediction**	**Thickness of ears (mm)**
***O*-Xylene**	4.9176	High-sensitizer	0.40±0.041***
Nicotinamide	1.3452	Low sensitizer	0.23±0.027^{ns}
Nicotinamide + *O*-Xylene	-	-	0.25±0.013**
Indomethacin + *O*-Xylene	-	-	0.25±0.030**
Normal group	-	-	0.23±0.010

Values expressed as mean ± SEM, n= 5 animals /group; ***: $p < 0.001$, **: $p < 0.01$,
ns: no significant differences

HYDROGEN PEROXIDE SCAVENGING EFFECT

Hydrogen peroxide (H_2O_2) is not a free radical with itself and is not very reactive, but it can sometimes be toxic to cells because it can form hydroxyl radical, which crosses cell membranes, and can oxidize and damage many cell compounds. In animal cells, its reaction with iron ions contributes to generation of the hydroxyl radicals which are damaging agents[xviii]. The principle of this method is to neutralize the H_2O_2 with an antioxidant which will facilitate its decomposition into water and oxygen according to the following reaction:

$$2H_2O2 \longrightarrow 2H_2O + O2.$$

The effect of Nicotinamide towards hydrogen peroxide is shown in **Fig4**. Results expose that this vitamin exhibited a scavenging effect with inhibition percentage of 69.69%, at the concentration of 496 µg/mL.

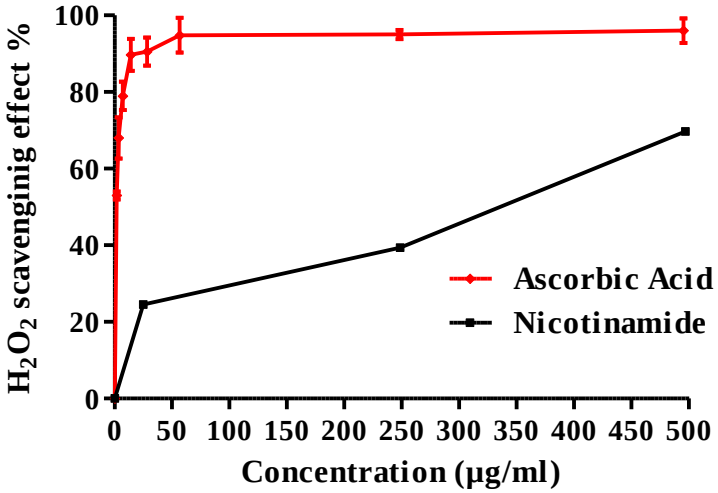

Figure4. Hydrogen peroxide scavenging effect of Nicotinamide and Ascorbic acid

The curves in the **figure4** were used to calculate the IC50 values of the tested compounds compared with Ascorbic acid's IC50 used as a standard.
The results obtained in **Table 3,** show that the two molecules present IC50 values of 333.44, 2.33 and 4.60 µg/ml for Nicotinamide, Ascorbic acid and indomethacin respectively.

Table 3. IC_{50} values of Nicotinamide, Ascorbic acid, and Indomethacin

Compound	**IC50 values (µg/mL)**
Nicotinamide	333.44 ± 2.25***
Ascorbic acid	2.33 ± 0.11
Indomethacin	4.60 ± 0.26***

Values expressed as mean ± SD, n= 3;*******: $p < 0.001$.

CONCLUSION

Topical anti-inflammatory results demonstrate the significant activity, and the safety of the tested heterocyclic compound (Nicotinamide) for the development of new topical formulation in inflammatory disease treatment. In addition, Theoretical finding are in good accordance with the achieved experimental results, which reveal that the use of computational method is an essential step before the experiment in the drug discovery process.

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CONFLICT TO INTEREST

The authors declare that there is no conflict of interest.

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