

2,4-Dichlorophenoxy Acetic Acid as an Antidiabetic Drug: *In silico*, Preformulation and *in vivo* Approaches

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ABSTRACT

Background: 2,4-Dichlorophenoxy acetic acid (2,4-D) was recently rediscovered as new anti-inflammatory agent through an *in silico* molecular modeling and *in vivo* anti-inflammatory inspection. Further computational investigations showed very close similarity between 2,4-D and pioglitazone in the mode of binding to PPAR γ ligand binding pocket, suggesting an antidiabetic activity. **Aim:** To evaluate the binding strength of 2,4-D to PPAR γ binding pocket and to improve the low water solubility of 2,4-D in formulation. **Methods:** particle size reduction via Nano Spray Dryer B-90 was chosen as possible technique to enhance the drug solubility and in turn facilitating its formulation. Chemical and physical properties of both raw and micronized forms of 2,4-D were investigated utilizing FTIR and DSC respectively. Additionally, dissolution profiles in different dissolution media were evaluated. Finally, the antidiabetic activity of micronized 2,4-D was investigated using streptozotocin (STZ)-induced animal model. **Results:** Revealed enhanced dissolution profiles for the micronized form of the drug on all media under investigation compared to its raw form. The *in vivo* antidiabetic activity for micronized 2,4-D form indicated no significant difference in its blood sugar lowering activity compared to pioglitazone as reference drug. **Conclusion:** the previous results could suggest micronized 2,4-D as a cheap antidiabetic agent with similar activity to pioglitazone.

Key words: 2,4-D, Micronized, Pioglitazone, Repositioning, Streptozotocin.

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INTRODUCTION

In silico, repositioning has been widely used in the last few years to find new and best target for old drugs or chemicals.^{1,2} This approach requires the application of some computational techniques for the validation of the target selection. Many successful stories have been reported for the repositioning of some compounds and were introduced in a novel dosage form to treat potential diseases.

Peroxisome Proliferator Activated Receptor gamma (PPAR γ) agonists constitute a well-known class of drugs for treatment of diabetes mellitus type II.³ Many modulators were reported to bind and activate

these receptors such as thiazolidinedione.⁴ Recently, non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as Ibuprofen, Flurbiprofen and Indomethacin have been reported to bind and strongly activate PPAR γ .⁵⁻⁸ Their binding was proven to be at the ligand-binding site of PPAR γ where thiazolidinediones normally bind. This resulted in the raising of the possibility of new pharmacological actions to these agents.

2,4-dichloro phenoxy acetic acid (2,4-D) is one of the most commonly used phenoxy herbicides due to its efficacy and cheap cost.⁹ The wide use of that herbicide made it one of the most scru-



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tinized herbicides regarding its effect on human. Although some reports indicated possible health hazards associated with use of the herbicide, including carcinogenic activities, the majority of studies revealed its safety.¹⁰ Our research group has recently discovered 2,4-D as a new potential anti-inflammatory agent; which is strongly related to NSAID.¹¹ 2,4-D was firstly reported by WHO in 1984 to produce hypoglycemic effect on humans when it was used to wipe up weeds. Additional investigation was performed by Mikov and coworker,¹² showed hypoglycemic effect in Albino BALB/C mice following 2,4-D IP administration. However, no further work was done on such molecule to investigate its anti-diabetic versus neither its hypoglycemic activity nor its suitable and effective dosage form.

Orally administered dosage forms represent the easiest way of drug delivery systems, since it is characterized by accuracy, stability and easy production.¹³ However, more than 40% of newly discovered active pharmaceutical ingredients (APIs) are poorly soluble in water and, according to biopharmaceutical classification system; could be categorized in class II or IV.¹⁴ Unfortunately, 2,4-D could be added to such categories of constituents showing poor aqueous solubility. 2,4-D showed water solubility of less than 0.9 mg/mL, which could represent a major obstacle for its oral formulation and administration. Hence, enhancement of solubility and dissolution character of 2,4-D represented important goal in order to achieve higher drug absorption and bioavailability. Several enhancement approaches were developed to overcome solubility and dissolution difficulties such as particle size reduction, solubilization, salt formation, inclusion complex, solid dispersion and nano-suspension.¹⁵⁻¹⁷ Particle size reduction represents the traditional method for solubility and dissolution enhancement. Decreasing the particle size of poorly soluble drug resulted in increasing its surface area hence; improve its dissolution properties allowing its oral administration.¹⁸ Jet milling, ball milling, high-pressure homogenization are considered as mechanical techniques practically used for particle size reduction.^{18,19} Micronization, can be defined as decreasing drug particle size less than 10 micrometer.¹⁸ Recently, Buchi pharmaceuticals introduced the Nano Spray Dryer B-90, through which powder micronization approach became accessible.²⁰ Straightforward preparation and higher collection efficiency are achieved through their patented piezoelectrically vibrating nozzle in addition to electrostatic particle collector.²¹ Recently, Nano Spray Dryer B-90 is investigated intensively for solubility and dissolution enhancement of several APIs^{20,22,23}

To the author's knowledge, the antidiabetic effect of pure 2,4-D following its oral administration had not been previously investigated. Accordingly, the main aim of the current study is the investigation of the antidiabetic activity of 2,4-D depending on its binding to PPAR γ *in silico*. The *in vivo* antidiabetic effect of the drug was inspected after improving its solubility and dissolution characters through micronization technology.

MATERIALS AND METHODS

Materials

Molecular Operating Environment (MOE) 2013.08 package.²⁴ Absolute ethanol, glycerol, streptozotocin, pioglitazone and 2,4-D were purchased from Sigma-Aldrich.

Molecular Docking Studies

Preparation of the Protein

The crystal structure of PPAR γ in complex with pioglitazone was downloaded from protein data bank (pdb code = 2XKW)²⁵ that was resolved by x-ray diffraction method (resolution 2.02 Å) and R-value free 0.22. All coordinates of the binding site were derived from protein data bank where the ligand-binding domain was identified. The protein has two identical chains A and B as a result we deleted Chain B and used only Chain A. Also, polyethylene glycol molecule that was co-crystallized with chain A was deleted to simplify the docking process.

The crystal structure of PPAR γ in complex with indomethacin was downloaded from protein data bank (pdb code = 4XUM) to obtain the best binding pose for indomethacin.

Molecular Docking Studies with MOE 2013.08.

The chemical structures of the studied drugs was sketched by MOE 2013 builder. London dG was used as the scoring method while, affinity was used as second rescoring, the placement method was selected to be "Triangle matcher".

Validation of the docking process.

Validation of the docking process was used by redocking the compounds using Leadit 2.1.5²⁶ software was purchased from BioSolveIT GmbH, Germany.

Pharmaceutical Preformulation of 2,4-D.

Pulverization and Size Adjustment of Raw 2,4-D.

Raw 2,4-D was pulverized using traditional porcelain mortar, followed by particle size adjustment through

sieving method (U.S. standard test sieve ASTM E-11 specification, Fisher Scientific Company, USA).

Preparation of Micronized Crystal using Buchi Spray Dryer B-90

100mg/mL clear solution of 2,4-D in absolute ethanol was prepared. The previous solution was pumped into Nano Spray Dryer B-90 through pump 1 with flow rate (7mL/ min). The drying gas was heated up to 68°C inlet temperature and 23°C out temperature. The spray mesh was (7 μ) and the nozzle was heated to 70°C with pressure 51 mbar. The drying gas flow rate was 85 ml/min and it was filtered before existing from the sprayer. The product was collected using a rubber special spatula. Microcrystal yield was calculated based on the original amount of raw drug utilized. % yield = (Weight of collected microcrystal/ Weight of raw drug) x 100

Microscopical Examination and Particle Size Analysis

Small amounts of raw and micronized 2-4 D were spread on a glass slide as a thin layer and examined for drug crystals using a inverted microscope with magnification power of 40 x. Photomicrographs were taken using a Olympus digital camera. One mg of either raw or micronized 2,4-D was suspended in 5 mL of 50% v/v glycerol in water and used to assess their particle size. The measurement was carried out through Zetasizer (Malvern Instruments Inc., Westborough, MA).

Angle of Repose

Funnel method was used for angle of repose determination. Briefly, two grams either raw 2,4-D or its microcrystal form are allowed to flow through the funnel. The tip of the funnel was adjusted where it just touches the apex of powder's heap. The powder heap's diameter was measured and the angle of repose was calculated according to the following equation; $\tan \theta = h/r$, where h represents the height of the powder's heap and r represents heap's diameter.

Physical and Chemical Investigations

Differential Scanning Calorimetry (DSC)

The DSC thermograms were recorded (DSCShimadzu- 50, Japan). Samples (3 mg) were heated in hermetically sealed aluminum pans over the temperature of 50-200°C at a constant rate of 10°C/min under a nitrogen purge (30 mL/min) as stated in.²⁷

Fourier Transform Infrared (FTIR)

FTIR spectra were obtained on a Perkin- Elmer (Perkin-Elmer- FTIR spectrophotometer, 1600 series,

Perkin-Elmer Corporation, Norwalk, USA) using KBr disk method. The scanning range was 200-4000 cm^{-1} and the resolution was 1 cm^{-1} .²⁷

Solubility Determination

Saturation solubility was measured, where, excessive powder of either raw 2,4-D or its microcrystal were added to 10 mL stoppered flask, followed by adding either distilled water, 0.1 N HCl buffer, phosphate buffer pH 5.5, phosphate buffer pH 6.8 and phosphate buffer pH 7.4. The prepared suspensions were placed at $37 \pm 0.5^\circ\text{C}$ and with shaking at 50 min^{-1} using GFL 1086 thermostatic shaker water bath (Labortechnik, Burgwedel, Germany). After 24 hr, suspensions were filtered, diluted with the same dissolution medium and assayed spectrophotometrically at 282 nm. Each experiment was carried out in triplicate.

Drug Dissolution

The dissolution of either raw 2,4-D or its microcrystal form was carried out according to the USP-24 rotating paddle method (dissolution tester, Erweka-DT-Frankfurt, Germany). Distilled water, 0.1 N HCl buffer, phosphate buffer pH 5.5, phosphate buffer pH 6.8 and phosphate buffer pH 7.4 (500 mL) were used as different dissolution medium. The stirring rate was 50 rpm and the temperature was maintained at $37 \pm 0.5^\circ\text{C}$. 250 mg of either raw 2,4-D or its microcrystal was placed on the surface of the dissolution medium. At specified time intervals, 3 mL aliquots were withdrawn, filtered, diluted with dissolution medium and assayed spectrophotometrically at 282 nm. Each experiment was carried out in triplicate.²⁸

In vivo Antidiabetic Activity

Animals

Male Wistar rats (200 \pm 10 g, 6–8 weeks) were used. Rats were obtained from the Animal Breeding Center, Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were housed with food and water and maintained in standard laboratory conditions, at temperature $25 \pm 1^\circ\text{C}$ and relative humidity $55 \pm 5\%$ with a 12-hr light/dark cycle.

Ethical statement

All animal experiments comply with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All animal experimental procedures and protocols were approved by the Animal Research Ethics Committee at Zagazig University, Egypt (number 3082015) and they were performed in accordance with

the Guidelines for the Ethical Conduct for Animals Handling in Research, Zagazig University (ECAH-RZU), Egypt.

Experimental Design

Antidiabetic activity was evaluated utilizing type II diabetic rats. Animals were rendered diabetic by intraperitoneal injection of streptozotocin (50mg/kg) freshly dissolved in 0.1 M citrate buffer at pH 4.5. Confirmed diabetes induction occurred 14 days following streptozotocin injection. Rats with serum glucose level over 250 mg/dL were selected for further experiments. Rats were randomly divided into 3 groups with 6 animals in each. 20mg/kg micronized 2,4-D and pioglitazone, were given orally to group 3 and 4 leaving group 2 as untreated. A group 1 was created to represent normal rat without STZ injection. Blood samples were collected from animal tail and assessed for blood glucose levels at specified time intervals after glucose challenge by using Accu-Chek® active laboratory equipment supplied with Accu-Chek® active strips Code 970 (Roche, Mannheim, Germany).²⁹

Statistics

For comparison, a one way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) as a *post-hoc* test was applied, using SPSS program version 14. The difference was considered as significant when $P < 0.05$.

RESULTS AND DISCUSSION

PPAR are members of nuclear receptor supergene family that are acting as key sensors or glucose and lipids homeostasis. They have three isoforms; α , β and γ . PPAR γ has a main role in the glucose metabolism and are used as targets for some anti-inflammatory, antidiabetic drugs. The ligand binding pocket of PPAR γ has a conserved α -helical core that is composed of 12 α -helices and 4 β -sheets. The α -helix 12 covers the ligand binding pocket and contains the most conserved residues; His323, His449 and Tyr473. Thiazolidinediones are well known PPAR γ agonists that act by binding to the ligand binding pocket by strong interaction with Tyr473.²⁵ The anti-inflammatory activity of 2,4-D recently discovered and its structure similarly to Ibuprofen, together with the lately detected strong binding of NSAID to the PPAR γ active binding site²⁵ in a mode similar to the thiazolidinedione class of antidiabetic drug. All the above reasons triggered the investigation of the antidiabetic activity of 2,4-D, both *in silico* and *in vivo*, which is the main purpose of this current study. The poor solubility of 2,4-D in aqueous

solution prompted its pharmaceutical formulation to a dosage form suitable for oral administration and thus facilitate the *in vivo* determination of its antidiabetic effect.

Molecular Docking

The binding mode of both indomethacin and pioglitazone were reported in protein bank (pdb 4XUM 2XKW respectively). The main interactions formed by indomethacin were; hydrophobic interactions with Phe363 through π - π stacking with the p-chlorobenzoyl moiety, three hydrogen bonding with His449, Tyr473 and His323. At physiological pH the histidine amino acid can form a positive charge therefore, a possible salt bridge formation with the carboxylate anion of indomethacin can occur that can strengthen the interactions (Figure 1A). On the other hand, the interactions formed by pioglitazone were three hydrogen bonds with His323, Tyr473 and Cys285 (Figure 1B). The protein data bank used the deprotonated state for the carboxylate group of indomethacin (Figure 1A).

In order to evaluate the binding mode of 2,4-D and to compare it to different PPAR γ agonists, the docking study was done for 2,4-D, indomethacin, ibuprofen, flurbiprofen and pioglitazone inside the PPAR γ ligand binding site. According to the docking results, both indomethacin and 2,4-D showed two hydrogen bonds formed by the carboxylic group with both Cys285 and Ser289 (Figure 2).

On the other hand, the docking of pioglitazone was not able to accommodate it and keep its interactions and showed no interactions with the specified residues. In addition, pioglitazone bulky structure resulted in some clashes and some parts to exit from the binding site (Figure 2C).

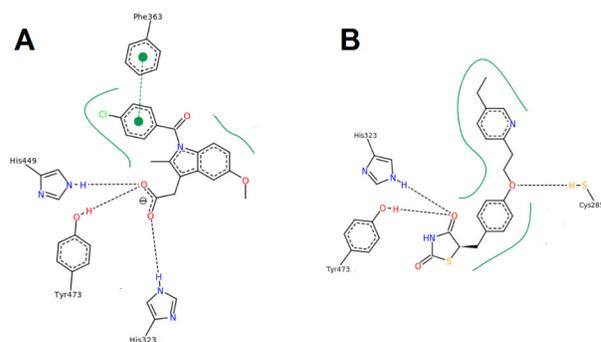


Figure 1: The main PPAR- γ agonists interactions that were derived from protein data bank for both A. Indomethacin showing its hydrogen bond with Cys285 B. Pioglitazone that illustrates three hydrogen bonds with Cys285, His323 and Tyr473.

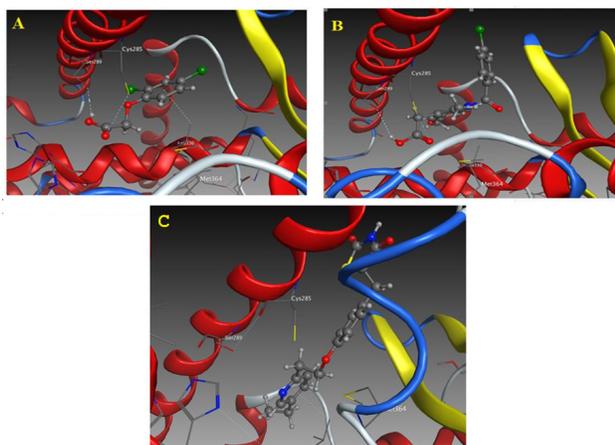


Figure 2: The best orientation mode of both 2,4-D (A) and indomethacin (B). It shows the interactions with both Cys285 and Ser289. (C) The docking of bulky structure of pioglitazone resulted in deviation from its original pose and affected the interactions.

The docking score of 2,4-D was -7.35 kcal/mol and for indomethacin was -7.37 kcal/mol (Table 1). The docking results were of high prediction that 2,4-D has a good fitting and binding mode with less RMSD toward binding to PPAR γ when compared to NSAIDs and thiazolidindione agent, accordingly, it was predicted to have antidiabetic activity. Nevertheless, to investigate the antidiabetic activities of 2,4-D, solubility and dissolution characters of the drug has to be improved through pharmaceutical formulations.

Validation of the docking process

In order to validate the docking, a second docking process was conducted using Leadit 2.5.1 program, the program that is widely used by protein data bank. The results showed that Indomethacin was the top ranked docking score (-24.56 kcal/mol), Ibuprofen and Fluoriprofen shared almost the same value (-23.15 and -23.11 kcal/mol respectively). 2,4-D showed -22.85 kcal/mol while pioglitazone was the last score -17.20 kcal/mol. This confirmed the results obtained by MOE 2013 docking (Table 1). Regarding the formed interactions, it was found that 2,4-D formed a number of interactions with the conserved residues like; His323, His449, Tyr473, Tyr327 and Ser289 when compared to the other ligands (Figure 3).

Pharmaceutical Preformulation of 2,4-D

Commercial 2,4-D powder was characterized by relatively large and non-homogenous particle size. Raw 2,4-D were well pulverized and particle size of the resulting raw crystalline powder were adjusted by sifting through standard sieve and the pulverized powder

Table 1: Molecular docking results of 2,4-D, DAIDs and pioglitazone.		
	ΔG Kcal/mol (MOE2013)	Docking score with lead it 2.5.1 Kcal/mol
2,4-D	-7.35	-22.85
Pioglitazone	-6.85	-17.20
Ibuprofen	-7.12	-23.15
Fluriprofen	-6.94	-23.11
Indometacin	-7.37	-24.56

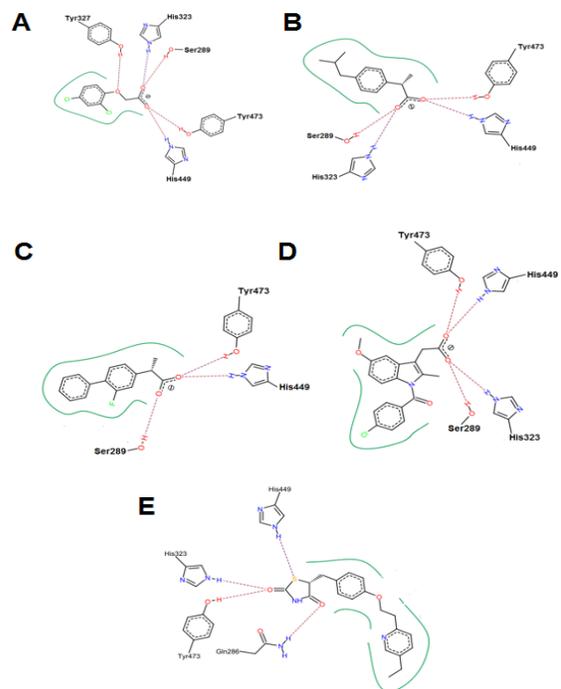


Figure 3: Molecular docking with leadit 2.5.1 showing the best pose of: A) 2,4-D, B) Ibuprofen, C) Fluoriprofen, D) Indomethacin and E) Pioglitazone.

successfully passed through sieve No. 45 (355 μ) and retained on sieve No. 100 (150 μ).

In order to prepare the micronized form of 2,4-D, a solution of the drug in absolute ethanol were pumped into spray head of Buchi spray dryer B-90. Piezoelectrically driven mesh vibrations in the spray head resulted in spraying drug-ethanol droplets in a homogenous and micronized size. Hot drying gas feed into the equipment chamber resulted in sudden drying of the sprayed particles in addition to electrically charging the resulting dried micronized particles. Dried fine particles were adhered on the oppositely charged collector and then collected using a particle scraper.³⁰

The percentage yield of particles was found to be $75 \pm 5\%$. The loss of yield could be attributed to the particles that are sucked into the glass-drying chamber before

reaching the collecting electrode, due to the smaller particle size and in turn smaller density.³¹

Physical and chemical investigations

In order to verify that enhancement of dissolution rate is not due to change the physico-chemical structure. DSC is a physical technique used to investigate the variation involved in phase change. In addition it is reflected the Crystallinity of the raw materials.³² DSC was performed and the results are shown in Figure 4.

The DSC thermograms, of both raw and micronized 2,4-D revealed a similar sharp endothermic peak at 141°C which is consistent with melting point of pure 2,4-D. There is no change in the melting point, indicating that the solid state of the particles did not exhibit any physical change in the crystalline structure of the raw drug following spray drying. FTIR results (Figure 5) indicated the presence of strong peak at 1725 cm⁻¹ peak which can be attributed to the C=O carbonyl band of carboxylic group. The peaks at 1600 and 1580 cm⁻¹ are assigned to the aromatic ring. CH stretching vibration in the aromatic ring is a weak peak at 3040 cm⁻¹. In addition, the peaks 2925 and 2854 cm⁻¹ stretching arise from the asymmetric and symmetric stretching vibration peaks of CH₂. The peaks at 1180 cm⁻¹ and 1100 cm⁻¹ are due to the C-O vibration in Ph-O-CH₂-. The C-C vibration in CH₂-C=O appears at 1420 cm⁻¹. Finally, the broad stretching band from 2500 – 3300 cm⁻¹ is due to the -OH group. It is clear that the two FTIR spectra are almost the same, which means that the chemical structure of the drug has not been affected.

Microscopic Examination, Particle Size Analysis and Angle of Repose

Microscopic examination of the prepared raw and micronized 2,4-D indicated a crystal shaped powders with a marked optically recognized difference of their particle size (Figure 6). The particle size of raw 2,4-D was previously adjusted to be in range of 150-355 μ. On the other hand, the average particle size of micronized 2,4-D was 5.1 μm measured with PDI 0.299. In order to evaluate enhancement of powder flowability, the angle of repose was measured and the results were 35.5°C (passable flow) and 26.4°C (good flow) for raw and micronized 2,4-D respectively, indicating free flowing powder,³³ which is a key requirement for a good pharmaceutical formulation and further processing of microcrystal 2,4-D could be straightforward.

Solubility Determination

Saturation solubility or thermodynamic solubility can be defined as the solubility of compound in

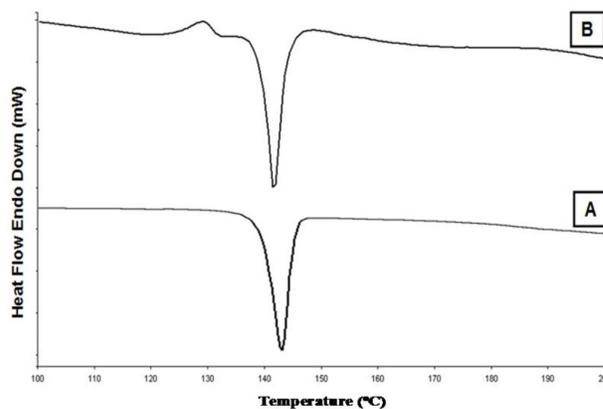


Figure 4: DSC spectra of 2,4-D; A. Raw crystalline form; B. Microcrystalline form.

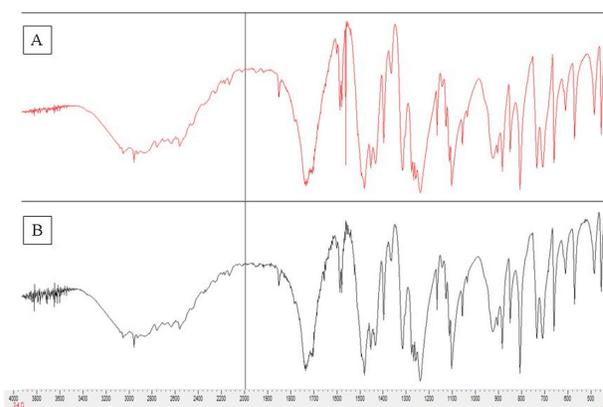


Figure 5: FTIR spectra of 2,4-D; A. Raw crystalline form; B. Microcrystalline form.

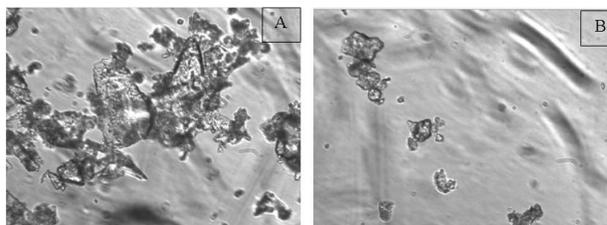


Figure 6: Optical Photomicrographs of 2,4-D A. Raw crystalline form; B. Microcrystalline form (magnification power is 40X).

equilibrium with excess undissolved materials at the end of the dissolution process.¹⁴ Several factors are affecting the saturation solubility of the compound including: polymorphism, particle size, particle shape, buffer pH and common ion effect. Saturation solubility represents a gold standard for product development, therefore, the solubility of raw and micronized 2,4-D was assessed and the results are shown in Table 2.

From the results obtained, it is clear that a significant enhancement in the water solubility of micronized

Table 2: Saturation solubility of raw crystalline 2,4-D and microcrystalline 2,4-D measured in different media.

Media	Crystalline 2,4-D (mg/mL) ± SD	Microcrystalline 2,4-D (mg/mL) ± SD
Dist. Water	0.84±0.07	1.59±0.06
pH 1.2	0.36±0.05	0.48±0.01
pH 5.5	1.73±0.11	1.99±0.05
pH 6.8	13.79±0.23	15.83±0.16
pH7.4	22.63±0.25	26.99±0.19

2,4-D 1.596 ± 0.012 mg/mL in comparison with 0.846 ± 0.014 mg/mL for raw drug. The improvement of drug solubility could be attributed to the marked reduction on the particle size as a result of the spray-drying process. On the other hand, as shown in Table 2. The solubility of micronized 2,4-D was 0.483 ± 0.011 , 1.894 ± 0.051 , 15.830 ± 0.161 and 24.993 ± 0.191 mg/mL for pH 1.2, buffer pH 5.5, buffer pH 6.8 and buffer pH 7.4 respectively. The solubility of raw 2,4-D was 0.3623 ± 0.009 , 1.736 ± 0.107 , 13.790 ± 0.226 and 22.635 ± 0.088 mg/mL for pH 1.2, buffer pH 5.5, buffer pH 6.8 and buffer pH 7.4 respectively. The solubility of the both raw and micronized 2,4-D can be arranged ascendingly in respect to the increase in medium pH. The lesser solubility of both raw 2,4-D and its micronized form in pH 1.2 represent an obstacle for drug dissolution following oral administration, which may result in reducing their bioavailability.³⁴ Fortunately, micronized 2,4-D showed about 25% increased solubility in pH 1.2 along with significant increase in other media under investigation.

Drug Dissolution

The rate of drug dissolution is a limiting step in the absorption process following oral drug administration. Figure 7. Represents the dissolution process of either raw 2,4-D or its microcrystal in distilled water, 0.1 N HCl buffer, buffer pH 5.5, buffer pH 6.8 and buffer pH 7.4. Interestingly, the data obtained showed that, within 120 min the dissolution in distilled water was 54% and 98% for raw and micronized 2,4-D respectively. Additionally, within 120 min the dissolution in 0.1 N HCl buffer was 49% and 73% for raw and micronized 2,4-D respectively. A significant dissolution rate enhancement was obtained for micronized 2,4-D in both distilled water and 0.1 N HCl buffer, The previous enhancements are of great importance during drug formulation in addition to increasing drug absorption following oral administration.³⁵ Moreover, 100%

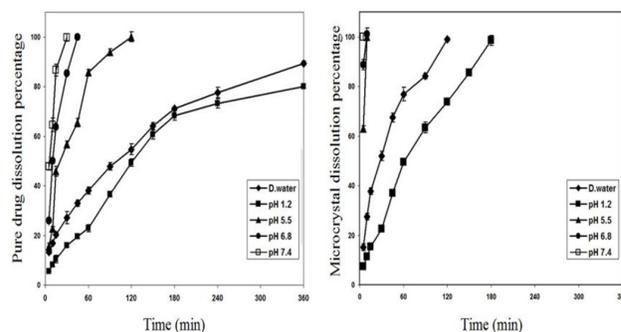


Figure 7: In vitro dissolution profiles of 2,4-D. A: Raw crystalline form; B: Microcrystalline form in different media at 37°C. Data represented as mean ± SD (n = 3).

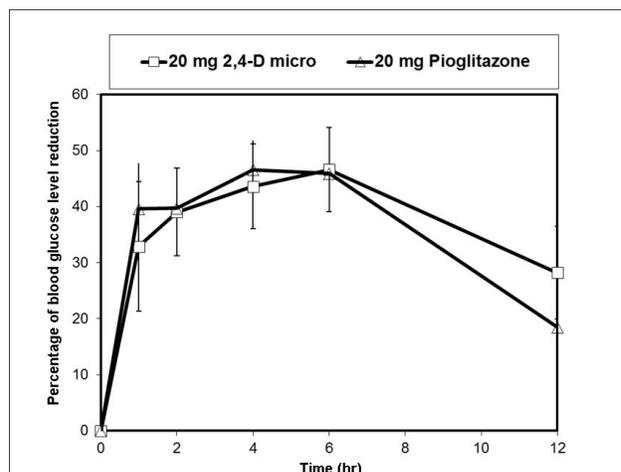


Figure 8: Blood glucose reduction level time profile following administration of 20 mg of either 2,4-D raw drug, 2,4-D micronized and pioglitazone orally into diabetic induced Wistar rats.

dissolution was obtained within 15 min and 120 min for of micronized and raw 2,4-D respectively.

In vivo Antidiabetic Activity

The antidiabetic activity of 20 mg of micronized 2,4-D was evaluated on STZ induced diabetic adult male Wistar rats. Pioglitazone was used as a positive control. The blood glucose level against time profile following oral administration of 20 mg of micronized 2,4-D or pioglitazone is shown in Figure 8.

A significant reduction in blood glucose level was obtained in all groups under investigation ($p < 0.05$). In addition, no significant difference in the blood glucose level was obtained among micronized 2,4-D-treated group and pioglitazone-treated group.

CONCLUSION

2,4-Dichlorophenoxy acetic acid was proven computationally to have good binding activity to PPAR γ ,

which suggested its antidiabetic activity. The molecular modeling and docking studies showed very close similarity between 2,4-D and pioglitazone in mode of binding to PPAR γ and its similarity to NSAIDs in affinity toward PPAR γ . The drug water solubility was enhanced through micronization techniques. Following oral administered into rats with induced diabetes, micronized 2,4-D showed significant reduction of the blood glucose level. Having the advantages of being cheap, low toxicity and easily chemically synthesized drug, 2,4-D could attract high attention as a potentially new anti-diabetic agent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

2,4-D: 2,4-Dichlorophenoxy acetic acid; **PPAR γ :** Peroxisome Proliferator Activated Receptor gamma; **FTIR:** Fourier Transform Infrared; **DSC:** Differential Scanning Calorimetry; **STZ:** Streptozotocin; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **APIs:** Active pharmaceutical ingredients; **MOE:** Molecular Operating Environment.

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PICTORIAL ABSTRACT



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SUMMARY

- 2,4-Dichlorophenoxy acetic acid was proven computationally to have good binding activity to PPAR γ , which suggested its antidiabetic activity.
- The molecular modeling and docking studies showed very close similarity between 2,4-D and pioglitazone in mode of binding to PPAR γ and its similarity to NSAIDs in affinity toward PPAR γ .
- The drug water solubility was enhanced through micronization techniques.
- Following oral administered into rats with induced diabetes, micronized 2,4-D showed significant reduction of the blood glucose level.
- Having the advantages of being cheap, low toxicity and easily chemically synthesized drug, 2,4-D could attract high attention as a potentially new anti-diabetic agent.

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