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RESEARCH ARTICLE

In Silico Pharmacokinetics, Molecular Docking, *In Vitro* and *In Vivo* Acute Toxicity Assessment of *Unani* Polyherbal Formulation: *Tiryaq-e-Wabai*

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Abstract

Background: *Tiryaq-e-wabai* is a *Unani* polyherbal drug that has been used as an antidote for a variety of systemic disorders and epidemic diseases, according to traditional *Unani* literature. Recently, this drug gained attention during the COVID-19 pandemic. **Aim:** In this study, both *in vitro* and *in vivo* systems were used to evaluate the acute toxicity of *Tiryaq-e-wabai*. **Methods:** Cytotoxicity was evaluated in L132 (human lung epithelial cell line) cells. Acute oral toxicity was determined in Sprague-Dawley rats administered a single dose of the drug at 2 g/kg body weight and observed for 14 days as per Organisation for Economic Co-operation and Development (OECD) guidelines. Histological examinations were performed to assess gross morphological changes in tissues. Collagen content and mast cell infiltrations in tissues were evaluated by imaging sections stained with Masson's trichrome and toluidine blue, respectively. **Results:** L132 cells remained significantly viable at various concentrations of the polyherbal drug at 24-hour and 48-hour intervals. *Tiryaq-e-wabai* had no harmful effects on experimental animals. No mortality occurred during the observation period. Neither gross biochemical, haematological, nor morphological changes were noticed. Furthermore, excessive collagen deposition and mast cell infiltrations in the tissues were not observed in control and experimental rats. To ascertain how the bioactive compounds in *tiryaq-e-wabai* interact with the Angiotensin-Converting Enzyme 2 (ACE2) complex, *in silico* molecular docking was employed. Ligands that exhibited their highest-scoring conformations with ACE2 were identified. Absorption, Distribution, Metabolism, Excretion, Toxicity (ADMET) and Density Function Theory (DFT) analyses were also performed. **Conclusion:** The present study shows that the tested *Unani* polyherbal drug does not cause cytotoxicity and can be well tolerated up to a dose of 2g/kg b. wt and highlights its efficacy. The outcomes justify further research into the antiviral potential of this specific *Unani* formulation.

Major Findings: *Tiryaq e wabai* has an LD_{50} value greater than 2000mg/kg b.wt. It can potentially prevent viral intrusion into host cells by blocking ACE2 (theoretically supported).

Keywords: Acute Oral Toxicity, ADMET, DFT, Molecular Docking, *Tiryaq-e-wabai, Unani* Polyherbal Formulation

1. Introduction

In the *Unani* system of medicine, some polyherbal formulations have been suggested for preventing and treating epidemic diseases[1]. *Tiryaq-e-wabai* is one of them, used primarily as a prophylactic drug for epidemic diseases and various systemic disorders. It is made in the form of pills and consists of three primary

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ingredients: *Aloe vera, Crocus sativus* and *Commiphora myrrh,* popularly known as *Sibr, Zafraan,* and *Mur* in the ratio of 2:1:1, respectively[2]. These ingredients possess a wide range of medicinal properties. Experimental and clinical studies conducted by researchers on *Aloe vera* (L.), *Crocus sativus* (L.), and *Commiphora myrrh* (T. Nees) have demonstrated their potential antioxidant and anti-inflammatory effects[3-5].

Commiphora myrrha (T. Nees) is a large shrub that secretes resin from its stem. It has been reported as an anti-inflammatory, astringent (disinfectant), antibacterial, anti-parasitic, and antispasmodic agent[6,7]. The dried red stigma of *Crocus sativus* (L.) contains bioactive phytochemical constituents such as carotenoids, sterols, terpenoids, flavonoids, etc., possessing medicinal properties and broad-spectrum activity[8]. *Aloe vera* (L.) is rich in vitamins, minerals, enzymes, lignin, saponins, amino acids, and salicylic acids. Phytochemicals present in *Aloe vera,* such as auxins and gibberellins, are believed to have wound-healing and anti-inflammatory properties[5].

The popularity of traditional herbal formulations can be attributed to their wide therapeutic range and negligible contraindications even at higher doses[1]. However, a safety assessment based on contemporary scientific guidelines was required. Therefore, this study was undertaken to evaluate the acute toxicity of this formulation both *in vitro* and *in vivo*.

It has been documented that the receptor-binding domain of the SARS-Cov spike protein can bind to the Angiotensin-Converting Enzyme 2 (ACE2) receptor, which is the mechanism by which the virus is transmitted from person to person[9]. Since *Tiryaq-e-wabai* was used as an antidote against Covid-19[10], using *in silico* molecular docking, it was possible to determine how the bioactive compounds in *Tiryaq-e-wabai* interacted with the ACE2. ADMET and DFT analyses were also performed.

2. Materials and Methods

All the analytical grade chemicals used were purchased from Sigma-Aldrich, St. Louis, MO, USA, SD Fine Chemicals, Mumbai, and Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Tiryaq-e-wabai was prepared according to the standard procedure of the *Unani* pharmacopoeia. Zafran (*Crocus sativus*) is dissolved in *Arq Gulab* (rose water) by slow grinding, followed by the addition of finely ground powder of *Mur Makki* (*Commiphora myrrha*) and *Sibr* (*Aloe barbadensis*) into the *Mahlool* (paste) of *Arq Gulab* and *Zafran* to make gram-sized pills[11].

2.1 Cell Viability Assay

Human lung epithelial cell lines (L132) were procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were maintained in a Dulbecco's Modified Eagle's Medium (DMEM) supplemented with Foetal Bovine Serum (FBS) (10%) and antimycotic solution (streptomycin and penicillin), and incubated at 37°C in a 5% CO_2 atmosphere. To determine the cell viability of *Tiryaq-e-Wabai* in L132 lung epithelial cells, the cells were cultured in 96-well plates and allowed to grow at around 90 % confluence and treated with *Tiryaq-e-Wabai* at various dose-dependent concentrations for 24 and 48 hours. Then 10µl of MTT solution (5mg/ml) in each well was added, followed by incubation for 3-4 hours. Later, 100µl Dimethyl Sulfoxide (DMSO) was added to each well, and finally, the absorbance was recorded at 570nm in a Bio-Rad iMark plate reader.

2.2 Acute Oral Toxicity

The experiment was performed according to the guidelines of the Committee for the Purpose of Control and Supervision (CPCSEA). The experimental protocol was approved by the Institutional Animal Ethics Committee vide BRULAC/ SDCH/ SIMATS/ IAEC/09 22/06. The animals were acclimatised to new environmental conditions. Sprague Dawley Rats were kept under standard conditions with access to standard feed and water ad libitum. Animals were fasted before the commencement of the test.

The polyherbal formulation was administered as a single dose (2000mg/kg b. wt.) to female Sprague Dawley rats (weighing approximately 250 g) using an oral gavage tube. Each phase of the study involved the use of three rats per step based on a "limit test" following the OECD 423 guidelines[12]. An additional group served as control and was administered with normal saline (2 ml/kg b. wt.). The animals under study were checked for any general behaviour changes and mortality/morbidity throughout the experimental period. Clinical observation was done to detect the

presence of toxicity signs. After a 14-day observation period, the animals were euthanised under light anaesthesia. Organ weights (Brain, Heart, Lungs, Liver and Kidneys) were recorded. To check the macroscopic morphology of the tissues and organs, the animals were dissected.

Haematological parameters were analysed in freshly collected blood samples in vacutainers containing anticoagulants. Haematological parameters were measured using a fully automatic haematology analyser (Horiba cell counter, Model- Abx Micros ES 60). Determination of White Blood Cells (WBCs), Red Blood Cell counts (RBCs), Platelets (PLTs), and Haemoglobin (Hb) concentrations was done. Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), and total protein in serum were determined using an automated turbochem biochemistry analyser.

The tissues (Brain, Heart, Lungs, Liver and Kidneys) were quickly excised and processed for the histological examinations. The tissues were fixed, sections (3mm) were cut and stained with hematoxylin and an eosin stain. Deparaffinised tissue sections (Brain, Heart, Lung, Liver, and Kidney) were stained with toluidine blue (0.1% toluidine blue in 1% sodium chloride solution) for 3 minutes. The tissues were washed and dehydrated quickly with different series percentages of alcohol, and finally, the slides were mounted with DPX and allowed to air dry. The collagen accumulation in tissues was examined using Masson's Trichrome staining method. The slides were deparaffinised by xylene and rehydrated in declining ethanol grades. The slides were treated with Hematoxylin Weigert, acid fuchsin, and phosphomolybdic-phosphotungstic acid mixture with repeated rinsing. Later, the slides were exposed to an aniline blue solution and cleared, dehydrated, and mounted. Finally, slides were visualised under the Olympus light microscope[13,14].

2.3 DFT, ADMET and Molecular Docking

The plausible stereochemistry of the structure was determined through a series of semi-empirical techniques and computational simulations, such as molecular mechanics, as it is vital for predicting molecular electronic properties of the system, some of which are non-covalent. These optimal geometrical parameters were then utilised to compute the optimised structure at the DFT's B3LYP level of theory and Time Dependent-Self Consistent Field (TD-SCF) for evaluating various excited states at the Molecular Orbital (MO) level theoretically using *Gaussian*. The Frontier Molecular Orbital (FMO) were visualised using *Gaussview*. The DFT calculations were done using Gaussian software at the 6-31D level of theory.

ADMETox analysis was performed using pkCSM[15]. Molecular docking was conducted using the GLIDE module of Schrödinger[16]. The compounds from the *Tiryaq-e-wabai* were docked with the ACE-2 enzyme. The protein preparation wizard was used to prepare the protein, adding missing hydrogens, assigning bond orders, and optimising the hydroxyl group, amino acids and hydrogen bonds. The OPLS3e force field was selected for energy minimisation, targeting non-hydrogen atoms with a Root Mean Square Deviation (RMSD) value of 0.3 Å. Using the centroid of a few chosen residues and their ligand binding mode, a receptor grid was generated in the region where the compounds were to be docked. Ligands were made with Ligprep, and OPLS3e force field was used to optimise their structures and generate structures within a specific ph range. Finally, docking was performed using Ligand docking, with derived output files from the grid and ligand as input, and the XP (Extra Precision) method selected for docking[17].

2.4 Statistical Analysis

All the data obtained were represented as mean ± SEM (n=6 rats/ group) and (n=3) for *in-vitro* assay. Statistical analysis was performed using the Student's t-test with

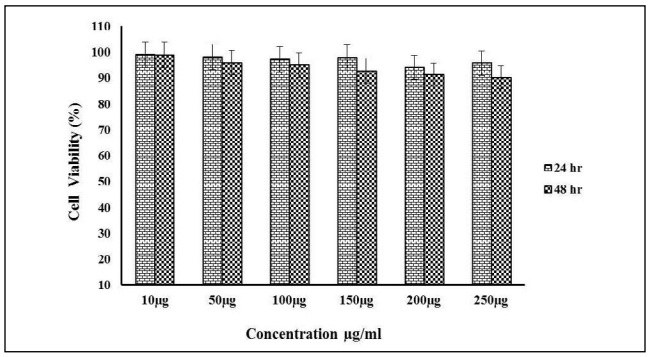


Figure 1. MTT assay of *Tiryaq-e-Wabai* treated with L132 (Human normal lung cell).

a significance level set at $P < 0.05$. (SPSS/16 software package; SPSS Inc.)

3. Results

3.1 Cell Viability

Tiryaq-e-wabai was treated with L132 cells at different concentrations (10µg, 50µg, 100µg, 150µg, 200µg, and 250µg) in a dose-dependent manner for 24 and 48 hours, respectively (Figure 1).

3.2 Acute Toxicity

No incidence of mortality was reported in *Tiryaq-e-wabai*-treated rats at a dose of 2,000 mg/kg b.wt. During the experimental period. No significant change in daily food intake pattern was observed in rats at the tested dose level compared to the control group. No adverse effects were noted at the single dose tested (2g/kg b. wt.). The drug-treated animals showed no abnormal clinical signs or behavioural changes throughout the experimental study period, such as aggression, dizziness, drowsiness, hyperactivity, or convulsions.

Table 1. Average body weight and organ weights of control and drug-treated rats

Parameters (gm)	Control	*Tiryaq-e-wabai*
Body weight	239.5 ±3.15	235.3±2.51
Brain weight	1.77±0.03	1.76±0.05
Heart weight	1.02±0.10	1.06±0.11
Lung weight	1.49±0.83	1.48±0.45
Liver weight	7.78±1.18	7.41±1.02
Kidney weight	1.76±0.05	1.77±0.03

Values are expressed as mean ± S.E.M. (n=6 rats). No significant difference between the control and experimental group.

Table 2. Effect of TW on haematological parameters in control and experimental rats

Parameters (gm)	Control	*Tiryaq-e-wabai*
WBC (10^3/mm3)	6.0±0.12	9.3±0.4*
RBC (10^6/mm3)	5.23±0.08	5.25±0.14
Hb (g/dl)	11.16±0.27	11.66±0.38
Platelets(10^3/mm3)	621±13.3	609.16±19.89

Values are expressed as mean ± S.E.M. (n=6 rats). No significant difference between the control and experimental group. *P < 0.05 compared to control.

Table 3. Effect of TW on biochemical parameters in control and experimental rats

Parameters (gm)	Control	*Tiryaq-e-wabai*
Total protein (g/dl)	5.95±0.1	5.98±0.15
SGPT IU/L	59.78±1.55	66.20±2.38
SGOT IU/L	147±7.33	143.16±5.03

Values are expressed as mean ± S.E.M. (n=6 rats). No significant difference between the control and experimental group.

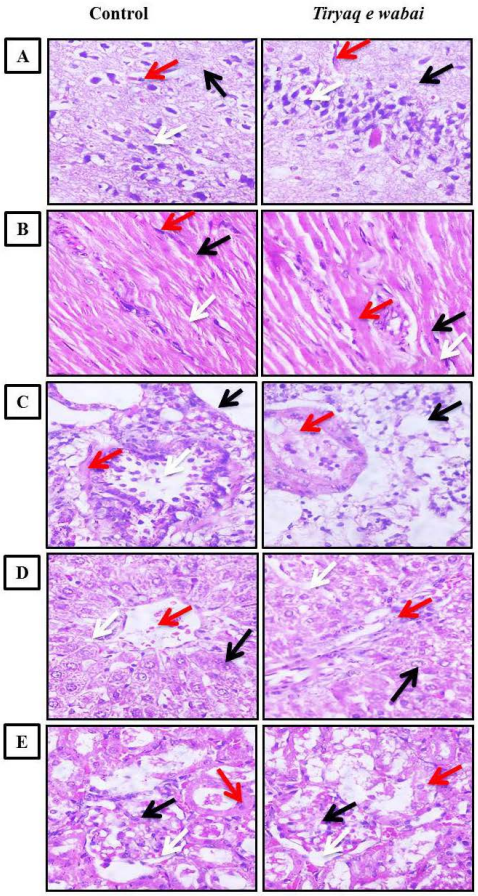


Figure 2. Haematoxylin and Eosin (H and E) staining of tissues.

The gain or loss of body weight and organ weight (brain, heart, lung, liver and kidney) was insignificant in animals administered with *Tiryaq-e-wabai* compared to the control. *Tiryaq-e-wabai* did not influence the mean body weight and organ weight of animals (Table 1).

No significant changes in hematological parameters such as hemoglobin, erythrocytes and platelet count were observed in the rats treated with *Tiryaq-e-wabai* compared to the control. An increase in the number

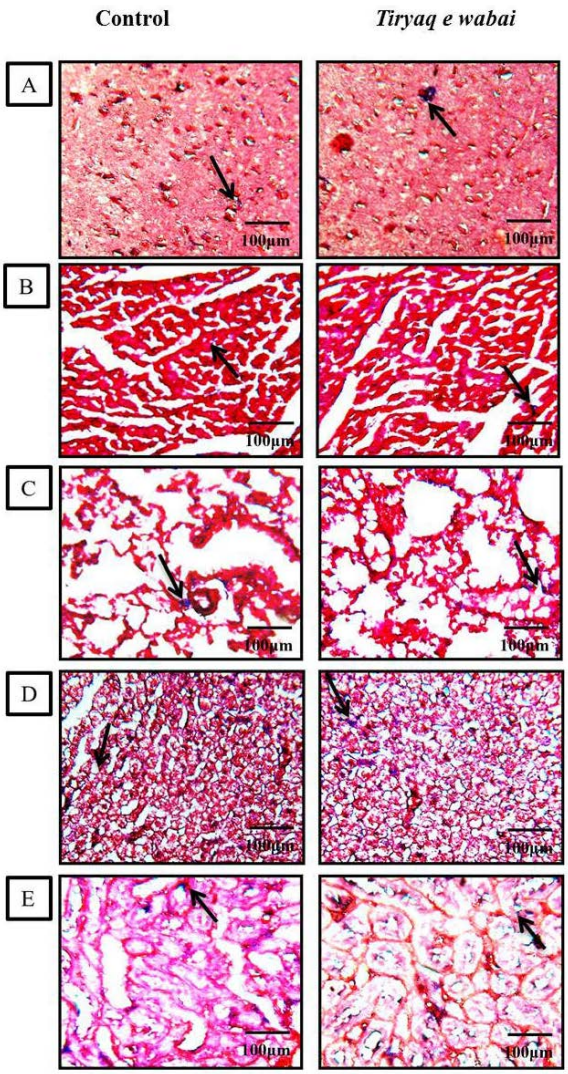


Figure 3. Collagen deposition in tissues.

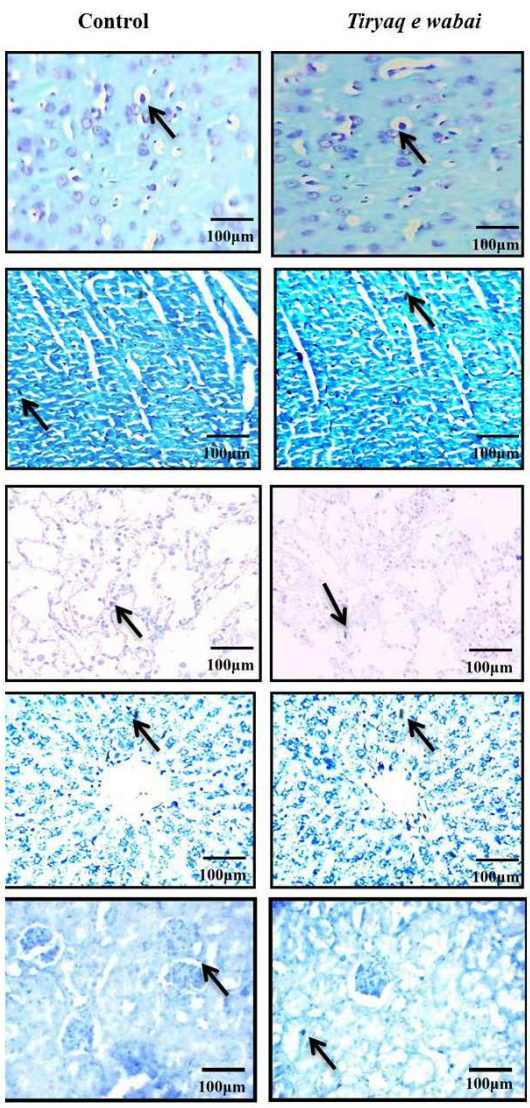


Figure 4. Mast cell infiltration in tissues.

of leukocytes was noted in the experimental group compared to control rats (Table 2).

Insignificant changes in SGOT, SGPT and total protein levels were found in the experimental group compared to the control group (Table 3).

3.2.1 Histological Examinations

Figure 2 represents Haematoxylin and Eosin (H and E) staining of (a) Brain tissue showing normal appearing neurons (white arrow), neuropil (black arrow), and blood vessels (red arrow). (b) Heart tissue with normal cardiac myocytes (black arrow) and intact myonuclei (white arrow), and intermyocardial connective tissue (red arrow). (c) Kidney tissue with normal glomeruli (black arrow) and intact Bowman's capsule (white arrow), and tubules (red arrow). (d) Liver tissue with normal central vein (red arrow) and portal vein (yellow arrow), hepatocytes (black arrow) with normal sinusoidal spaces (white arrow). (e) Lung tissue with normal alveoli (black arrow), bronchioles (white arrow), and normal blood vessels (red arrow) of control and experimental groups of rats.

Masson's trichrome staining was carried out to assess collagen deposition in the tissue. All vital tissues of the control and experimental rats exhibited normal architecture without excessive collagen deposition (Figure 3).

Toluidine blue staining was carried out to assess mast cell infiltration in tissues. The mast cell count

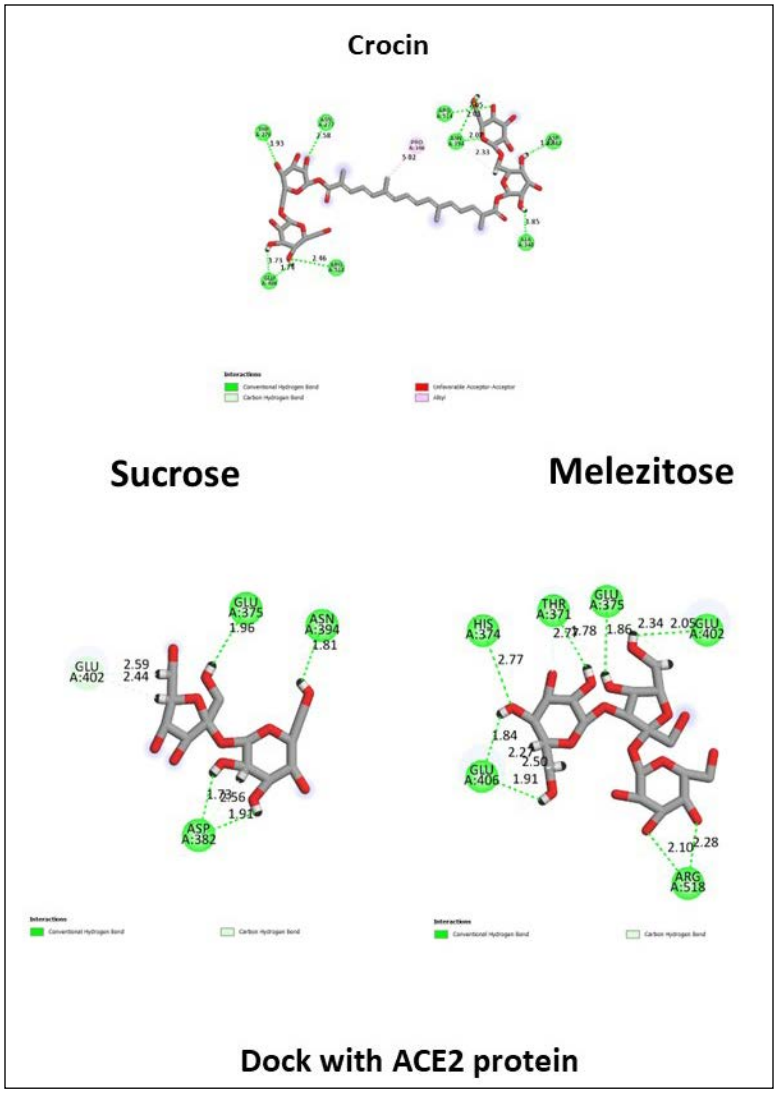


Figure 5. Molecular Docking with ACE2.

was not higher in any of the experimental group's vital tissues than in the control group (Figure 4).

4. Discussion

4.1 *In Vitro and In Vivo* Evaluation of *Tiryaq-e-wabai*

Although *Tiryaq-e-wabai* has been mentioned in *Unani* literature for its use as prophylactic medicine against epidemic diseases, it recently gained attention during the global outbreak of a respiratory pandemic called COVID-19, caused by severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2)[11,18]. *Unani* medicine is known for its wide spectrum efficacy and negligible contraindications[19]. However, assessment of safety aspects is mandatory. This study aimed to determine the acute toxicity of *Tiryaq-e-wabai*. However, long-term exposure to this drug should be carried out to assess the chronic toxicity aspect.

L-132 normal cells treated with *Tiryaq-e-wabai* remained viable at various concentrations (Figure 1). Therefore, it does not induce *in vitro* cytotoxicity at given concentrations.

According to the OECD 423 guideline, the acute toxicity range of the test substance depends on the mortality status of the rodents, which helps in finding the toxicity category of the tested drug[12]. Since there was no mortality during the observation period, the polyherbal drug can be classified as a category V substance.

Table 4. Molecular docking-ACE2 protein

S. No.	Compound Name	Dock Score (kcal/mol)	Interacting Residues	Bond Length (Å)
1	Crocin	-10.6	Thr 276, Asn 277, Pro 346, Ala 348, Asp 382, Asn 394(3), Glu 406(2), Arg 518	1.93, 2.58, 5.02, 1.85, 1.72, 2.01, 2.07, 2.33, 1.73, 1.74, 2.46
2	Melezitose	-8.5	Thr 371(2), His 374, Glu375(2), Glu 402, Glu 406(4), Arg 518(2)	1.78, 2.77, 2.77, 1.86, 2.34, 2.05, 1.84, 1.91, 2.27, 2.50, 2.10, 2.28
3	Sucrose	-7.2	Glu 259(2), Glu 375, Asp 382(2), Asn 394	2.44, 2.59, 1.96, 1.73, 2.56, 1.81
4	Glycero galacto heptose	-6.9	Ala 348(3), Glu 402(2)	1.81, 1.82, 2.24, 1.71, 2.01
5	Aloin A	-4.6	Phe 274, Thr 371, Glu 402, Glu 406(2), Arg 518	5.37, 2.18, 1.94, 1.76, 1.77, 2.77
6	Pyranone	-3.1	Arg 273, Tyr 515	2.93, 2.22
7	Glycerin	-2.7	Glu 398, Tyr 515	1.76, 2.18
8	-2-Hydroxyoctanoic acid, acetate	-2.6	Arg 273(2)	2.72, 1.67
9	5-Hydroxymethylfurfural	-2.5	Glu 406, Arg 518	1.82, 2.14
10	Caryophyllene oxide	-2.1	Ala 348	1.92

Table 5. Pharmacokinetics (pKCSM)

Molecule	Water Solubility	Caco2 Permeability	Intestinal Absorption	Skin Permeability	Log P	Rot Bond	Surface Area
1-Crocin	-2.849	-1.311	0	-2.735	-5.225	18	389.9
2-Melezitose	-1.533	-0.339	0	-2.735	-7.571	8	192.2
3-Sucrose	-0.125	-0.160	0	-2.735	-5.395	5	130.4
4-Gly Gal He	0.122	-0.548	6.46	-3.246	-4.017	6	79.85
5-Aloin	-2.893	-1.092	42.07	-2.735	-0.891	3	170.5
6-Pyranone	0.021	0.539	84.49	-4.339	-0.264	0	57.73
7-Glycerin	0.919	1.068	74.24	-3.950	-1.668	2	35.85
8-H-Oct Acid	-1.106	0.961	93.02	-2.625	1.973	7	84.25
9-Me Furfural	-0.623	1.171	97.98	-3.209	0.584	2	51.91
10-Caryophyl	-4.382	1.506	94.90	-3.058	3.936	0	99.25

This stimulatory influence on leukocyte count is consistent with a previous study[20]. Measurement of haematological parameters can play a crucial role in establishing the toxicity induced by the drugs and offers a predictive assessment of safety evaluation.

Typically, alterations in SGOT, SGPT, and protein levels in tissues result from a compensatory physiological adaptive mechanism to overcome stress, toxicity, or tissue injury[21,22]. However, in our study, the normal SGOT, SGPT and protein levels (Table 3) in the treatment group reflect the normal physiological state of the animals and suggest that *Tiryaq-e-wabai* does not cause hepatotoxicity and maintains normal hepatocellular functions.

The histopathological analysis can be decisive in examining the toxicity of a substance, and it could be considered a gold standard[8]. Normal tissue architecture of experimental tissue sections indicates that administration of *Tiryaq-e-wabai* in a single dose did not result in any necrotic or histopathological changes in any of the vital tissues (Figure 2).

Collagen is the most abundant component of the extracellular matrix that provides structural and biochemical support to cells. Collagen homeostasis

Table 6. DFT data

S.No	HOMO eV DFT	LUMO eV DFT	Band GapDFT	Total Energy DFT Hartrees	Dipole D	Symmetry
1-Crocin	-4.938	-2.487	-2.451	XXX	10.1930	C1
2-Melezitose	-6.953	1.151	-8.105	'	4.3771	C1
3-Sucrose	-6.590	1.149	-7.741	'	2.0215	C1
4-Gly Gal Hept.	-6.725	-1.604	-5.121	'	2.9788	C1
5-Aloin	-5.768	-1.146	-4.621	'	1.3137	C1
6-Pyranone	-5.962	-1.636	-4.326	'	1.8033	C1
7-Glycerin	-6.876	1.599	-8.476	'	3.4555	C1
8-H-Oct Acid	-7.373	-0.371	-7.001	'	4.5998	C1
9-Me Furfural	-6.407	-1.551	-4.856	'	4.9101	C1
10-Caryophyllene	-6.366	0.285	-6.651	'	2.1704	C1

Table 7. Docking score noted against electronegativity, dipole moment (DFT) and log P (pharmacokinetics)

S.no	Molecule	DS	Ω Electro nega tivity	Log P	Dipole
1	Crocin	-10.6	5.623	-5.225	10.1930
2	Melezitose	-8.5	1.038	-7.571	4.3771
3	Sucrose	-7.2	0.956	-5.395	2.0215
4	Glycero galacto	-6.9	3.386	-4.017	2.9788
5	Aloin A	-4.6	2.585	-0.891	1.3137
6	Pyranone	-3.1	3.336	-0.264	1.8033
7	Glycerin	-2.7	0.821	-1.668	3.4555
8	2-Hydroxy OA	-2.6	2.141	1.973	4.5998
9	5-Hydroxy MF	-2.5	3.260	0.584	4.9101
10	Caryophyllene	-2.1	1.389	3.936	2.1704

is crucial and can be altered by injury, repair, and/or pathologic change[23,24]. The absence of excessive collagen deposition in any of the vital tissues of the experimental group excludes the possibility of fibrotic changes due to the administration of *Tiryaq-e-wabai* (Figure 3).

Mature mast cells do not circulate in the bloodstream or infiltrate excessively under normal circumstances. Such a scenario indicates underlying pathophysiology, especially allergic inflammation[25,26]. The results rule out the possibility of inflammatory changes due to the administration of *Tiryaq-e-wabai*.

Table 8. Frontier molecular orbital (MO) values, lowest unoccupied MO, highest occupied MO (computed using DFT), hardness and softness values based on FMO values

Molecule	LUMO	HOMO	η~ I-A E_L-E_H	S = 1/(L-H)
1-Crocin	-4.938	-2.487	2.451	0.407
2-Melezitose	-6.953	1.151	8.104	0.123
3-Sucrose	-6.591	1.149	7.739	0.129
4-Gly Gal He	-6.725	-1.604	5.121	0.195
5-Aloin	-5.768	-1.146	4.622	0.215
6-Pyranone	-5.962	-1.636	4.326	0.236
7-Glycerin	-6.876	1.599	8.475	0.117
8-H-Oct Acid	-7.373	-0.371	7.002	0.142
9-Me Furfural	-6.407	-1.551	4.856	0.205
10-Caryophyl	-6.366	0.285	6.651	0.150

4.2 *In Silico* Evaluation of *Tiryaq e wabai*

Adopting an *in-silico* approach might give novel leads on the antiviral potential of *Unani* drugs in a time-efficient and cost-effective manner[27]. Therefore, the present study investigated the effects of some phytocompounds present in *Ttiryaq-e-wabai* on the key druggable targets of SARS-Cov-2 through a molecular docking approach. SARS-CoV-2 is a coronavirus that caused the COVID-19 pandemic. ACE2 is expressed in all vital tissues and functions as both an enzyme and a functional receptor on cell surfaces, allowing SARS-Cov-2 to enter host cells. Regulation of the Renin-Angiotensin-Aldosterone System (RAAS) is largely

Table 9. Band gap, electronegativity, chemical potential, global hardness, global softness and electrophilicity index (based on Fukui reactivity parameters) using DFT's FMO values

Molecule	LUMO	HOMO	Band Gap E_L-E_H	Electro Negativity, χ -½(E_L+E_H)	Chem. Pot μ= - χ	Glob Hard, η=½(E_L-E_H)	Glob Soft S= 1/2η	Electro Phil. index ω=$μ^2$/2η
1-Crocin	-4.938	-2.487	2.451	3.712	-3.712	1.225	0.407	5.623
2-Melezitose	-6.953	1.151	8.104	2.901	-2.901	4.052	0.123	1.038
3-Sucrose	-6.591	1.149	7.739	2.720	-2.720	3.869	0.129	0.956
4-Gly Gal He	-6.725	-1.604	5.121	4.164	-4.164	2.560	0.195	3.386
5-Aloin	-5.768	-1.146	4.622	3.457	-3.457	2.311	0.216	2.585
6-Pyranone	-5.962	-1.636	4.326	3.799	-3.799	2.163	0.231	3.336
7-Glycerin	-6.876	1.599	8.475	2.638	-2.638	4.237	0.117	0.821
8-H-Oct Acid	-7.373	-0.371	7.002	3.872	-3.872	3.501	0.142	2.141
9-Me Furfural	-6.407	-1.551	4.856	3.979	-3.979	2.428	0.205	3.260
10-Caryophyl	-6.366	0.285	6.651	3.040	-3.040	3.325	0.150	1.389

mediated by ACE2. Research has demonstrated that the novel coronavirus and similar coronaviruses directly interact with ACE2 through their spike S proteins[9,28,29]. Thus, ACE2 expression can limit viral infection.

Compounds from the *Tiryaq-e-wabai* polyherbal drug were downloaded from the PubChem database and analysed for ADMETox properties.

The ACE2 protein (PDB ID: 1R42) was downloaded from the Protein Data Bank. This protein includes the domain region of Peptidase family M2, spanning positions 28 to 599. Key functional residues of ACE2 include 345H, 346P, 347T, 348A, 368D, 374H, 375E, 378H, 382D, 401H, 402E, 406E, 449T, 503L, 504F, 505H, 510Y, 512F, and 515Y (Figure 5).

Further, these compounds were docked with the ACE2 enzyme. Molecular docking studies revealed that Crocin interacted with the active site residues Ala348, Asp382, and Glu406 of ACE2, achieving a dock score of -10.6 kcal/mol. Additionally, Melezitose, Sucrose, and Glycero galacto heptos docked with ACE2, with dock scores of -8.5, -7.2, and -6.9 kcal/mol, respectively (Table 4).

Combining multiple herbs in compounded formulations can form various byproducts that may result from interactions between the different phytochemicals. Depending on their nature and concentration, these chemical constituents may exert therapeutic action in concert and act on multiple targets simultaneously[30]. We have found some interesting correlations in the pharmacological aspect of our combination. One is the favourable binding score of various constituents, and the other is the possible mechanism that can be explained based on the reactivity parameters according to chemical intuition.

The Caco-2 cell monolayer permeability model is a leading *in vitro* tool for assessing the potential bioavailability of drugs in the human body. Here, the Pyranone's value is different compared with Crocin in the sense that the latter has a negative value and thus relatively good permeability, while Pyranone has a positive value, suggesting that it is involved in the surface interaction without penetration. The lower solubility (in water) of this molecule makes it more oriented towards the epithelial parts of the receptors and thus, without entering the interior part of the macromolecule, it stabilises the receptor, facilitating an efficient binding of Crocin (Table 5). This aspect is absent when Crocin alone is administered. This is supported by the docking studies as well, where the Pyranone part is just at the bottom exterior part of the ACE2 (Figure 5).

With about 13 ligands in the mixture, Crocin effectively binds to ACE2. The rest of the molecules probably make the macromolecule rigid to effectively facilitate the binding of Crocin efficiently (by penetrating easily). This can be attributed to synergism due to ligands[31]. Based on the type of interaction, two broad categories of synergy have been identified: pharmacodynamic and pharmacokinetic. When two compounds are targeted at the same

receptor or physiological system, pharmacodynamic synergy occurs[31,32]. Drug absorption, distribution, biotransformation, or elimination processes all contribute to pharmacokinetic synergy, thus shedding light on the toxicity aspects. For instance, combined administration of drugs that can compete for albumin binding will elevate the free drug concentrations, thereby potentiating their actions.

It is for this reason that we have evaluated some descriptors that can describe these properties. Some of them are chemical descriptors like the hardness or softness of the molecule and the electrophilic nature of the molecule. A glance at the table reveals that Crocin has a Global softness value of about 0.407 (Table 9). The softness represents the reactivity nature of the molecule or ligand; the higher the value greater is the capability to bind or react.

The next one is Pyranone, followed by Aloin and Me-Furfural. While Pyranaone plays the metabolic role of sugar moiety, the other small molecules probably make the macromolecule more rigid, a factor that could affect proper binding. Thus, looking at the Pharmacokinetics factors (Table 5), we can conclude that Pyranone has the highest skin permeability and intestinal absorption. It has a negative logP value of about -0.264. A negative logP value indicates that a compound is more hydrophilic, or has a higher affinity for the aqueous phase, than the lipid phase. With 0 rotatable bonds, this is a kind of rigid ligand that does not go into the organic or cellular aspects of the cell but binds efficiently at the surface of the ACE2 and lets other species too bind in the right place effectively.

A molecule's dipole moment is a measurement of the polarity of a chemical bond formed between two atoms. It is the product of the charge and distance between two equal and opposite charges[33]. A more active compound is found to have a higher dipole moment than one that is relatively inactive. The higher cellular interaction of the more polar molecule may account for the superior biological activity. Going by this aspect, Crocin and Melezitose have higher dipole moments and have greater interaction. While Pyranone and Aloin have lesser dipole moments (Tables 6-8), they make up for the effective binding of other molecules as discussed in the light of chemical reactivity descriptors.

5. Conclusion

No mortality, gross haematological alterations, biochemical perturbations, and morphological or pathological changes were noted in animals due to single-dose oral administration of *tiryaq-e-wabai*. It is therefore suggested that the tested *Unani* polyherbal drug has an LD_{50} value greater than 2000mg/kg b.wt. It does not induce *in vitro* cytotoxicity at given concentrations. The *Unani* drug can potentially prevent SARS-Cov-2 viral intrusion into host cells by blocking ACE2 (theoretically supported). While *in vivo* and *in vitro* experiments have helped set up the biological aspects, *in silico* calculations have assisted in formulating mechanisms of probable bioactivity.

As *tiryaq-e-wabai* is utilised during respiratory pandemics, investigating the antiviral and anti-inflammatory potential of this *Unani* medicine based on modern scientific guidelines will offer new insights into understanding its mechanism of action.

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8. Ethics Approval

Animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) vide BRULAC/SDCH/SIMATS/IAEC/09-22/06.

9. Author Contributions

Ashar Waheed: Conceptualisation, funding acquisition, project administration, writing original draft; Zaheer Ahmed: Conceptualisation and supervision (*Unani* perspective); Imran Predhanekar: Investigation and writing: original draft (Pharmacokinetics and docking);

Aadhil Ashwaq: Formal analysis and validation, data curation and visualisation; Mohammed Zaid: Investigation; Shabeer Ahmed: Writing: review and editing; Unaiza Usmani: Methodology. All authors have read and agreed to the published version of the manuscript.

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