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Research Article

QUINIC ACID AS A POTENT DRUG CANDIDATE FOR PROSTATE CANCER – A COMPARATIVE PHARMACOKINETIC APPROACH

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ABSTRACT

Objective: Phytotherapy is growing importance with the emergence of deadly diseases. Prostate cancer is one such disease which is the most prevalent cancer afflicting men. Therapeutic application of plant is better understood with the pharmacological investigation of its phytoconstituents. Quinic acid is a prominent bioconstituent of flowers of *Moringa oleifera* Lam., a traditional plant of high nutritional and medicinal values. The present study analyzes the pharmacokinetic properties of this phytocomponent for its therapeutic application in prostate cancer.
Methods: *In silico* tools were used to screen the physicochemical, Lipinski-type and drug properties of Quinic acid from the other compounds used for comparison. The *M. oleifera* flower compound, Quinic acid and the standard therapeutic Curcumin were the ultimate compounds selected for further studies on their Adsorption-Distribution-Metabolism-Excretion (ADME) characteristics and toxicity parameters.
Results: The overall pharmacokinetics results indicated that Quinic acid had more number of advantages over Curcumin and the other compounds studied. Quinic acid possessed greater values of drug score and drug likeness with lesser brain penetration and lesser toxicity effects.
Conclusion: The study suggested that the pharmacokinetic properties of Quinic acid were more preferable to be used as a potent drug candidate to combat prostate cancer.

Keywords: Prostate cancer, Quinic acid, *Moringa oleifera*, Pharmacokinetics, *In silico* tools, Drug candidate

INTRODUCTION

Drug compounds are guided by several physicochemical properties. The medicinal chemist Christopher Lipinski suggested that a compound was more likely to be membrane permeable and easily absorbed by the body if its molecular weight was less than 500 Daltons, partition coefficient between n-octanol and water (logP) less than 5, hydrogen bond donors less than 5 and hydrogen bond acceptors less than 10, all being multiples of 5 and thus framed Lipinski's Rule of Five [1,2]. It is expected for a good drug molecule, atleast 3 out of the 4 criteria should be satisfied. It has also been suggested earlier that molar refractivity between 40 and 130 is the acceptable range of compounds to be associated with drug likeness [3].

Adverse drug reactions have become the major cause of failure of drugs. Thus, besides analyzing their physicochemical and Adsorption-Distribution-Metabolism-Excretion (ADME) properties, it is highly necessary to perform risk assessments and analyze the toxicity of drugs. Application of *in silico* tools with toxicology techniques that are fast and cost efficient, enables the researcher to investigate the impact of such chemicals at an early stage of product development.

Prostate cancer is the most common type of cancer afflicting men and is the leading cause of death next to lung cancer and colorectal cancer [4]. The risk increases when the cancer cells metastasize from the prostate to bones and lymph nodes. The high costs and risks associated with the current therapies have compelled the need for alternative cost-effective and non-toxic measures to minimize the complications. Medicinal plants and their products being more convenient and greatly accepted by the users for their minimum side effects, low cost and easy availability, seem to offer a promising solution to this problem. One such highly valued plant is *Moringa oleifera* Lam., a multifunctional versatile plant with an impressive range of economic, health and nutritional potentials. The flowers of this traditional plant have been used in the folk medicine to treat cancerous tumors [5]. The methanolic extract of *M. oleifera* flowers has displayed significant antioxidant potentials [6] and possesses a list of compounds as identified by Gas Chromatography-Mass Spectrometry (GC-MS), with Quinic acid being the second most prevalent compound next to Ethyl oleate, in the flower extract [7].

Quinic acid is a cyclic polyol and forms phenolic acid derivatives. It is a major biochemical intermediate in the shikimate pathway, a biosynthetic pathway of many aromatic compounds that exist in plants and microorganisms [8,9]. It cannot be synthesized by mammals including humans. Quinic acid supplements through diet, nutritionally support the synthesis of tryptophan and nicotinamide in the gastro intestinal tract (GIT). This in turn leads to DNA repair enhancement and Nuclear Factor-kappa B (NF-κB) inhibition *via* increased nicotinamide and tryptophan production [10]. It has potent broad spectrum antioxidant, anti-inflammatory, hepato protective and several other medicinal properties [11,12,13].

With this known list of significant biological activities associated with Quinic acid, it is essential to analyze the drug properties driving them. To be a potent drug, a chemical compound needs to be efficient in its pharmacokinetic properties. Thus, this study is undertaken to investigate the pharmacokinetic properties of Quinic acid, a prominent candidate compound of *M. oleifera* flowers and thereby to interpret its therapeutic application against prostate cancer.

MATERIALS AND METHODS

For a comparative study to observe the effect on the disease, Quinic acid along with the other candidate compounds present in the methanol extract of *M. oleifera* flowers as predicted by the GC-MS analysis [7], were analyzed. Two standard therapeutic agents for prostate cancer: a natural compound, Curcumin and a chemotherapeutic drug, Estramustine, and the database ligands of the drug targets, Glucosaminyl (N-acetyl) transferase 1 (GCNT1) and Prostatic acid phosphatase (PAP), involved in prostate cancer as studied earlier [14,15] were also used for comparison.

Three dimensional (3D) Structure Data Format (SDF) chemical file formats of the compounds were obtained from PubChem Compound database [16]. For those with only two dimensional (2D) SDF

chemical file formats available, Simplified Molecular Input Line Entry Specification (SMILES) translator was used [17]. But for those compounds whose structure file formats were unavailable in PubChem Compound database, their structure as obtained from the GC-MS studies was utilized. These structures were drawn using ChemSketch [18], 3D optimized and saved as Molecular Design Limited (MDL) mol files [19]. These file formats were used for pharmacokinetics studies using ACD/I-Lab 2.0 [20] while for calculations involving OSIRIS property explorer [21], compounds were directly drawn.

Basic Physchem Properties Module of ACD/I-Lab 2.0 was exploited for a quick check on several useful physicochemical properties characterizing the compounds of interest. The properties analyzed were molar refractivity, molar volume, parachor, refractive index, surface tension, density and polarizability.

Lipinski had described a few molecular properties important for a drug's pharmacokinetics in the human body. These properties namely molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, topological polar surface area (TPSA) and number of rotatable bonds as included in the Basic Physchem Properties Module of ACD/I-Lab 2.0 were checked for the compounds.

Various parameters like molecular weight, partition coefficient between n-octanol and water (logP), solubility, drug likeness and drug score that constituted the drug properties of the compounds were calculated by the Bioinformatics tool OSIRIS property explorer.

The ADME module of ACD/I-Lab 2.0 was utilized to analyze the bioavailability, absorption, volume of distribution (Vd) and penetration across the blood brain barrier (BBB) of the selected compounds with favourable drug properties.

The selected compounds were analyzed for their toxicities by employing various toxicity modules of ACD/I-Lab 2.0. The compounds were investigated for mutagenicity (AMES test), genotoxicity hazards, Human Ether-a-go-go-Related Gene inhibition (hERG), acute toxicity, Organization for Economic Cooperation and Development toxicity (OECD) categories, aquatic toxicity, endocrine system disruption, health effects and Maximum Recommended Daily Dose (MRDD).

RESULTS AND DISCUSSION

Studies on physicochemical properties

Except a few compounds, all other *M. oleifera* flower compounds including Quinic acid (40.04 ± 0.3 cm^3) exhibited molar refractivity in the acceptable range. The other physicochemical properties studied were also in the desirable limit for Quinic acid.

Among the GCNT1 inhibitors, molar refractivity of only Calcitriol, 5,5'-dithiobis-2-nitrobenzoic acid and 6 Deoxy N-acetyl galactosamine were in the preferable range while 6 Methyl mercapto purine was observed as the only PAP activator with the favourable molar refractivity.

The least molar volume was expressed by Melamine among the *M. oleifera* flower compounds. Among the GCNT1 inhibitors, Iodoacetate expressed the least value and Tunicamycin expressed the maximum value. Dithiothreitol possessed the largest molar volume among the PAP activators. Out of all the compounds analyzed, 2 Mercapto ethanol, the PAP activator possessed the least value and n-Hexatriacontane from *M. oleifera* flowers exhibited the maximum value.

Among the *M. oleifera* flower compounds studied, Melamine possessed the least parachor while n-Hexatriacontane exhibited the maximum value. Iodoacetate expressed the least parachor among the GCNT1 inhibitors. 6 Methyl mercapto purine possessed the largest parachor among the PAP activators. Out of all the compounds analyzed, 2 Mercapto ethanol, the PAP activator possessed the least value and Tunicamycin, the GCNT1 inhibitor exhibited the maximum value.

Of all the compounds studied, Diethyldicarbonate, the GCNT1 inhibitor possessed the least value for index of refraction and Melamine from *M. oleifera* flowers exhibited the maximum value. Methyl palmitate expressed the least refractive index among the *M. oleifera* flower ligands. 5,5'-dithiobis-2-nitrobenzoic acid expressed the largest refractive index among the GCNT1 inhibitors. Among the PAP activators, 2 Mercapto ethanol possessed the least value while 6 Methyl mercapto purine possessed the largest value. The refractive index values of the compounds correlated significantly with their densities.

Among the GCNT1 inhibitors, Diethyldicarbonate had the lowest surface tension and 5,5'-dithiobis-2-nitrobenzoic acid had the highest value. Among the PAP activators, 2 Mercapto ethanol showed the least value while Ascorbic acid showed the maximum value. The *M. oleifera* flower compounds, n-Tetracosane and Melamine exhibited the lowest and the greatest values respectively out of all the compounds considered for the study.

Melamine had the maximum density among the *M. oleifera* flower ligands. Calcitriol had the least density among the GCNT1 inhibitors. Among the PAP activators, 2 Mercapto ethanol showed the least value while Ascorbic acid showed the maximum value similar to the surface tension results. Out of all the compounds analyzed, n-Tetracosane from *M. oleifera* flowers possessed the least value and Iodoacetate, the GCNT1 inhibitor exhibited the maximum value.

Among the *M. oleifera* flower compounds studied, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one possessed the least polarizability while n-Hexatriacontane exhibited the maximum value. Iodoacetate expressed the lowest polarizability among the GCNT1 inhibitors while 6 Methyl mercapto purine expressed the maximum value among the PAP activators. Tunicamycin, the GCNT1 inhibitor exhibited the greatest polarizability while 2 Mercapto ethanol, the PAP activator exhibited the lowest value out of all the compounds analyzed.

The physicochemical properties of the standard therapeutics Curcumin and Estramustine upon analysis indicated that both of these compounds exhibited physicochemical properties in the moderate range of desirability.

Studies on Lipinski-type properties

The Candidate drugs that conform to the Rule of Five tend to have lower attrition rates during clinical trials and hence have an enhanced opportunity of reaching the market [22]. In addition to the typical 4 criterial properties suggested by Lipinski, other properties such as molecular flexibility and polar surface area have also been recognized to exert an impact on oral bioavailability of drugs [23], hence have been included.

Though the molecular weight of the compound is expected to be less than 500 as suggested by Lipinski, the range within 180-500 Daltons seems to be closely associated with drug likeness [3]. In the current study, apart from Quinic acid (192.17 Daltons), molecular weight of all other compounds identified from the methanolic extract of *M. oleifera* flowers except for Melamine, 3,5-Dihydroxy- 6- methyl- 2, 3-dihydro-4H-pyran-4-one, (4-Hydroxy phenyl) acetonitrile, n-Hexatriacontane and alpha-Tocopherol-beta-D-mannoside, fitted into the required range. Among the GCNT1 inhibitors, except for 5,5'-dithiobis-2-nitrobenzoic acid, Iodoacetate, 6 Deoxy N-acetyl galactosamine and Calcitriol, molecular weight of all other compounds was not in the expected range. Among the PAP activators, Citric acid was the only compound whose molecular weight seemed to be favourable. In a study, a majority of the tested compounds with high permeability had molecular weight lower than those of low permeability and bioavailability [24].

Hydrogen bonding descriptors include a count of the number of potential hydrogen bond donors and acceptors. The number of

hydrogen bond donors and acceptors of Quinic acid were 5 and 6 respectively. Among the compounds studied, all except Melamine and Tunicamycin had hydrogen bond donors less than 5. Again, Tunicamycin seemed to be the only compound with hydrogen bond acceptors more than 10. Some of the compounds displayed zero value for the number of hydrogen bond donors or acceptors or both. This was prominently observed in the *M. oleifera* flower compounds, n-Tetracosane, n-Tetratriacontane and n-Hexatriacontane, signifying the incapability of these compounds to form hydrogen bonds.

It had been found that compounds with polar surface area equal to or less than 140 Å^2 and 10 or fewer rotatable bonds were predicted to have good oral bioavailability [23]. Matching to this criterion TPSA of Quinic acid was found to be 118.22 Å^2. Amazingly, TPSA of all the compounds studied except 5,5'-dithiobis-2-nitrobenzoic acid and Tunicamycin was less than 140 Å^2. TPSA of the compounds which showed zero value for both the number of hydrogen bond donors and hydrogen bond acceptors, was obviously zero, reflecting the absence of polar surface in these compounds and its association with the existence of hydrogen bonds. Similar observation was made while analyzing the physicochemical properties of Central Nervous System (CNS) drugs [25].

Molecular flexibility was dependent on the number of rotatable bonds in the molecule structure and could be obtained simply by counting the non-terminal, non-cyclic, single bonds except C-N amide bond [26]. On analyzing the ligands, the least number of rotatable bonds (1) was found in Quinic acid among the *M. oleifera* flower compounds, Iodoacetate, 6 Deoxy N-acetylgalactosamine and N-ethylmaleimide among the GCNT1 inhibitors, and 2 Mercapto ethanol and 6 Methyl mercapto purine among the PAP activators. The *M. oleifera* flower compounds, Melamine and 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one and the GCNT1 inhibitor N-Bromosuccinimide did not possess any rotatable bond indicating lack of molecular flexibility in these compounds.

In general, compounds with high molecular weight show more number of rotatable bonds. Usually, the polar surface area and hydrogen bond count tend to increase with increase in molecular weight [27]. Similarly, a correlation exists between molecular flexibility and molecular weight indicating that larger compounds seem to be more flexible [23,28]. On considering the molecular properties of model drugs, it is observed that higher intestinal permeability and drug absorption are associated with reduced molecular flexibility which in turn reflects the decrease in the number of rotatable bonds and lower polar surface area or lower hydrogen bond counts [24].

Lipinski-type properties of Curcumin and Estramustine were all in the required range. Among the *M. oleifera* flower compounds Quinic acid, 4-Hydroxy-3,5,6-trimethyl – 4 - [(1E)-3-oxo-1-butenyl] - 2-cyclohexen-1-one, Ergost-5-en-3 beta-ol, Stigmasterol and Gamma-Sitosterol, among the GCNT1 inhibitors, Iodoacetate, 6 Deoxy N-acetylgalactosamine and Calcitriol, and Citric acid among the PAP activators also exhibited Lipinski-type properties in the favourable range.

Studies on drug properties

Drug properties of the compounds were predicted and tabulated as in Table 1. It has been shown for compounds to have a reasonable probability of being well absorbed; their logP value must not be greater than 5.0 [29]. It is widely used to predict pharmacokinetics in Lipinski's Rule of 5, as compounds with logP values greater than 5 tend to have poor absorption or permeation [2]. The value has been extended to a range of −0.4 to +5.6 in an attempt to improve the prediction of drug likeness [3].

The calculated logP (clogP) value of the *M. oleifera* flower compounds Melamine, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, (4-Hydroxy phenyl) acetonitrile, 4-Hydroxy-3,5,6-trimethyl -4-[(1E)-3-oxo-1-butenyl] -2-cyclohexen-1-one, was found to be within the range as mentioned above. Among the remaining *M. oleifera* flower compounds, Quinic acid showed clogP value little lesser (negative) than the expected range indicating its inclination towards hydrophilicity while the other compounds had higher values (positive) beyond the range reflecting their extensive lipophilicity, the highest value being expressed by n-Hexatriacontane. From the clogP values of the GCNT1 inhibitors, it could be inferred that N-Bromosuccinimide and 6 Deoxy N-acetylgalactosamine were hydrophilic, Calcitriol and 12-O-Tetradecanoylphorbol-13-acetate were highly lipophilic while the remaining compounds were in the expected range. Among the PAP activators, clogP values of Citric acid and Ascorbic acid denoted their hydrophilicity while for the other compounds; they were within the expected range.

Solubility is one of the important parameters to achieve preferable concentration of drug in systemic circulation for exhibiting desired pharmacological response [30]. LogS value is a unit stripped logarithm (base 10) of the solubility measured in Mol/litre. The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with a bad absorption. More than 80% of the drugs on the market have an estimated logS value greater than -4. Fitting according to the solubility criterion of the marketed drugs, the *M. oleifera* flower compounds, Melamine, 3,5-Dihydroxy- 6- methyl-2,3-dihydro-4H-pyran-4-one, (4-Hydroxy phenyl) acetonitrile, Quinic acid and 4-Hydroxy-3,5,6-trimethyl -4-[(1E)-3-oxo-1-butenyl] -2-cyclohexen-1-one showed good aqueous solubility, the best being exhibited by Quinic acid. N-Bromosuccinimide, Diethyldicarbonate, Hydroxyphenylglyoxal, Iodoacetate, 6 Deoxy N-acetylgalactosamine and N-ethylmaleimide possessed significant solubility among the GCNT1 inhibitors with latter two exhibiting equal and maximum solubilities. Surprisingly all the PAP activators taken for the study were soluble, the highest solubility being expressed by Citric acid out of all the compounds analyzed. Interestingly, all those compounds which exhibited negative clogP value showed remarkable aqueous solubility except for Tunicamycin which was on the border-line.

Table 1: Drug properties of the ligands

Compound	clogP	Solubility	Drug Likeness	Drug score
Melamine	-0.54	-2.03	-2.22	0.07
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	-0.47	-0.93	-1.38	0.35
(4-Hydroxyphenyl) acetonitrile	1.9	-1.97	-7.9	*0.14
Quinic acid	-1.93	-0.14	0.51	0.48
4-Hydroxy-3,5,6-trimethyl -4-[(1E)-3-oxo-1-butenyl] -2-cyclohexen-1-one	1.86	-1.96	-3.15	0.31
(2E)-3,7,11,15-Tetramethyl -2-hexadecen-1-ol	8.06	-4.63	-3.77	*0.16
Methyl palmitate	6.97	-4.37	-35.36	*0.22
Palmitic acid	6.52	-4.24	-25.22	0.09
Ethyl palmitate	7.41	-4.67	-39.36	*0.17
Methyl cis-7-octadecenoate	7.54	-4.68	-39.14	*0.20
cis-9-Hexadecenal	6.57	-4.24	-23.15	*0.14
Methyl linoleate	7.19	-4.45	-35.73	*0.22
Ethyl Oleate	7.98	-4.98	-43.1	*0.15
Ethyl stearate	8.34	-5.21	-39.36	*0.14
n-Tetracosane	11.59	-6.92	-20.4	*0.13
Ethyl docosanoate	10.19	-6.29	-39.36	*0.11
n-Tetratriacontane	16.23	-9.62	-20.4	*0.10
9-Octadecenamide	6.55	-4.63	-27.66	*0.23
Malonic acid, di(10-chlorodecyl) ester	8.7	-6.18	-30.04	*0.10
n-Hexatriacontane	17.15	-10.16	-20.4	0.09
alpha-Tocopherol-beta-D-mannoside	8.3	-7.03	-5.7	0.09
Ergost-5-en-3 beta-ol	7.78	-6.4	-8.19	*0.14

Stigmasterol	7.89	-6.44	1.22	*0.19
Gamma-Sitosterol	8.24	-6.67	-4.48	*0.11
12-Oleanen-3-yl acetate, (3alpha)	7.33	-7.06	-2.66	*0.13
Curcumin	2.97	-3.62	-3.95	0.39
Estramustine	5.31	-5.83	0.23	0.3
N-Bromosuccinimide	-1.28	-1.09	-4.52	0.49
Calcitriol	5.98	-5.15	-5.11	*0.16
Diethyldicarbonate	1.9	-2.1	-12.3	*0.28
5,5'-dithiobis-2-nitrobenzoic acid	2.99	-4.51	-9.72	0.34
Hydroxyphenylglyoxal	0.66	-1.71	-1.78	*0.27
Iodoacetate	0.6	-0.58	-4.19	*0.11
6 Deoxy N-acetylgalactosamine	-1.34	-0.53	-0.96	0.63
N-ethylmaleimide	0.12	-0.53	-4.22	0.3
12-O-Tetradecanoylphorbol-13-acetate	7.07	-5.92	-27.3	0.02
Tunicamycin	-0.04	-4.16	-17.6	0.08
Ascorbic acid	-1.92	-1.53	0.6	0.64
Citric acid	-2.15	0.07	3.56	0.58
Dithiothreitol	0.53	-2.04	-5.23	*0.23
2 Mercapto ethanol	0.55	-1.13	-6.35	*0.14
6 Methyl mercapto purine	0.5	-2.28	-4.33	*0.17

Shaded values: Fit into the favourable range of drug properties
Shaded ligands: Satisfy 3 out of the 4 criteria
* Moderate range of drug score from 0.10 to 0.29

A positive value of drug likeness states that the molecule analyzed contains predominantly fragments which are frequently present in commercial drugs. Among the *M. oleifera* flower compounds, Quinic acid and Stigmasterol alone expressed positive values of drug likeness. None of the GCNT1 inhibitors exhibited positive value while among the PAP activators, Citric acid and Ascorbic acid possessed significant drug likeness with Citric acid showing the highest value. High solubility and less molecular weight are desirable attributes for drug likeness.

The drug score as calculated by OSIRIS property explorer was the combination of drug likeness, clogP, logS, molecular weight and toxicity risks in one handy value that might be used to judge the compound's overall potential to qualify for a drug. Molecular weight of the compounds predicted by OSIRIS property explorer was same as that by ACD/I-Lab 2.0. Among the *M. oleifera* flower compounds, Quinic acid gave the highest score followed by 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one and 4-Hydroxy-3,5,6-trimethyl – 4 - [(1E)-3-oxo-1-butenyl] -2-cyclohexen-1-one. Melamine gave the least value followed by Palmitic acid, n-Hexatriacontane and alpha-Tocopherol-beta-D-mannoside with the same value. Among the GCNT1 inhibitors, 6 Deoxy N-acetylgalactosamine expressed the maximum score followed by N-Bromosuccinimide, 5,5'-dithiobis-2-nitrobenzoic acid and N-ethylmaleimide. 12-O-Tetradecanoyl phorbol-13-acetate showed the least score followed by Tunicamycin. Among the PAP activators, Citric acid and Ascorbic acid possessed higher scores with the highest score being expressed by Ascorbic acid out of all the compounds. The other compounds exhibited moderate scores.

On analyzing the drug properties of Curcumin and Estramustine, while clogP, solubility and drug score seemed to be favourable in case of Curcumin, drug likeness posed a negative value. Similarly, for Estramustine solubility was found to be lesser than -4 while the remaining drug properties seemed to be favourable.

Screening of compounds

The compounds were screened based on their physicochemical properties, Lipinski-type properties and drug properties, in order to select two major competing compounds for further analyses on their ADME and toxicity. The compounds, Quinic acid, 4-Hydroxy-3,5,6-trimethyl-4-[(1E)-3-oxo-1-butenyl]-2-cyclohexen-1-one, Ergost-5-en-3 beta-ol, Stigmasterol, Gamma-Sitosterol, Curcumin, Estramustine, 6 Deoxy N-acetylgalactosamine and Calcitriol not only possessed physicochemical properties but also Lipinski properties in the favourable range. When these compounds were analyzed for their drug properties, Quinic acid, 4-Hydroxy- 3,5,6- trimethyl- 4-[(1E)-3-oxo-1-butenyl]- 2-cyclohexen-1-one, Curcumin and Estramustine ruled out the other compounds. Though Citric acid had favourable range of Lipinski as well as drug properties, its molar refractivity was not in the permitted level, hence couldn't be selected for the next step. Similarly, the compounds which posed to be selective for any one of these properties and not for the others were not considered for the final selection. Ultimately, the screened list of compounds was checked for the drug score. Quinic acid and Curcumin with higher drug scores were finally selected. Interestingly, the former happened to be the *M. oleifera* flower compound and the later, the standard therapeutic used for comparison.

Studies on ADME

Results indicated that oral bioavailability of both Curcumin and Quinic acid were less than 30% with the probabilistic value of Curcumin being slightly greater than that of Quinic acid. This might be due to the possibility of passive absorption in Curcumin which seemed to be very less in Quinic acid as a result of decreased lipophilicity in the latter. But still, Quinic acid had an advantage over Curcumin in escaping first-pass metabolism. Once again, the contributing factor for the increased first-pass metabolism in Curcumin seemed to be its lipophilicity [31,32]. But in this compound, the effect of passive absorption was more than that of first-pass metabolism on bioavailabilty which was the cause for the minor difference observed in the bioavailabilty between the two compounds.

Being lipophilic, Curcumin showed 100% intestinal passive absorption contributed only by transcellular route while Quinic acid due to its hydrophilicity showed only 4% intestinal passive absorption, the majority of which was contributed by paracellular route (99%). Similar effect was observed in bisphosphonates whose poor absorption was speculated to have been attributed to their poor lipophilicity [33]. Jejunum permeability and absorption rate were also more for Curcumin than Quinic acid. The pK_a values represented the acidic or basic strength of the compounds. The values indicated that Quinic acid ($2< pK_a <7$) was a weak acid while Curcumin ($7< pK_a <10$) was a weak base. Decreased jejunum permeability and absorption rate of Quinic acid than Curcumin pointed out the possibility that Quinic acid might be highly ionized at jejunum pH which might have prevented it to cross the lipid membrane.

Quinic acid being an acid, had less Vd value compared to Curcumin which showed moderate value as it was a hydrophobic compound. Curcumin was also predicted as a neutral compound with no base groups. Also the note that Vd values could be larger for very hydrophobic drugs suggested that Curcumin was only moderately lipophilic. The greater Vd value of Curcumin compared to Quinic acid might be due to its lipophilicity [31,32]. A previous study on administration of 15 basic drugs to dogs also supported this concept [34].

The extent of brain penetration (Log PB) seemed to be less in case of Quinic acid and denoted that its concentration in brain was less than that in plasma which was opposite in case of Curcumin. Further, from Brain/plasma equilibration rate of the two compounds given by the combination of permeation rate and fraction unbound in brain [Log (PS * fu, brain)], it could be interpreted that the unbound fraction of Quinic acid in brain tissue was more than that of Curcumin. Also, the unbound fraction of Quinic acid in plasma seemed to be greater than that of Curcumin. An earlier study had also demonstrated that Quinic acid did not accumulate in the blood stream even after repeated oral administrations [10]. The results suggested that Quinic acid could cross BBB and penetrate the brain only with great difficulty which was easier for Curcumin. This might be attributed to the lipophilic nature and transcellular passive

diffusion observed in Curcumin. In fact Curcumin's greater ability to cross BBB had been applied in treating Alzheimer's disease, a neurodegenerative disease [35]. Though this property of Curcumin might be of more value than Quinic acid in CNS related diseases, its implication in other conditions might exert an adverse effect on brain.

Toxicity analysis

The probability of Quinic acid to yield a positive AMES test result was 4.7 times lesser than that of Curcumin which in turn implied the number of times the former was less risky than the latter. The difference might be due to the difference in the chemical structures of these two compounds. The results also implied the absence of hazardous fragments in both Quinic acid and Curcumin. Though both the compounds were not genotoxic, Quinic acid with a lesser value for positive AMES test seemed to be safer than Curcumin.

Human Ether-a-go-go-Related Gene is an essential gene that codes for the alpha subunit of potassium ion channel which mediates the electrical activity of the heart. Analyzing the inhibition of this ion channel constitutes an emerging field in drug toxicity studies. The inhibition can lead to long QT syndrome [36]. The probabilities of the selected compounds being hERG inhibitors with IC_{50} lower than the cut-off value of 10 µM were calculated. The results indicated that the probability of Quinic acid was nil while Curcumin showed a probability value of 0.5. They were indicative of the possible cardiotoxicity associated with Curcumin which might be absent in Quinic acid. This was supported by a previous study which reported that Curcumin inhibited hERG potassium ion currents in Human Embryonic Kidney (HEK) 293 cells stably expressing hERG channels in a dose-dependent manner, with IC_{50} value of 5.55 µM [37].

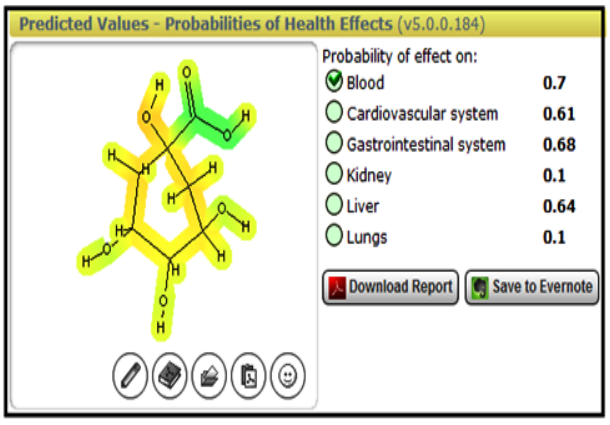
Lethal dose 50 (LD_{50}), is usually considered as the standard measure of acute toxicity. The Acute Toxicity module employed predicted LD_{50} values of the selected compounds in rats and mice based on various routes of administration. LD_{50} values obtained for Quinic acid were all reliable with the highest reliability observed in mouse subcutaneous administration which showed the largest LD_{50} value. Higher LD_{50} values indicated less toxicity. The values were much higher than that of Curcumin except for intraperitoneal route in rat which was almost close to each other in both the cases. However, this value was of less reliability as like the Curcumin LD_{50} for mouse oral route. Neverthless, the LD_{50} for rat oral route which was of high significance in pharmacokinetics studies, was found to be almost twice with Quinic acid to that found with Curcumin. Also, mouse intraperitoneal route Curcumin LD_{50} which was of high reliability was much less than the respective Quinic acid LD_{50}. Results also indicated that mouse subcutaneous route followed by rat oral route showed minimum toxicity with Quinic acid while it was the rat oral administration which displayed the least toxicity with Curcumin. They were also suggestive of the less oral toxicity associated with the drug candidates, with Quinic acid on the preferable side.

The OECD toxicity module classified the query compounds into various categories [38]. Predictions were made in terms of probabilities of their respective LD_{50} for rat oral administration in the range between <5 mg/kg up to >5000 mg/kg. Based on these values, the most probable OECD hazard category for the compounds was detected. Quinic acid showed the higher probability of LD_{50}>2000 mg/kg which meant the value could also be >5000 mg/kg. Hence the compound could be either non-toxic (LD_{50}>5000 mg/kg) or be placed in the least toxic category V (LD_{50}=2001-5000 mg/kg). The probability of Curcumin's LD_{50} was >300 but <5000 mg/kg. Thus it could belong to either category V or to more toxic category IV (LD_{50}=301-2000 mg/kg). The results supported the observations made in the acute toxicity tests as described above and further suggested the less toxicity of Quinic acid over Curcumin.

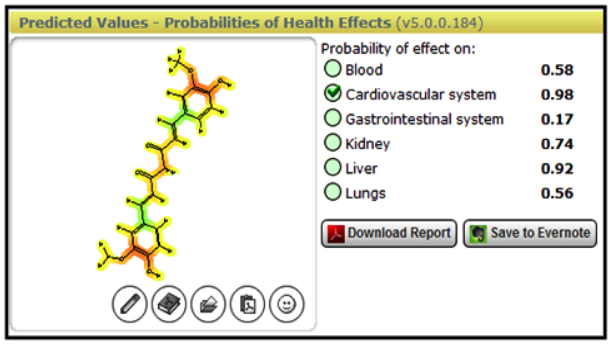
Concentration of chemicals in water lethal to 50% of exposed organisms (LC_{50}) is considered as the standard measurement of their aquatic toxicity. The two typical biological models used in aquatic toxicity assays are fathead minnow (*Pimephales promelas*) and water flea (*Daphnia magna*). Though reliability seemed to be less, the LC_{50} values of Quinic acid were found to be several times more than that of Curcumin, which suggested the aquatic toxicity of the former was far much less than that of the latter. The results also indicated that while the toxicity towards fathead minnow was less than that towards water flea in case of Quinic acid, it was the opposite in case of Curcumin. The aquatic toxicity of Curcumin could be confirmed with an earlier report which had illustrated excess accumulation of Curcumin in fish bodies and their consequent death due its overdoses [39].

Estrogen receptor alpha (ER-α) is a nuclear receptor activated by the sex hormone estrogen [40,41]. Compounds binding to ER-α can result in endocrine disruption producing adverse effects like reproductive toxicity or cancer. The module measured the estrogen receptor binding affinity (LogRBA) which was an estimate of the relative affinity of the test compounds to the receptor compared to its normal ligand estradiol. Compounds with LogRBA > 0 were classified as strong binders and those with LogRBA < -3 were classified as non-binders. Results indicated that both Quinic acid and Curcumin were non-binders of ER-α with LogRBA < -3. But on closely observing their values, it was spotted that unlike Quinic acid which showed zero for LogRBA > -3, Curcumin exhibited a probability value of 0.25 which suggested the likelihood of Curcumin to become a weak binder of ER-α (-3<LogRBA<0).

Health Effects module evaluated the likelihood of the query compounds to produce any adverse effects on a number of organs and their systems within the therapeutic dose range as given in Fig. 1. The structural features contributing to these effects were represented as color-maps with red associated with toxicity and green with non-toxic effect. Though the ethanobotanical, pharmacognostic, phytochemical and pharmacological properties of turmeric and Curcumin have been extensively reviewed [42], the results of the current study indicated that except for blood and gastrointestinal system, for all other systems studied, Curcumin's adverse effects were more than that of Quinic acid. The least effects were seen on kidneys and lungs by Quinic acid while the most adverse effect was on cardiovascular system by Curcumin. This might be due to the cardiotoxicity produced by Curcumin by inhibiting hERG potassium channels as observed earlier in this study. Quinic acid had been found to exert beneficial effects on kidney by providing enough acidity in the urine to decrease the chances of kidney stones formation [43]. A number of previous reports had explained the adverse effects of Curcumin which could be the reason for the observed results. It could be toxic to liver and gallbladder when consumed for a long period in very high doses. It eased the flow of bile, flushing the gallstones through bile duct and blocking it [44]. It might produce side effects like stomach ache, nausea, diarrhea and gastrointestinal bleeding. It exhibited antithrombotic activity and hence not advisable in bleeding conditions. It could chelate iron strongly and hence lead to anemia [45]. It stimulated the over production of stomach acid and could lead to hypoglycemia and hypotension. It could increase the risk of hyperoxaluria. It might also lead to other complications such as uterine contractions, allergic reactions and drug interactions [46].



(a)

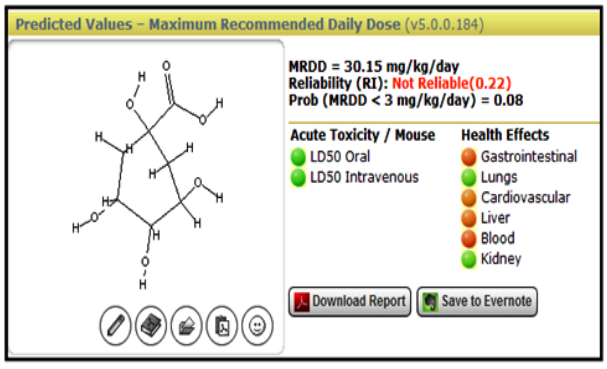


(b)

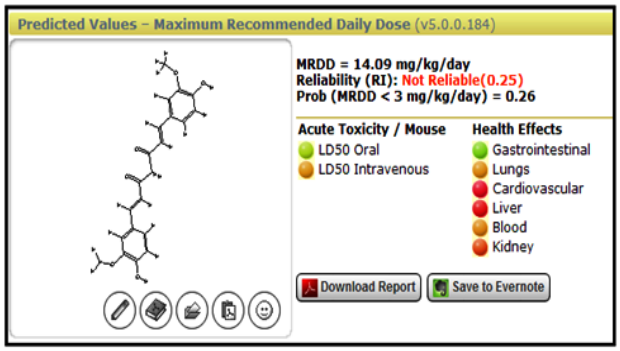
Fig. 1: Health effects of (a)Quinic acid (b)Curcumin. The ticked ones are the respective systems experiencing the largest probability of adverse health effects. The fragmental contribution maps on the structure pane illustrate the role of individual atoms and fragments of the query compounds in a color-coded manner. Red color indicates a positive contribution to the final predicted value Green color indicates a negative coefficient in the regression equation. Yellow substructure highlights usually accompany the knowledge based "expert-like" models.

MRDD, a complex property based on various complicated mechanisms, evaluated the potential of the query compounds to produce long-term adverse effects as depicted in Fig. 2. Usually, MRDD that did not exceed 3 mg/kg/day could be of greater practical value as it could be indicative of potential safety problems. But, the probability for this condition seemed to be less for Quinic acid than Curcumin and both the values seemed to be of very less reliability. Nevertheless, acute toxicity and health effects results associated with these values suggested that Quinic acid was less toxic than Curcumin as indicated by more number of green signals in the former while the latter displayed red signals also besides orange signals. These observations coincided with the results of previous sections of toxicity interpretations. Thus it could be considered that while the body could produce lesser toxic effects with greater MRDD of Quinic acid, it was the opposite in case of Curcumin which once again reflected the lesser toxicity of Quinic acid compared to Curcumin.

Earlier studies have suggested the significant role of certain bioactive compounds derived from medicinal flower extracts as drug candidates. Phenolic compounds of *Phlomis crinite* flower extracts have been analyzed as useful candidates for chemoprevention studies [47]. Similarly, another study has indicated that rutin and quercitrin from the flowers of *Hypericum montbretti,* possess antidepressant-like effects [48]. In view of their reported phytochemical, pharmacological and toxicological properties, anthocyanins and protocatechuic acid from the calyx extracts of *Hibiscus sabdariffa* have been found to be useful as antihypercholesterolaemic, antinociceptive and antipyretic agents [49]. Similarly, the current findings support that Quinic acid, a major bioactive compound of *M. oleifera* flowers, is a potent drug candidate of high therapeutic value.



(a)



(b)

Fig. 2: Maximum recommended daily dose of (a)Quinic acid (b)Curcumin are expressed along with the traffic–light buttons representing their respective LD_{50} values and adverse health effects. Though MRDD of Quinic acid is more, it exhibited greater number of green lights (non-toxic) than Curcumin.

CONCLUSION

The study clearly demonstrated that the pharmacokinetic properties of Quinic acid were favourable as a drug candidate. The comparative analysis with the other compounds, besides emphasizing the greater drug potential and lesser toxicity of Quinic acid over Curcumin, illustrated the application of this predominant phytocomponent of *M. oleifera* flowers in combating the manifestation of prostate cancer.

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