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Lead finding from whole plant of *Marrubium vulgare* L. with Hepatoprotective Potentials through *in silico* methods

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ABSTRACT

Objective: In the present study an attempt has been made to study the antihepatotoxic activity of active compounds in this plant through *in silico* methods. **Methods:** We have taken 12 compounds from this plant. All the compounds were further subjected to molecular property prediction and drug likeness by Molinspiration and found in compliance with Lipinski's rule of five. Biochemical parameters like SGOT and SGPT were determined by Reitman and Frankel, ALP by Kind and King, TP by reported methods of Wooton. **Results:** All the compounds were showed expected similar bioactivity especially in case of enzyme inhibition. Compound Vulgarin showed no violation with good drug likeness score and biological activity as compare to standard drug Silibinin. Vulgarin exhibited a significant antihepatotoxic activity by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase (SGOT) serum glutamate pyruvate oxaloacetate transaminase (SGPT) and alkaline phosphatase (ALP) while the total protein (TP) levels were increased when compared with standard drug silymarin against CCl₄-induced toxicity in Wistar rats. These biochemical observations were also supplemented by histopathological examinations of the liver sections. **Conclusions:** We found that Vulgarin one of the twelve compounds is showed better drug likeness and biological activity against Silibinin. So this particular compound can be taken as lead compound for further drug discovery for hepatotoxic activity.

1. Introduction

Liver injury can be induced by various factors, and hepatotoxins, such as CCl₄, ethanol and acetaminophen which are metabolized by cytochrome P450 (CYP2E1) CCl₄, the classic hepatotoxin, is widely used to induce liver damage in animal models and to investigate the role of lipid peroxidation as a mediator of hepatic injury. The mechanism of CCl₄-induced acute liver injury is accepted widely that CCl₄ was metabolized to a highly reactive trichloromethyl radical (CCl₃·) by cytochrome P450 in liver. CCl₃· in liver can induce lipid peroxidation and lead to hepatocellular membrane damage[1]. Natural antioxidants could prevent

the deleterious effects of toxic agents by scavenging free radicals and other reactive oxygen species[2].

In this paper we compare different compounds of plant *Marrubium vulgare* L. with the standard drug Silibinin on the basis of Lipinski's rule of 5 and physiological interpretation by Molinspiration software.

Marrubium vulgare L. (Lamiaceae) also called as Paharigandana (Hindi), White horehound (English), Troper (Kashmiri). Whole plant grows in waste ground throughout Europe. It thrives almost in any soil, but does best in light calcareous, rather dry, soil and sunny situations. It is naturalized in North and South America, the Mediterranean district and Western Asia as far as India. This plant was frequently employed as folk medicine to treat a variety of ailments, exhibits antispasmodic and antinociceptive effects in different experimental models. It possesses tonic, aromatic, stimulant, expectorant, diaphoretic and diuretic

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properties. It is helpful for bronchial asthma and non-productive cough. It was formerly much esteemed in various uterine, visceral and hepatic affections and in phthisis[3]. The plant is reported to possess hypoglycemic[4], antibacterial[5], antidiabetic[6], Gastroprotective activity[7] and many other reported biological activities. Essential oils extracted by distillation from aromatic plants are appreciated for their bioactive efficacy as fungicides, bactericides[8], antioxidant[9] and other biological activities. In the present study we evaluate the hepatoprotective activity of *Marrubium vulgare* L. compounds through in silico analysis.

On the basis of literature survey we find many compound isolated from plant *Marrubium vulgare* L. Some of them are phenylethanoid glycoside, marruboside, (+) (E)-caffeoyl-malic acid, acteoside, forsythoside B, arenarioside and ballotetroside [10].

vulgarol, β -sitosterol, lupeol, marrubiin, vulgarin and apigenin-O-glucoside [11]. natural lactoyl (2-hydroxypropionyl) flavonoids, luteolin and apigenin-7-lactates together with their 2''-O-glucuronides and 2''-O-O-glucosides. The known flavonoids, vicenin II, vitexin, luteolin 7-glucoside, apigenin-7-O-glucoside, apigenin-7-(6''-p-coumaroyl) glucoside, chrysoeriol, quercetin 3-rhamnoglucoside and apigenin [12], stachydrine, flavonoids, anthocyanins, ascorbic acid and caffeinic acid [13]

2. Materials and Methods

The structure of these chemical compounds were obtained from many research articles and each chemical compound was drawn with chemical drawing tools such as chem Draw ultra 7.0 and saved in the 'mol' file format. The biological activities of the compounds were predicted individually with the help of Molinspiration's biological activity calculator. Drug likeness of the compounds was tested Lipinski's rule of 5 and this is also done with Molinspiration's property calculator [14].

2.1. Experimental animals

Male Albino Wistar rats weighing 150–200 g were employed for assessing the antihepatotoxic activity. They were procured from the Central Animal House of Jamia Hamdard, New Delhi (Sanction Letter No. 173/CPCSEA), after approval under the project proposal number-326. They were fed with a standard pellet diet and water ad libitum.

2.2. Antihepatotoxic activity

The rats were divided into four groups, six rats in each group. Group I served as normal control, which received normal saline only. Group II as toxic control received CCl₄ diluted with liquid paraffin in a ratio of (1:1) [1.5 ml/kg b

w, per oral (p.o.)] on the first day[15]. Group III was given a single dose of CCl₄ on the first day (1.5 ml/kg b w, p.o.) and then silymarin (Slybon-70, 10 mg/kg bw p.o.) once a day for 6 days. Group IV received a single dose of CCl₄ (1.5 ml/kg bw, p.o.) on the first day and then methanolic extract at the dose of 500 mg/kg b w, p.o. for 6 days. Group V received a single dose of CCl₄ (1.5 ml/kg bw, p.o.) on the first day and then compound Vulgarin at the dose of 50 mg/kg b w, p.o. for 6 days. On day 8, the blood samples were withdrawn by puncturing the orbital plexus first, and then the rats were killed by decapitation. The blood samples were allowed to clot for 30–40 min at room temperature.

2.3. Assessment of liver function

Biochemical parameters like SGOT and SGPT were determined by Reitman and Frankel[16], ALP and TP were determined by reported methods of Kind and King [17]and Wooton [18].

2.4. Statistical analysis

The data of biochemical estimations were reported as \pm SE. For determining the statistical significance, one-way analysis of variance and Dunnett's test were employed. P-values of less than 0.05 were considered significant [19].

2.4. Histopathological studies of the liver

Rat livers were quickly removed after autopsy and fixed in 10% formalin. The sections were cut and then stained by hematoxylin and eosin. These were observed under microscope [20].

3. Result

3.1. Drug likeness calculation on the basis of Lipinski rule of five

On the basis of literature survey we have taken twelve compounds from the plant and with the help of Molinspiration software we calculate different properties of these compounds. The twelve compounds showed different drug likeness score and compare with standard drug Silibinin (Table 1).

3.2. Biological activity of compounds

Nine compounds of the plant which fulfill the requirements of Drug likeness are taken for biological activity calculation with the help of Molinspiration software and compared with standard drug Silibinin Table 2.

3.3 Antihepatotoxic activity

Table 1.

Properties of compounds at different parameters, based on Lipinski's rule of five

Compd no.	Compounds	mllog P	TPSA	n atoms	MW	nON	nOHNH	n violations	n rotb	volume
I	Marrubiin	2.945	59.673	25	346.46	4	1	0	3	335.179
II	Premarrubiin	2.777	44.773	25	346.46	4	0	0	0	331.81
III	Vulgarin	2.164	46.533	20	276.37	3	1	0	0	271.435
IV	Luetol	7.64	40.456	33	456.75	2	2	1	1	485.53
V	Vulgarol	5.184	40.456	23	322.53	2	2	1	5	351.591
VI	Apigenin-7-glucronide	0.25	187.118	33	462.40	11	6	2	4	381.125
VII	Vitexin	0.518	181.041	31	432.38	10	7	1	3	355.203
VIII	Apigenin	2.463	90.895	20	270.24	5	3	0	1	224.049
IX	Chryseriol	2.021	120.357	23	316.26	7	4	0	2	257.612
X	Stachydrine	-3.999	37.299	10	144.19	3	1	0	1	145.368
XI	Acetoside	0.114	236.059	44	624.63	14	9	3	12	546.5
XII	1-Caffeoyl-L malic acid	0.036	141.359	20	282.20	8	4	0	6	226.294
XIII	Silibinin (Standard Drug)	1.465	155.147	35	482.44	10	5	0	4	400.862

Table 2.

Biological activity of compounds on different parameters

Compd.No.	Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
I	Marrubiin	0.17	0.30	-0.37	0.40	0.24	0.39
II	Premarrubiin	0.02	0.04	0.38	0.39	0.03	0.16
III	Vulgarin	0.26	0.17	-0.39	0.58	0.58	0.40
IV	Vulgarol	0.27	0.33	-0.20	0.90	0.06	0.58
V	Vitexin	0.13	-0.14	0.19	0.23	0.03	0.46
VI	Apigenin	-0.07	-0.09	0.18	0.34	-0.25	0.26
VII	Chryseriol	-0.10	-0.24	0.24	0.18	-0.31	0.19
VIII	Stachydrine	-0.03	1.07	-1.67	-2.05	-1.12	0.09
IX	1-Caffeoyl-L malic acid	-0.01	-2.92	-0.26	0.38	-0.12	0.19
X	(Std. Compound) Silibinin	0.07	-0.05	0.01	0.16	0.02	0.23

Table 3.

Effect of methanolic extract and compound no. III (Vulgarin) of Marrubium vulgare L. on serum enzymatic activity in CCl4 induced liver damage in rats.

Groups(n=5)	Treatment	Dose	SGOT units/ml	SGPT units/ml	ALKP units/ml	TP gm/dl
I	Normal (control)	---	34.48 ± 1.45	25.54 ± 0.70	27.59 ± 0.56	7.37 ± 0.16
II	Toxic (control)	1.5 mg/kg (p.o.)	117.21 ± 4.05	93.71 ± 4.03	54.09 ± 2.39	4.41 ± 0.16
III	Silibinin (standard drug)	10 mg/kg (p.o.)	57.98 ± 1.61**	42.71 ± 2.90**	34.33 ± 1.05**	7.56 ± 0.12**
IV	Methanol extract	500 mg/kg (p.o.)	92.33 ± 2.30**	72.92 ± 3.37**	36.58 ± 2.34**	6.14 ± 0.28**
V	Vulgarin	50 mg/kg (p.o.)	59.12 ± 3.74**	45.24 ± 3.05**	42.54 ± 2.35**	6.93 ± 0.12**

SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvate transaminase; ALP, alkaline phosphatase; TP, total protein; s.c., subcutaneous; p.o., per oral.

** $P < 0.01$; * $P < 0.05$ vs CCl4; $P > 0.05$ ns.

Values are mean ± S.E. of five animals. One way analysis and Dunnett's test.

Methanolic extract & compound vulgarin from Marrubium vulgare L showed antihepatotoxic activity on wistar rat against CCl4 induced toxicity (Table 3).

4. Discussion

These properties are calculated on the basis of Lipinski's rule of five, which states that any compound considered as drug should have partition coefficient less than 5, its polar surface area within 140 Å², it should have H bond acceptor less than 10, it should have H bond donor less than 5 and its molecular weight within 500 dalton. Nine compounds out of twelve fulfill the Lipinski rule of five these are Marrubiin,

Premarrubiin, Vulgarin, Vulgarol, Vitexin, Apigenin, Chryseriol, Stachydrine, 1-Caffeoyl-L malic acid. Only these nine compounds further consider for biological activity.

On the basis of mechanism of action of Silibinin i.e. enzyme inhibition, protease inhibition and kinase inhibition we compare compound for their hepatoprotective activity and after comparison with Silibinin we find that five compounds, Marrubiin, Vulgarin, Vulgarol, Vitexin and Apigenin showed better enzyme inhibition than Silibinin. Vulgarin showed best activity as compared to standard drug, so this compound is taken for antihepatotoxic activity.

As shown in Table 3 the activities of liver enzymes serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate oxaloacetate transaminase (SGPT), and alkaline phosphatase (ALP) were markedly increased and

total proteins (TP) were decreased in CCl₄ treated rats as comparison to normal values. Silymarin (10 mg/kg, b.w., p.o.) had significantly decreased the level of SGOT, SGPT and ALP 57.98, 42.71, 34.33 Units/ml and increased total protein by 7.56 g/dl, respectively, whereas methanolic extract (500 mg/kg) had considerable decrease in SGOT 92.33, SGPT 72.92, ALP 36.58 Units/ml and an increase of TP 6.14 g/dl, was observed. Compound treated animals (50 mg/kg) had considerable decrease in SGOT 59.12, SGPT 45.24, ALP 42.54 Units/ml and an increase of TP 6.93 g/dl was observed. The histopathological studies also showed significant recovery of liver cells in the standard drug, methanolic extract & compound treated animals.

In this study we gone through 12 compounds of plant *Marrubium vulgare* L. for their Drug likeness and Biological activity in silico manner. On the basis of Lipinski's rule of five and comparison with standard drug Silibinin, we found that Vulgarin one of the twelve compounds is showed better drug likeness and biological activity against Silibinin. Vulgarin showed significant antihepatotoxic activity against CCl₄-induced toxicity in Wistar rats. So this particular compound can be taken as lead compound for further drug discovery for Hepatotoxic activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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