Research Article

# Lead Molecule from Whole Plant of *Ligustrum vulgare* L by Hepatoprotective Potentials Through *in Silico* Methods

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**Received:** 11-09-2022; **Revised:** 20-11-2022; **Accepted:** 26-11-2022; **Published on:** 15-12-2022.

**ABSTRACT**

**Objective:** In the present study an attempt has been made to study the antihepatotoxic activity of active compounds in this *Ligustrum vulgare* L plant through in silico methods.

**Methods:** We extracted 12 compounds from this plant. All the compounds were further subjected to molecular properties prediction and drug likeness by Molinspiration and found according to Lipinski's rule of five. Biochemical parameters SGPT and SGOT were determined by Reitman and Frankel, ALP was determined by Kind and King, TP was determined by reported methods of Wooton.

**Results:** All the 12 extracted compounds were showed expected bioactivity especially in case of enzyme inhibition. Among other Compounds Vulgarin showed no violation and good drug likeness score and biological activity as compare to standard drug Silimarin. Vulgarin compound exhibited a significant antihepatotoxic activity by inhibiting the elevated levels of serum enzymes such as SCOT, SGPT and ALP while the total protein (TP) levels were increased when compared by standard drug silymarin against paracetamol-induced liver toxicity in Wistar rats. These biochemical observations were also determined by histopathological examinations of the liver sections of the rats.

**Conclusions:** We found that Vulgarin is one of the active compounds among twelve compounds which showed better drug likeness and biological activity against standard drug silymarin. So, these particular compounds can be taken as lead compound for further drug discovery for hepatotoxic screening.

**Keywords:** *Ligustrum vulgare* L, Hepatoprotective Potentials, In silico methods, Lipinski's rule of five, SGPT and SGOT.

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**DOI:** 10.47583/ijpsrr.2022.v77i02.016

**DOI link:** http://dx.doi.org/10.47583/ijpsrr.2022.v77i02.016

## INTRODUCTION

Liver injury can be induced by various factors including hepatotoxins, such as Paracetamol, $CCl_4$, Ethanol and Acetaminophen which are metabolized by cytochrome P-450 (CYP2E1) CCl4, the classic hepatotoxin, which is widely used to induce liver damage in animals and to explore the role of lipid peroxidation as a mediator of hepatic injury in selected animals. The mechanism of $CCl_4$-induced acute liver toxicity is accepted widely that $CCl_4$ was metabolized to a highly reactive trichloromethyl radical ($CC1_3$-) by cytochrome P450 in liver. $CC1_3$- in liver can induce lipid peroxidation and leads to hepatocellular membrane damage[1]. Natural antioxidants can prevent the deleterious effects of toxic agents by scavenging free radicals and other reactive oxygen species present in the affected animal models[2].

In this research we compare the different compounds present in the plant Ligustrum vulgare L with the standard drug silymarin on the basis of Lipinski's rule of five and physiological interpretation by Molinspiration software.

Different Ligustrum species have been used in traditional Chinese and Japanese medicine due to its liver-protecting antiviral and anti-mutagenic activities. In the folk medicine of Azerbaijan, common privet leaves (Ligustrum vulgare) are used in hypertension therapy, which has been supported by recent studies of hypotensive and diuretic effects of common privet[3].

Ligustrum vulgare L belongs to the family of Oleaceae, also called as common privet. Ligustrum vulgare (common privet) plant leaves have been used for treatment of diuretic, oropharyngeal inflammations, as antirheumatic and hypotensive agents in folk medicine in southern Europe. Plant *Ligustrum* species were used in prevention and treatment of hepatitis and acute to chronic bronchitis due to their different triterpenoid glycosides present and its liver-protecting, antiviral and antimutagenic capacity[3]. An ethanolic extract of common privet leaves exhibited weak antibacterial activity against Gram-positive bacteria and cytotoxic activity against HeLa cells. The ethyl acetate extract of *L. vulgare* leaves significantly inhibited the angiotensin converting enzymes (ACE) and neutral endopeptidase[4].

On the basis of literature survey we find many compound isolated from plant Ligustrum vulgare L. Some of them are phenylethanoid glycoside, ligustrin, (+) (E)-caffeoyl 1-malic acid, acteoside, forsythoside B, arenarioside and ballotetroside[5], vulgarol, B-sitosterol, lupeol, ligustrin, vulgarin and apigenin-O-glucoside[6]. Natural lactoyl (2-hydroxypropionyl) flavonoids, luteolin and apigenin 7-lactates together with their 2'-O-glucuronides and 2"-0-0-glucosides. The known flavonoids, vicenin II, vitexin, luteolin 7-glucoside, apigenin-7-0-glucoside, apigenin-7-(6"-p-coumaroyl) glucoside, chrysoeriol, quercetin 3-rhamnoglucoside and apigenin[7] stachydrine, flavonoids, anthocyanins, ascorbic acid and caffeinic acid[8].

## MATERIALS AND METHODS

The structure of these chemical compounds was 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 pharmacological activities of the compounds were predicted individually by using 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 biological property calculator[9].

### Experimental animals

Male and female Albino Wister rats weighing 180-220 gms were taken for assessing the antihepatotoxic activity. They were purchased from the Biogen Animal House, Bangalore Sanction Letter No. 008/CP/IAEC/2020), after approval under the project proposal number-JKKMMRACP/IAEC/ 2020/008. They were fed with a standard pelleted diet along with glucose water ad libitum.

### Antihepatotoxic activity

The wister rats were divided into four groups, each group in six rats. Group I marked as normal control, which received 0.9% normal saline. Group II marked as negative control received a CCl4 which is dilute with liquid paraffin in a ratio of (1:1) that is [1.5 ml/kg bw, per oral (p.o.)] on the first day of study[10].

In first day, Group III was received single dose of CCl4 (1.5 ml/kg body weight, p.o.) and then silymarin (Slybon-70, 10 mg/kg body weight p.o.) given 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 collected and allowed to stand for clot 30-40 mins at room temperature.

### Assessment of liver function

Biochemical parameters SGOT and SGPT were determined by Reitman and Frankel[11], ALP and TP were determined by reported methods of Kind and King[12] and Wooton[13].

### Statistical analysis

The data of biochemical estimations were reported as + SE. For determining the statistical significance, one-way analysis of variance and Dunnett's test were studied. P-values less than 0.05 were considered as significant for study[14].

### Histopathological studies of the liver

Wister Rat livers were quickly removed after autopsy and fixed with 10% formalin. The sections were cut and then stained with hematoxylin and eosin. These were observed under microscope[15].

**Table 1:** Biological Properties of compounds at different parameters, based on Lipinski's rule of five:

| Compound no | Compound name | Milog P | TPSA | N atoms | MW | nON | nOH-NH | Volume |
|---|---|---|---|---|---|---|---|---|
| I | Ligustrin | 2.945 | 59.673 | 25 | 346.46 | 4 | 1 | 335.179 |
| II | Preligustrin | 2.777 | 44.773 | 25 | 346.46 | 4 | 0 | 331.81 |
| III | Vulgarin | 2.164 | 46.533 | 20 | 276.37 | 3 | 1 | 271.435 |
| IV | Luetol | 7.64 | 40.456 | 33 | 456.75 | 2 | 2 | 485.53 |
| V | Vulgarol | 5.184 | 40.456 | 23 | 322.53 | 2 | 2 | 351.591 |
| VI | Apigenin-7-glucronide | 0.25 | 187.118 | 33 | 462.40 | 11 | 6 | 381.125 |
| VII | Vitexin | 0.518 | 181.041 | 31 | 432.38 | 10 | 7 | 355.20 |
| VIII | Apigenin | 2.463 | 90.895 | 20 | 270.24 | 5 | 3 | 224.049 |
| IX | Chryseriol | 2.021 | 120.357 | 23 | 316.26 | 7 | 4 | 257.612 |
| X | Stachydrine | -3.999 | 37.299 | 10 | 144.19 | 3 | 1 | 145.368 |
| XI | Acetoside | 0.114 | 236.059 | 44 | 624.63 | 14 | 9 | 546.5 |
| XII | Caffeory-L malic acid | 0.036 | 141.359 | 20 | 282.20 | 8 | 4 | 226.294 |
| XIII | Silymarin | 1.465 | 155.147 | 35 | 482.44 | 10 | 5 | 400.862 |

**Table 2:** Biological activities of compounds on different parameters were follows by:

| Compound no | Compound name | GPCR ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor | ligand Protease inhibitor | Enzyme inhibitor |
|---|---|---|---|---|---|---|---|
| I | Ligustrin | 0.17 | 0.30 | -0.37 | 0.40 | 0.24 | 0.39 |
| II | Preligustrin | 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 |
| V | Vulgarol | 0.27 | 0.33 | -0.20 | 0.90 | 0.06 | 0.58 |
| VII | Vitexin | 0.13 | -0.14 | 0.19 | 0.23 | 0.03 | 0.46 |
| VIII | Apigenin | -0.07 | -0.09 | 0.18 | 0.34 | -0.25 | 0.26 |
| IX | Chryseriol | -0.10 | -0.24 | 0.24 | 0.18 | -0.31 | 0.19 |
| X | Stachydrine | -0.03 | 1.07 | -1.67 | -2.05 | -1.12 | 0.09 |
| XII | Caffeory-L malic acid | -0.01 | -2.92 | -0.26 | 0.38 | -0.12 | 0.19 |
| XIII | Silymarin | 0.07 | -0.05 | 0.01 | 0.16 | 0.02 | 0.23 |

**Table 3:** Effects of methanolic extract and compound no. III (Vulgarin) of *Ligustrum vulgare* L. on serum enzymatic activity in CCl4 induced liver damage in wistar rats were follows:

| 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 | Negative (control) | 1.5mg/kg (p.o.) | 117.21 ±4.05 | 93.71±4.03 | 54.09 ±2.39 | 4.41 ± 0.16 |
| III | Silymarin (standard drug) | 10 mg/kg (p.o.) | 57.89±1.61** | 42.75 ±2.90** | 34.38±1.05** | 7.51± 0.12** |
| IV | Methanol extract | 500 mg/kg (p.o.) | 92.63± 2.30** | 78.92±3.37** | 36.95±2.34** | 6.10± 0.28** |
| V | Vulgarin | 50 mg/kg (p.o.) | 59.10±3.74** | 45.21±3.05** | 43.51± 2.35** | 6.91±0.12** |

## RESULTS

### 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 of ligustrum vulgare and with the help of Molispiration software biological evaluator. We calculated different biological properties of these twelve compounds. The twelve compounds showed different drug likeness score and compare with standard drug Silymarin (Table 1).

### Biological activity

Nine compounds of the plant ligustrum vulgare which were fulfill the requirements of Drug likeness, were taken for biological activity calculation by using Molinspiration software and compared with standard drug Silymarin Table 2.

### Antihepatotoxic activity

The compound vulgarin isolated from Methanolic extract of ligustrum vulgare L and the plain methanolic extract showed antihepatotoxic activity on wistar rate against $CCl_4$ induced toxicity (Table 3).

## DISCUSSION

These biological properties are calculated on the basis of Lipinski's rule of five, these rule states that any compound considered as drug should have partition coefficient less than 5 or equal, these polar surface area should be within 140 $A_2$, it should have Hydrogen bond acceptor less than 10, it should have Hydrogen bond donor less than 5 and its molecular weight (MW) within 500 doltan. Out of twelve compounds nine compounds fulfill the Lipinski rule of five these are Ligustrin, Preligustrin, Vulgarin, Vulgarol, Vitexin, Apigenin, Chryseriol, Stachydrine, 1-Caffeory-L malic acid. These nine compounds further consider for biological activity.

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

Table 3 showed that activities of liver enzymes serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate oxaloacetate transaminase (SGPT), and alkaline phosphatase (ALP) were increased rapidly and total

proteins (TP) levels were decreased in $CCl_4$ treated rats were compared with normal values. Silymarin (10 mg/kg, body weight, p.o.) had significantly decreased the level of SGOT, SGPT and ALP 57.89, 42.75, 34.38 Units/ml and increased total protein by 7.51 g/dl, respectively, whereas methanolic extract of *Ligustrum vulgare* L (500 mg/kg) had considerable decrease in SGOT 92.63, SGPT 78.92, ALP 36.95 Units/ml and an increase of Total Protein 6.10 g/dl, was observed. Compound treated animals (50 mg/kg) had considerable decrease in SGOT 59.10, SGPT 45.21, ALP 43.51 Units/ ml and an increase of Total Protein 6.91 g/dl was observed. The histopathological investigation also showed significant recovery of liver cells in the standard drug silimarin, methanolic extract & compound vulgarin treated animals.

## CONCLUSION

In this study we went through 12 compounds of plant Ligustrum 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 Silymarin, we found that Vulgarin was one of the twelve compounds is showed better drug likeness and biological activity against Silymarin. Phytoconstituent Vulgarin showed significant antihepatotoxic activity against $CCl_4$-induced hepatotoxicity in Wistar rats. So, this particular compound can be taken as lead compound for further drug discovery for Hepatotoxic activity.

**Acknowledgment:** We are very thankful to the Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy for providing necessary funds & facility.

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**Source of Support:** The author(s) received no financial support for the research, authorship, and/or publication of this article.

**Conflict of Interest:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.