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Integrating *in Silico* and *in Vivo* Approaches for Investigating the Role of Tonoliv Syrup in the Prevention and Treatment of Hepatoprotective Activity

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ABSTRACT

The liver can be harmed by carbon tetrachloride (CCl4) because it is converted by an enzyme called CYP450 into a highly reactive trichloromethyl radical. This radical causes the fatty acids present in the cytoplasmic membrane to undergo auto-oxidation, leading to changes in the regulation of Ca2+ ions. As a result, the morphology and functionality of the cell membranes are altered, ultimately leading to cell death. Tonoliv syrup is a polyherbal formulation that contains poly-phenolic compounds or flavonoids. These compounds act as free radical scavengers, seizing the radicals that contribute to the breakdown of CCl4 by microsomal enzymes. This helps to reduce liver damage caused by CCl4 exposure. All 10 active phytochemical constituents that target the protein "Cytochrome P450 2E1" (PDB ID-3E4E) underwent protein-ligand docking. Wedelolactone, saponin, sennoside a and b, andrographolide, ß-sitosterol, Kutkin, chicoric acid, berberine, chebulinic acid, and fumarine molecules exhibit binding energy close to the standard drug silybin A.

1. INTRODUCTION:

In recent years, there has been growing interest in leveraging both *in silico* and *in vivo* approaches to study the potential hepatoprotective activity of natural compounds. This research aims to bridge the gap between computational modeling and experimental validation by integrating *in silico* and *in vivo* methods to investigate the role of Tonoliv syrup in the prevention and treatment of liver damage. The integration of computational simulations with real-world experiments holds promise for a more comprehensive understanding of the mechanisms underlying the hepatoprotective effects of Tonoliv syrup. This interdisciplinary approach could provide valuable insights into the development of novel therapeutic strategies for liver disorders.

1. Computational Modeling of Tonoliv Syrup's Molecular Interactions

2. *In Silico* Prediction of Hepatoprotective Mechanisms

3. *In Vivo* Evaluation of Tonoliv Syrup's Efficacy in Liver Protection

4. Integrative Analysis of *In Silico* and *In Vivo* Data

5. Comparative Assessment of Tonoliv Syrup with Standard Hepatoprotective Agents

6. Implications for Translational Research and Clinical Applications

The liver, one of the largest organs in the human body, plays a crucial role in detoxification, excretion of endogenous and exogenous compounds, and the regulation of homeostasis. Its involvement in biochemical pathways related to growth, disease resistance, nutrient supply, energy provision, and reproduction underscores its significance in maintaining overall health and well-being. However, the liver is constantly exposed to environmental toxins and can be affected by unhealthy habits such as excessive alcohol consumption and misuse of over-the-counter drugs, leading to various liver conditions including hepatitis, cirrhosis, and alcoholic liver disease. Traditional and synthetic medications for liver problems have shown limited effectiveness and potential adverse effects, prompting a global shift towards the use of natural medicinal plants. Many herbal products and Ayurvedic preparations have been utilized for the treatment of liver ailments. However, due to the complex nature of severe liver diseases, a single phytoconstituent may not be sufficient to achieve the desired therapeutic effect. Therefore, the development and evaluation of an effective formulation, such as Tonoliv

syrup, using various indigenous medicinal plants, with proper pharmacological experiments, is paramount in addressing the challenges associated with liver disorders. This research aims to investigate the development and evaluation of Tonoliv syrup as a potential treatment for liver disorders, utilizing indigenous medicinal plants and conducting comprehensive pharmacological experiments to assess its efficacy and safety.[1,2]

Cytochromes P-450 (CYPs) are a superfamily of hemoproteins involved in the metabolism of medicines, chemicals, and endogenous substrates. They are found in various tissues and organs, with the liver containing the highest concentration of these enzymes. While CYP-dependent synthesis of reactive metabolites of parent chemicals has been implicated in the pathophysiology of toxic responses, the mechanism of medication hepatotoxicity often remains unexplained. Reactive metabolites generated by CYPs can lead to toxic hepatitis, particularly after significant doses of the drug, or immunoallergic hepatitis, when the substance triggers an adverse immunological reaction in the liver. Understanding the role of CYPs in medication hepatotoxicity is crucial for developing strategies to predict and prevent adverse drug reactions related to liver function.[3]

Liver damage is a significant health concern, and the use of Tonoliv syrup, a polyherbal remedy, has gained attention for its purported effectiveness in addressing this issue. Unlike allopathic drugs that primarily suppress symptoms, Tonoliv is claimed to target the root cause of liver ailments. The syrup comprises a blend of natural ingredients, including Eclipta alba, Cichorium intybus, Phyianthus niruri, Picrorhiza kurroa, Fumaria officinalis, Boerhaavia diffusa, Rheum emodi, and Tinospora cordifolia, which are believed to work synergistically to promote the healing of liver damage.

1.1. Andrographis paniculata:

Andrographis paniculata, commonly known as "kalmega", belongs to the Acanthacea family. It is found to be one of the most active ingredients in Indian polyherbal preparations used to treat liver ailments. A number of researchers have isolated flavonoids, sesquiterpenes, lactones and other compounds from the plant. The active antihepatotoxic principle is likely the diterpene 5 lactone andrographolide, which has been shown to protect against alcohol and carbon tetrachloride-induced hepatic microsomal lipid peroxidation.[4]

***1.2. Eclipta alba*:**

The Compositae family consists of Eclipta alba, also known as Bhringaraj. It is often included in herbal formulations used to treat liver diseases. The leaf juice is used as a liver tonic. It acts as an antioxidant, scavenging free radicals that are produced during the metabolism of CCl4 by microsomal enzymes. By capturing oxygen-related free radicals, the extract prevents the initiation of lipid peroxidation and protects polyunsaturated fatty acids from oxidative damage.[5]

1.3.Cichorium intybus:

Cichorium intybus, commonly known as Kasni, is a hepatoprotective herb. It contains polyphenols and flavonoids with significant antioxidant activity, which protect the liver by preventing lipid peroxidation of cell membranes.[6]

1.4. Phyianthus niruri:

Phyllanthus niruri is a member of the Euphorbiaceae family and has been found to have hepatoprotective effects. It works by reducing the levels of thiobarbituric acid reactive substances (TBARS), increasing the levels of reduced glutathione, and enhancing the activities of antioxidant enzymes such as glutathione peroxidase (GPx), glutathione transferase (GST), superoxide dismutase (SOD), and catalase (CAT).[7]

1.5. Picrorhiza kurroa

Picrorhiza kurroa, also known as kutki, belongs to the Scrophulariaceae family. Its hepatoprotective properties may be attributed to the presence of picroliv. This compound helps to reduce the elevated levels of bilirubin, lipoprotein-X (LP-X), alkaline phosphatase, glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) in the serum of infected animals. Picroliv also helps to decrease the amounts of lipid and hydroperoxide peroxides in the liver, and promotes the restoration of superoxide dismutase and glycogen.[8]

1.6. Fumaria officinalis:

Pittapapada, also known as Fumaria officinalis, belongs to the Fumariaceae family. Research has shown that Fumaria extracts have potent antihypertensive, diuretic, hepatoprotective, and

laxative properties, mostly due to the presence of isoquinoline alkaloids. Additionally, due to their high phenolic contents, it is claimed to have considerable antioxidant activity. Antioxidants have been employed as essential preventive agents for human health to fight against oxidative stress disorders and food system oxidative damage.[9]

1.7. Boerhaavia diffusa

The Nyctaginaceae family includes a plant called Punarnava, or Boerhaavia diffusa L., which is used to treat ascites and jaundice. It is reported to have diuretic and laxative properties, as well as hepatoprotective effects on the liver. Herbal medications often work by inhibiting CYT P450 2E1 and providing antioxidant effects, which is also the case with Punarnava. This plant has been recognized for its therapeutic benefits, including hepatoprotective, hypoglycemic, and anti-diabetic qualities, as well as its ability to scavenge free radicals.[10]

1.8. Rheum emodi

Amalaparni, also known as Rheum emodi, is a plant belonging to the family Polygonaceae. It has been used in traditional medicine for a variety of purposes, including as a laxative, tonic, diuretic, and treatment for fever, cough, indigestion, and menstrual disorders. Multiple studies have demonstrated that Rheum emodi possesses a range of beneficial properties, including anticancer, antioxidant, anti-inflammatory, antimicrobial, antifungal, antidyslipidemic, antiplatelet, antidiabetic, antiulcer, hepatoprotective, immunoenhancing, and nephroprotective activities.[11]

1.9.Tinospora cordifolia:

Guduuchi, also known as Tinospora cordifolia, belongs to the Menispermaceae family and possesses antipyretic, antiperiodic, anti-inflammatory, anti-rheumatic, spasmolytic, hypoglycemic, and hepatoprotective properties.[12]

2. MATERIALS AND METHOD:

IN VIVO STUDIES:

2.1 TREATMENT PROTOCOL

For this experiment, 36 rats were divided into 6 groups. The first group, Group A, was the normal control. The second group, Group B, was the intoxicated control. The third group,

Group C, was the standard control and received silymarin at a dose of 50 mg/kg b.w. orally. The remaining three groups, D, E, and F, received Tonoliv syrup at doses of 100 mg/kg b.w., 200 mg/kg b.w., and 400 mg/kg b.w. respectively. All groups except Group I were intoxicated with 1ml/kg b.w. of CCl4. Following intoxication, the respective drugs were administered for 5 days, and the serum was analyzed for marker enzymes on the 6th day. Additionally, another 36 rats were divided into 6 groups (following the same pattern as above, with the same drug doses administered), and a sleeping time study was conducted after administering 1ml/kg b.w. of CCl4, followed by Thiopental Sodium (40 mg/kg b.w. i.p). Sleeping time was recorded for each group.

2.2 Animals:

Experimental methodology

The Female Swiss albino mice weighing 140±20 g were obtained from the animal house of the Department of Plant Resources (Banaspati Vibhag) in Nepal. The mice were housed in polypropylene cages and kept under standard conditions, with access to standard diet and water as per protocol. The animals were maintained in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) for the care and use of laboratory animals.[13]

2.3 Acute toxicity study:

An acute toxicity study was conducted on Tonoliv syrup using the up & down method and guidelines from the Committee for the Purpose of Control and Supervision on Experiment on Animals (CPCSEA) and the Organisation of Economic Cooperation and Development (OECD). The study used AOT425Statpgm (Version: 1.0) and its results and recommendations were based on the statistical program for acute oral toxicity (OECD guidelines 425). Female Swiss albino mice weighing 20-25g were used in the study and were given all the necessary facilities as per the experiment protocol. Tonoliv syrup was administered orally, and the animals were continuously observed for 12 hours to detect any changes in autonomic or behavioral response. This observation continued for 24 hours, and the mortality rate was monitored for 48 hours. The study began with a dose of 500 mg/kg b.w p.o, followed by 1000 mg/kg b.w.p.o and was completed with the administration of 2000 mg/kg.b.w p.o with a limit dose of 2000 mg/kg. The animals were observed for 14 days for any signs of toxicity.[14]

2.4 Sleeping time study:

A total of 36 rats were randomly divided into six groups of six rats each. Group A will serve as the normal control group, and after the injection of thiopental sodium (25 mg/kg b.w) its normal sleeping time will be determined. Group B will serve as the negative control group, and will receive an administration of CCl4 (1 ml/kg b.w, 50% v/v with olive oil) via oral route. Group C will receive silymarin, a standard drug, at a dose of 50 mg/kg b.w via oral route. Groups D, E, and F will receive Tonoliv syrup via oral route at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight respectively. After 24 hours, all groups except the normal control group (Group A) will receive CCl4. The sleeping time of each group will then be determined by injecting thiopental sodium and measuring their sleeping time 2 hours after CCl4 administration. [15-17]

2.5 Biochemical parameter study:

In this experiment, 36 rats were chosen and randomly divided into 6 groups of 6 rats each. Group A was kept as the control group and given normal water daily. Group B was given CCl4 (1 ml/kg b.w, 50% v/v with olive oil; p.o) to serve as the control group. Group C was given the standard drug silymarin (50 mg/kg b.w: p.o) along with CCl4. Group D, E, and F were given Tonoliv syrup at doses of 100mg/kg, 200mg/kg, and 400mg/kg body weight, respectively. All groups except for Group A were given CCl4 (1 ml/kg b.w, 50% v/v with olive oil; p.o) on the 2nd and 4th day of the experiment. On the 6th day of the experiment, all animals in each group were anaesthetized with chloroform, and nearly 3 to 5 ml of blood was extracted by cardiac puncture using disposable syringes. The blood was allowed to clot, and the serum was separated by centrifugation at 3000 rpm for 15 minutes. The supernatant layer of serum was collected in a sample bottle and used for biochemical estimations by spectrophotometry method. The results were expressed as mean values.

2.6 Venue:

All experiments were conducted in the pharmacology laboratory of the Department of Plant Resources (Banaspati Vibhag) in Nepal. The department's terms and conditions were strictly followed.

3. Docking studies:

Docking program requires three computation steps to carry out docking study these are as follows:

(1) Preparation of the receptor

(2) Preparation of the ligand

(3) Setup of the parameters of the docking program

The following subsections describe these three steps in detail.[18]

3.1 Receptor preparation:

The three-dimensional structure of cytochrome P450 2E1, with PDB code 3E4E, was obtained from the Protein Data Bank (PDB) at http://www.rcsb.org/pdb/home/home.do. The RCSB is a global archive that contains information about the 3D structure of macromolecules, such as proteins, DNA, and their complexes, as determined by X-ray crystallography, NMR spectroscopy, and cryoelectron microscopy.[18]

3.2 Ligand preparation:

Tonoliv syrup is a polyherbal formulation that contains 10 different herbs, each with its own active chemical constituent responsible for therapeutic activity. These active chemical constituents, also known as ligands, were downloaded from the ChEMBL Database and loaded into Pyrex for molecular docking. We used Data Warrior to predict the ligand molecular properties, such as log P value, hydrogen bond donors and acceptors, surface area, molecular weight, and absorption, distribution, metabolism, and excretion (ADME) analyses for solubility, intestinal absorption, and toxicity.[18]

3.3 Target protein and active site prediction:

The various literature surveys were taken into consideration for the evaluation of protein and the active sites.[18]

3.4 Molecular docking:

To conduct a molecular docking study, we use Pyrex software. In this study, we download the ligand from the ChEMBL Database and the protein from Protein Data Bank (PDB), which can be accessed at http://www.rcsb.org/pdb/home/home.do. For example, to download Cytochrome P450 2E1 (PDB CODE-3E4E), hydrogen is added to interact with the amino acid present in the particular protein, as seen in the 2D structure. To add hydrogen, open Discovery Studio Visualizer, click on "Chemistry," then "Hydrogen Add." Save the file in PDB mode. To prepare both the ligand and protein, Pyrex software is used. For protein preparation, open Pyrex software, click on the "File" menu, choose "Load Molecule," select the protein, then left-click on it and choose "Auto-Dock," followed by "Make Macromolecule." To prepare the ligand, click on "Open Babel," choose "Insert New Item" (represented by an excel format with a plus sign on the left-hand side), select the ligand, left-click on it, and choose "Minimize Selected," followed by "Convert Selected to Auto Dock Ligand (PDBQT)." To interact with the protein and ligand, click on "Vina Wizard," click on "Start" (located on the left-hand side). To add the ligand and macromolecule, press "CTRL" and select the ligand and macromolecule files in PDBQT format. Click on "Forward" (a new dialog box appears), then click on "Forward" again. Docking will start after that. Once docking is complete, save the file by clicking on "Save As Comma-Separated Value" (represented by an excel format with a save icon on the left-hand side). To analyze the report (for example, a 2D image), open Discovery Studio Visualizer, load the ligand, press "CTRL + H," followed by opening the protein and pressing "CTRL + H." Copy all the protein files and paste them into the ligand file. Select the protein, click on "Define Receptor," select the ligand, click on "Define Ligand," followed by ligand interaction and click on "Show 2D Diagram.".[18]

4.0 RESULTS AND DISCUSSION

4.1 Acute toxicity study:

During the acute toxicity studies, no deaths were recorded in the treated groups that received 500 mg/kg or 1000 mg/kg b.w of Tonoliv syrup orally. However, two mice died within 24 hours after receiving 2000 mg/kg b.w. of Tonoliv syrup. Therefore, Tonoliv syrup was considered safe up to 1000 mg/kg b.w. As a result, doses lower than 2000 mg/kg b.w. were chosen for pharmacological studies.

4.2 Barbiturate sleeping time:

Thiopentone sodium was administered at a dose of 25 mg/kg (i.p) to induce sedation in rats from the control group for a brief period. However, in rats with CCl4-induced liver injury, sedation lasted longer. Pre-treatment of rats from the toxic group with Tonoliv Syrup at doses of 100 mg/kg b.w, 200 mg/kg b.w, and 400 mg/kg b.w, as well as Silymarin (50 mg/kg.b.w), resulted in a shorter duration of thiopentone sodium-induced sedation when compared to the toxic group. The Tonoliv Syrup's ability to reduce the prolonged duration of thiopentone sodium-induced sedation in CCl4-treated rats confirms its hepatoprotective effect.

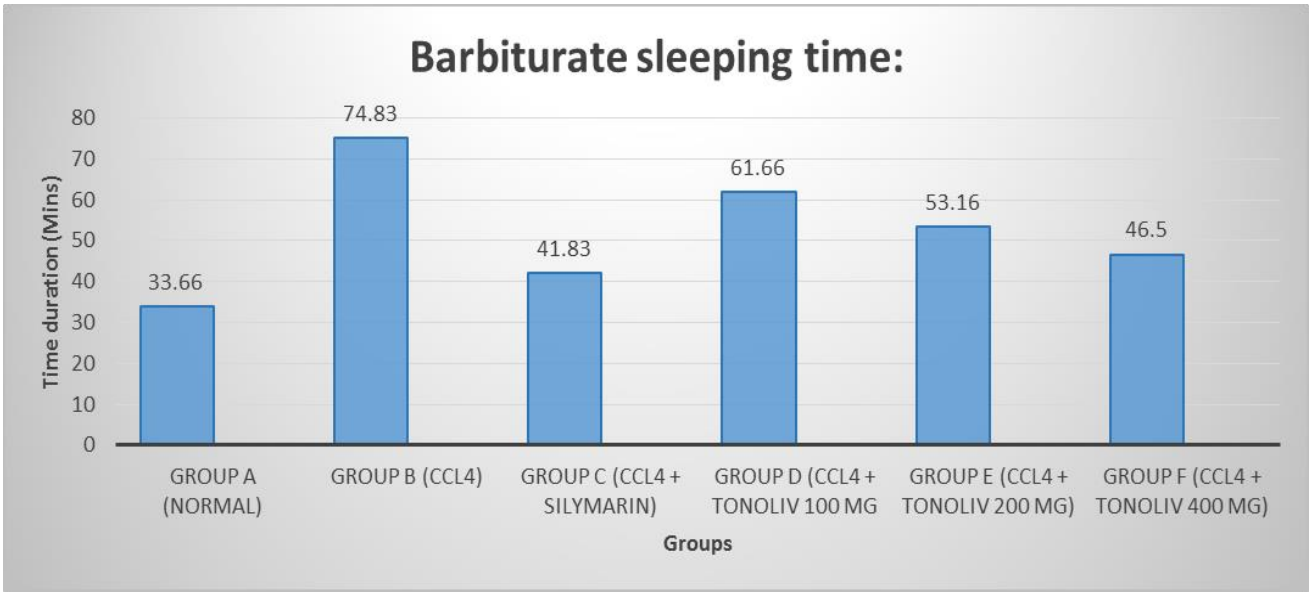
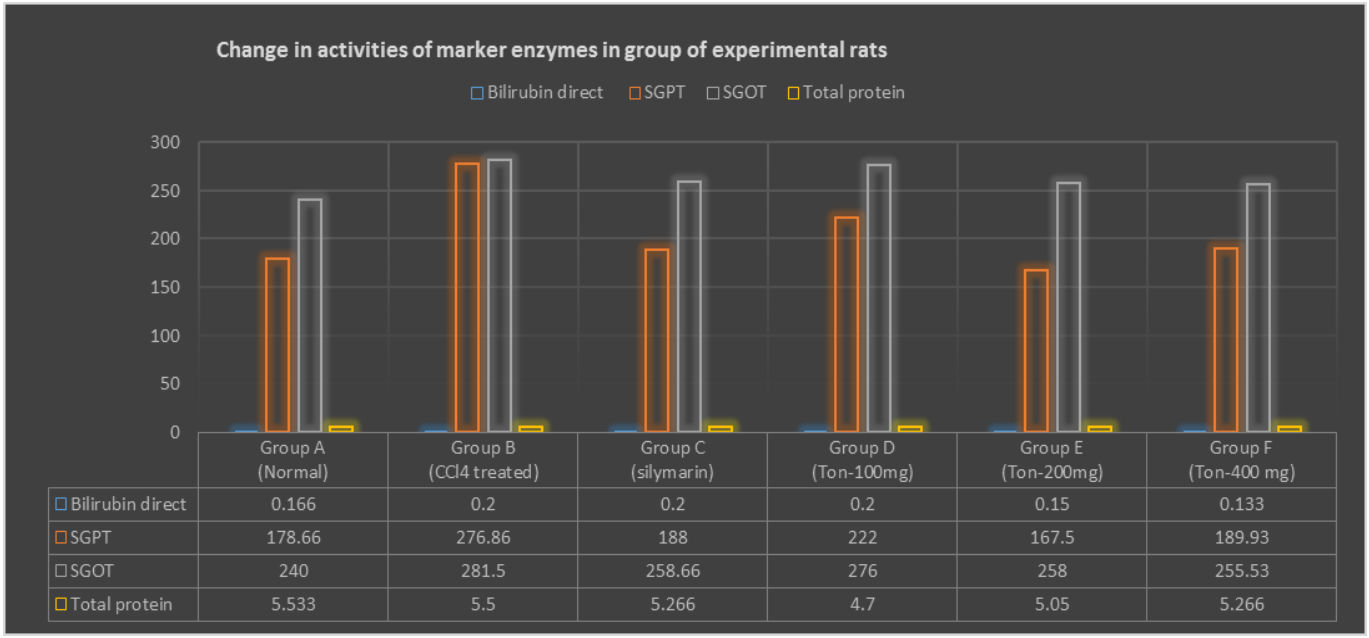


Fig. 1: Bar diagram of Groups against Time duration (Mins)

4.3 Biochemical parameters: The serum levels of SGPT, SGOT, and direct bilirubin were measured in different groups of animals. Group B, which was treated with CCl4, had higher levels of these enzymes compared to group A (normal). However, when group B was compared with group C (treated with silymarin at a dose of 50 mg/kg.b.w), a decrease in enzyme activity was observed. In the case of groups D (treated with Tonoliv at a dose of 100 mg/kg b.w), E (treated with Tonoliv at a dose of 200 mg/kg b.w) and F (treated with Tonoliv at a dose of 400 mg/kg b.w), the serum levels of SGPT, SGOT, and direct bilirubin decreased gradually compared to group B. The results of the experiment are shown in the table below.



	Group A (Normal)	Group B (CCl4 treated)	Group C (silymarin)	Group D (Ton-100mg)	Group E (Ton-200mg)	Group F (Ton-400 mg)
Bilirubin direct	0.166	0.2	0.2	0.2	0.15	0.133
SGPT	178.66	276.86	188	222	167.5	189.93
SGOT	240	281.5	258.66	276	258	255.53
Total protein	5.533	5.5	5.266	4.7	5.05	5.266

Fig. 2: Change in activities of marker enzymes in group of experimental rats

Table 1: Docking results with 3E4E

Compound name	Binding Affinity (-)	Interacting amino acids	Interaction Ligand-residue
Wedelolactone	8	THRB:362	Oxygen
Saponin	9	PHEB:360 HISB:81	Hydrogen Oxygen
Sennoside a And b	9.6	TYRB310 SERB:472 GLUB:407	Hydrogen Oxygen Hydrogen
Andrographolide	7.9	THRB:58 TYRB:398	Oxygen Hydrogen
ß -Sitosterol	8.2	-	-
Kutkin	9	ARGB:100 PROB:429 THRB:307	Oxygen Hydrogen Oxygen
Chicoric Acid	8.5	ARGB:126 ALAB:438	Oxygen Oxygen

Berberine	8.1	-	-
Chebulinic Acid	8.1	ARGB:126 CYSB:437 GLUB:440 SERB:431 ARGB:444	Oxygen Hydrogen Hydrogen Hydrogen Oxygen
Fumarine	5.2	ARGB:435 TRPB:122 ARGB126 ALAB:438	Oxygen Oxygen Oxygen Oxygen
Silybin a (Std)	9.3	ASPB:394 THRB:362 ILEB:469	Hydrogen Oxygen Hydrogen

2D image of docking ligand with binding interaction between active phytochemical constituent and 3e4e protein

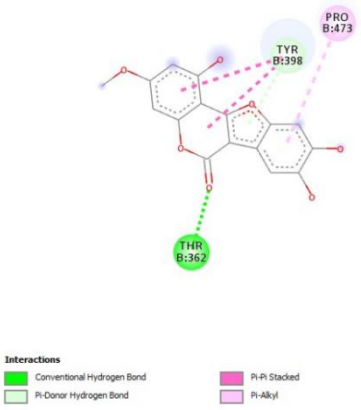


Fig. 3: 2D image of wedelolactone

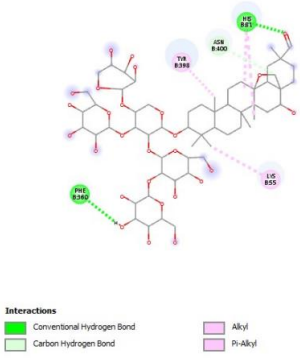


Fig. 4: 2D image of saponins

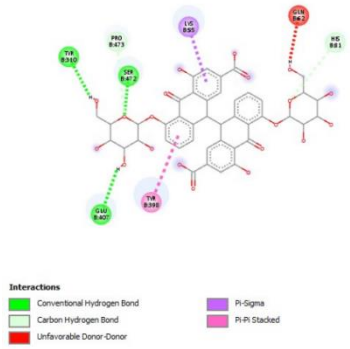


Fig. 5: 2D image of Sennoside A and B

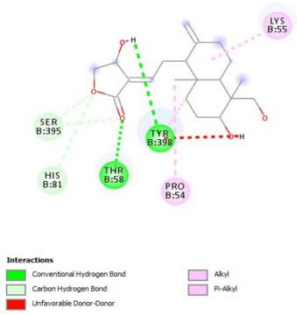


Fig. 6: 2D image of Andrographolide (diterpene lactone)

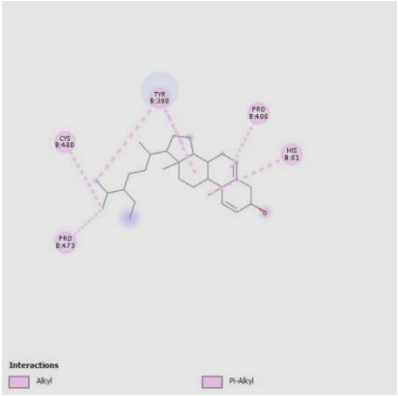


Fig. 7: 2D image of ß -sitosterol

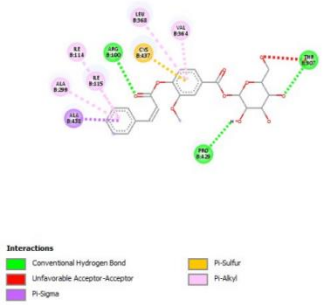


Fig. 8: 2D image of kutkin

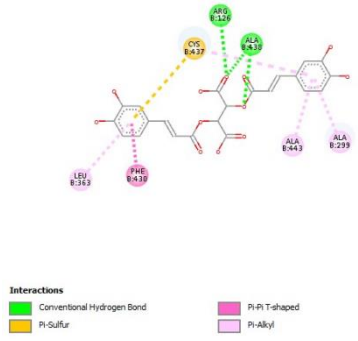


Fig.9: 2D image of chicoric acid

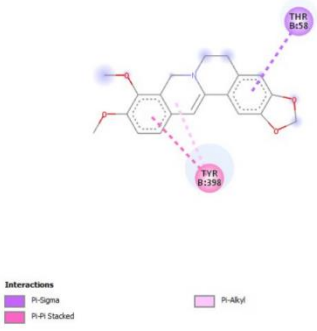
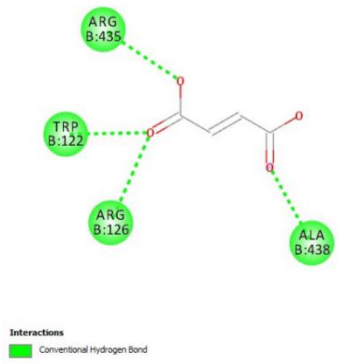


Fig.10: 2D image of Berberine



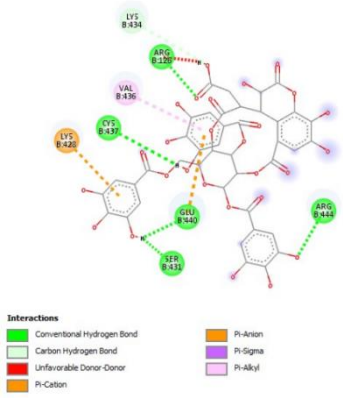


Fig.11: 2D image of chebulic acid **Fig.12: 2D image of fumarine**

Table 2: Properties of compound

Compound name	Number of H-bond (Donor)	Number of H-Bond (Acceptor)	CLogs (-)	Total surface areas	Drug likeness (-)	Mutagenic	Tumorigenic
Wedelolactone	13	27	3.532	616.36	0.40401	None	None
Saponin	15	27	4.508	806.38	15.93	None	None
Sennoside a And b	12	20	6.194	563.26	4.2468	None	None
Andrographolide	6	12	2.392	340.12	2.8439	None	None
ß -Sitosterol	4	10	3.187	335.44	5.8493	None	None
Kutkin	3	7	4.067	212.87	0.24304	None	None
Chicoric Acid	3	5	2.953	258.92	4.5926	None	None
Berberine	0	6	4.72	255.39	4.7237	None	None
Chebulinic Acid	0	5	4.669	243.72	2.2467	None	None
Fumarine	1	1	6.669	336.3	4.475	None	None

5. DISCUSSION:

Carbon tetrachloride is a commonly used hepatotoxin in experimental studies of liver diseases. The toxic effects of CCl4 are mainly due to its active metabolite, trichloromethyl radical. These radicals bind covalently to macromolecules and cause peroxidative degradation of membrane lipids in the endoplasmic reticulum, which is rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn produce products such as malondialdehyde (MDA) that damage the hepatic cell membrane by disrupting Ca++ homeostasis and ultimately causing liver cell damage. The extent of liver damage can be assessed by measuring the activities of serum enzymes SGOT, SGPT, bilirubin, and total protein, which are originally present in higher concentrations in the cytoplasm. When there is hepatopathy, these enzymes leak into the bloodstream in proportion to the extent of liver damage.

The results of this experiment indicate that the hepatic cell has been damaged in group B, as shown by the elevation of the marker enzyme. However, in groups C, D, E, and F, the level of serum enzyme decreased, indicating less damage to the hepatic cell than in group B. Additionally, the level of protein gradually increased with the dose of drug, suggesting that Tonoliv syrup is hepato-protective.

The hepatoprotective effect of Tonoliv Syrup was also tested in a thiopentone sodium sleeping time experiment in rats. This experiment demonstrated that barbiturates are metabolized almost exclusively in the liver by hepatic microsomal drug metabolizing enzymes (or CYP2E1). The duration of sleeping time in rats is considered a reliable index for the activity of hepatic MDMEs, and in CCl4 intoxication, the sleeping time after a given dose of barbiturate will be prolonged because the amount of hepatic metabolism per minute will be less.

The treatment of Tonoliv Syrup at doses of 100mg/kg, 200mg/kg, and 400mg/kg b.w significantly reduced the sleeping time in CCl4 intoxicated rats. This ability of Tonoliv Syrup to reduce the prolongation of thiopentone sodium-induced sleep in CCl4 poisoned rats is indicative of its hepatoprotective effect.

The herbs used in Tonloiv syrup contain polyphenolic compounds or flavonoids. Tonoliv may protect against CCl4-induced liver damage by acting as a free radical scavenger, intercepting the radicals involved in CCl4 metabolism by microsomal enzymes. By trapping

oxygen-related free radicals, the extract could prevent their interaction with polyunsaturated fatty acids and prevent the enhancement of lipid peroxidative processes. It also protects hepatic cells against the reduction in activities of hepatic antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase), depletion of hepatic glutathione, and increased activities of hepatic γ-glutamyl transpeptidase, glutathione-S-transferase, and lipid peroxidase. The extracts of the herbs may stimulate liver regeneration in rats, possibly by stimulating nucleic acid and protein synthesis.

In silico studies, ligand-protein interaction analysis revealed that Wedelolactone, saponin, sennoside a and b, andrographolide, β-sitosterol, Kutkin, chicoric acid, berberine, chebulinic acid, and fumarine show binding energy similar to standard drug silybin A.

6. CONCLUSION:

The dose of the drug was estimated to be up to 2000 mg/kg b.w. Tonoliv Syrup has been found to be hepatoprotective and has not caused any side effects. Sleeping time was reduced in rats treated with Tonoliv Syrup compared to those intoxicated with CCl4. Additionally, Tonoliv Syrup has been shown to decrease the enzyme levels of SGOT, SGPT, and direct Bilirubin, while increasing the level of total protein. This indicates a clear manifestation of the hepato-protective effect of Tonoliv Syrup, as the marker enzymes tend to return towards near normalcy after treatment. Insilco studies have shown that the phytoconstituents of Wedelolactone, saponin, sennoside a and b, andrographolide, ß-sitosterol, Kutkin, chicoric acid, berberine, chebulinic acid, and fumarine molecules have inhibitory activity against "Cytochrome P450 2E1" (PDB ID-3E4E). Therefore, this polyherbal formulation proves to have beneficial effects for the treatment of hepatoprotective.

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Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Future Research

More experimental work such as Western blot analysis, Real-time PCR, Flow cytometer, ELISA analysis, histologic analysis and Immunofluorescence, Chromatin Immuno precipitation and Immuno cytochemistry is needed to carry out to find the more pharmacological feature required for the drug to possess good hepatoprotective activity.

Abbreviation

CCl4:- carbon tetrachloride

PDB ID: Protein data bank

CYP: Cytochrome

PCR: Polymerase chain reaction

ELISA: Enzyme-linked immunosorbent assay

CPCSEA: Committee for the Purpose of Control and Supervision of Experimental Animals

OECD: Organization of economic cooperation and development

8 REFERENCES

1. Harshitha DRR, Maheswara Rao U. Evaluation of polyherbal formulation, livomyn for it's hepatoprotective and antioxidant activity. *Der Pharmacia Lettre* .2013, 5(6):135-141.
2. Ahsan KMI R, Haque M: Hepatoprotective Activity of Methanol Extract of Some Medicinal Plants Against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats. *Global Journal of Pharmacology.* 2009, 3(3)
3. Villeneuve JP. and Pichette V. cytochrome p450 and liver diseases.*Current Drug Metabolism,* 2004, *5,* 273-282.
4. Nasir MGA, Shehu R.A, Aliyu U, Toge BK: Hepatoprotective Effect of the Aqueous Leaf Extract of Andrographis paniculata Nees Against Carbon Tetrachloride Induced Hepatotoxicity in Rats. *Nigerian Journal of Basic and Applied Science.* 2013, 21(1):45-54.
5. Vasuki .R Rh, Pandian S, Arumugam G: Hepatoprotective action of ethanolic extracts of Eclipta alba and Piper longum and their combination on CCl4 induced hepatotoxicity in rats. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2012, 4(1).
6. Naseem N: Hepatoprotective effect of Cichorium Intybus linn (Kasni) Extracts against Carbon Tetrachloride induced Liver Damage. *Journal of Rawalpindi Medical College.* 2009, 13(2):53-55.

7. Chetterjee MPCS: Hepatoprotective effect of aqueous extract of Phyianthus niruri on nimesulide induced oxidative stress *in-vivo*. *Indian Journal of biochemistry and biophysics.* 2006, 43:299-305.
8. Kumar V TS, Kumar SP. Ani: A Review on Hepatoprotective
Activity of Medicinal Plants. *IJARPB.* 2012, 2(1):31-38.
9. IIvanov IG , Vrancheva RZ, Marchev AS, Nadezhda T. Antioxidant activities and phenolic compounds in Bulgarian Fumariaspecies. *IntJCurrMicrobiolAppSci.* 2014, 3(2):296-306.
10. Jayavelu AN, Sundaresan S.K. Devi B.: Hepatoprotective Activity of Boerhavia Diffusa Linn. (Nyctaginaceae) against Ibuprofen Induced Hepatotoxicity in Wistar Albino Rats. *International Journal of Pharma Research & Review, April. 2013; 2(4):1-8* 2013, 2(4):1-8.
11. Rehman WB, Anjum F, Tabasum H: Rheum emodi (Rhubarb): A Fascinating Herb. *Journal of Pharmaco gnosy and Phytochemistry.*2014, 3(2):89-94.
12. Khare CP: *Indian Medicinal Plant*.
13.Yadav.A.CPCSEA guidelines for laboratory animal facility.International Journal of Pharmacology and Clinical Research.Volume 2; Issue 1; 2020; Page No. 09-13.
14. Test guideline OECD 425. Acute oral toxicity-up-and-down procedure 2008,http//ntp.niehs.nih.gov/iccvam/suppodoc/feddocs/oecd_g1425-508.
15. Gupta NK: Evalutaion of hepatoprotective activity of Cleome viscosa Linn. extract. *Indian J Pharmacol* 2009, 1(41):36-40.
16. Ghosh AK, Seth A, Singh A: Preliminary evaluation of hepatoprotective potential of the polyherbal formulation. *J Intercuit Ethnopharmacoal.*2015, 4(2).
17. Yadav N.P: Hepatoprotective activity of leaves of Kalanchoe pinnate Pers. *Journal of Ethnopharmacology.* 2003, 86:197-202.
18.Samani et al.World Journal of Pharmaceutical Research.Vol 9, Issue 12, 2020.

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