An Assessment of Potential *In Vivo* Hepatoprotective Properties of *Vitis vinifera* in Experimental Rat Model

Abstract

Many civilizations have long employed herbal treatments to treat ailments. It has been shown that these medicinal plants contain a number of phytochemicals that can treat a variety of ailments, from minor to serious. A focused search for alternative natural therapeutic agents was inspired by the shortcomings of traditional medications and their peculiar adverse effects. The availability of natural products and their lack of negative side effects could be another explanation for this goal.

Human healthcare has historically benefited greatly from the grape plants and the ingestion of grape fruits. *Vitis vinifera* is known to have astringent, demulcent, carminative, diuretic, laxative, wound-healing, stomachic characteristics. *V. Vinifera* are widely used to treat bronchitis, allergies, the common cold, the flu, anemia, and other conditions. The blooms are also known to have expectorant and hematinic effects. Moreover, there are broad spectrum anti-inflammatory, anti-hypertensive, antineoplastic, anti-carcinogenic, anti-fibrotic, anti-viral, anti-fungal, anti-bacterial, anti-ulcerant, anti-aging, anti-asthma, anti-obesity, hyperpigmentation, wound-healing (cicatrizing) properties as well as immunomodulatory, cardio-protective, and neuroprotective roles have been reported.

The present study looked into the unexplored hepatoprotective and anti-oxidant potentialities of ethanolic fruit extract of *V. vinifera* against toxic chemical CCl_4-induced hepatotoxicity in male Wistar rats. In the present study, the extract shows a tremendous result as a hepato-protective and an antioxidant agent considering ($p^{*}<0.05$) in a dose-dependent manner. In lower doses the grape extract shows reversing physiological effects and in higher doses, it shows better therapeutic effects comparing the marketed drugs for hepatoprotective response.

Keywords: Hepato-protective, *V. vinifera,* CCl_4, cardio-protective, Wister rat

1. Introduction

The liver is the largest, most complicated, an essential and resilient internal organ performing an array of crucial roles in metabolism, hormonal regulation, nutrient absorption, glycogen storage, protein synthesis, bile secretion, heat distribution [3-4], detoxification and immunomodulation [5]. Hepatic diseases remain to possess major public health threat since an estimated 1.5 billion people [6] suffer globally from Chronic Liver Diseases (CLD); the percentage has increased by 33% in the US among senior citizens aged 45–64yrs. [7]. The liver is particularly susceptible to cellular injuries [8] due to excessive alcohol consumption, drug overdose (e.g., acetaminophen, thalidomide), chemotherapeutics, toxic chemical exposure, viral or parasitic infection [9-11] that generate and activate free radicals (ROS) affecting cell signaling pathways, apoptosis, gene expression, and certain metabolic cascades [12]. Scientific research provides large evidence of oxidative stress being involved and playing a key role in the pathophysiology of various liver diseases such as ALD, NAFLD, NASH, NASH cirrhosis, Hepatitis type C, HCC etc. [13-15]

2018 had over 840,000 liver cancer diagnoses and nearly-780,000 associated fatalities [16]. Jaundice, yellowish skin, abdominal pain, skin rashes, itching, rapid abnormal weight loss, persistent exhaustion, confusion, mental disorientation, nausea-vomiting, light-colored feces and dark urine are commonly reported symptoms of the disease. [17-19].

Despite enormous advancements in the modern medicine, there are no medications that stimulate and maintain hepatic function, provide broad defense against diseases, or repair and regenerate damaged hepatic cells [20]. Hepatoprotective medicines available include curcumin, ademetionine, N-acetylcysteine, penicillamine, melatonin, L–glutathione, β-carotene, silymarin, and resveratrol [21-23]. Often administered as oral dietary supplementation, they possess valuable detoxifying and anti–oxidant properties; protect against chemical, cholestatic, alcohol-mediated liver damage [24]; reduce fibrosis and steatosis [25]. These drugs have several downsides including narrow efficacy, undesired effects, toxicity, and high costs therefore making innovative replacements necessary with better safety margins, greater bioavailability, mass-people accessibility, and reduced expenditure. [26].

Medicinal plants serve as vast, unique, diverse and stupendous reserve [27-29] of novel bioactive compounds; these molecules play a crucial role in drug discovery and synthesis operations

serving as starting materials or reaction intermediates [30-33]. Due to their enhanced efficacy and minimal side effects, this promising sector has garnered significant scientific interest over recent decades [34-35]. Around 60-70% of the total population in developing countries are dependent on medicinal plants for fulfilling their primary healthcare requirements [36]. Plant-derived drugs have proven to possess beneficial effects in treating TB, skin conditions, cancer, diabetes, jaundice, AIDS, hypertension, blood diseases, strangury, mental health difficulties, and a variety of infectious diseases. [37]. Aspirin, digoxin, quinine, morphine, papaverine, atropine, caffeine, and others are widely-prescribed, common plant-derived medications. Illustrating the history of natural plants as a part of treatment for hepatotoxicity, *Aloe vera, Cichorium intybus, Cynara cardunculus, Silybum marianum, Solanum nigrum,* etc have shown tremendous experimental results. [38] There are many plants that possess valuable hepatoprotective properties (180 phytoconstituents isolated from 110 plants belonging to 55 families) only a small proportion of them being explored and applied for their therapeutic potentials [39-43].

The perennial woody, climbing vine can reach up to 35 m when growing in its free state; however, due to it often remains reduced to a small, 1-m shrub due to the human action of annual pruning [44-45]. *V. vinifera* has both seedless and non-seedless varieties, Vitaceae being comprised of 3000 species distributed, although not all are equally known or appreciated [46-49]. Moreover, there are broad spectrum anti-oxidant, anti-inflammatory, anti-hypertensive, antineoplastic, anti-carcinogenic, anti-fibrotic, anti-viral, anti-fungal, anti-bacterial, anti-ulcerant, anti-aging, anti-asthma, anti-obesity, hyperpigmentation, wound-healing (cicatrizing) properties as well as immunomodulatory, cardioprotective, hepatoprotective and neuroprotective roles have been reported. [50-52].

Various phytochemicals are known to occur at the root, stem, cane, cordon, leaf, flower, bud, seed, fruit, pomace, and skin. Grapefruits possess tremendous nutritional value; thorough investigations have revealed the presence of sugars (glucose, fructose, saccharose, dextrose, levulose), flavonoids (quercetin, myricetin, catechin, epicatechin), phenolic acids and polyphenols (ellagic acid, gallic acid, tannins), , aromatic acids, organic (maleic, oxalic, linoleic, tartaric, cinnamic, hydroxycinnamic, hydroxybenzoic) acids, anthocyanins, pro-anthocyanidins (mostly hexamers), stilbene-derived trans-resveratrol [53-55], vitamins and precursors (β-carotene, Vit A, Vit B1, Vit B6, Vit C), minerals salts (K, Mg, Ca, S, Fe, Cu, Mn, Zn) [56-57].

Significant concentrations of phenols, tocopherols, sterols, flavonoids and such other bioactive constituents serve as powerful functional ingredients that hold antioxidative, anti-inflammatory and [58-60] stress-combating capabilities [61-62]. In light of the preceding, our current study aims to assess the pharmacological impact, therapeutic efficacy, and safety profile of the ethanolic fruit extract of *V. vinifera* as an anti-oxidant, hepatoprotective agent against carbon tetrachloride (CCl_4)-induced liver toxicity in a dose dependent manner. Satisfactory output regarding therapeutic activity may provide justification towards further study using more

accurate and precise tools in the isolation of therapeutic constituents in search of a newer, safer, affordable and more effective medicine.

2. Methods and Materials

2.1 Drugs, Chemicals and Instruments

Carbon tetrachloride (CCl_4) was procured from Sigma Aldrich, Germany. The standard hepatoprotective drug API named silymarin, also known as silybin, had been obtained for the experiment from Square Pharmaceuticals Ltd., Kaliakoir, Gazipur–1750. The blood serum analyzing kits were purchased from Plasmatic Laboratory Products Ltd, UK.

In order to examine organ activities among different diagnostic systems, marker enzyme levels such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP);, serum creatinine and urea for renal function; cardiovascular parameters such as HDL, LDL, triglycerides (TG) and total cholesterol (TC) levels in the blood were monitored and assessed using a blood analyzer (Humalyzer 3000, semiautomated clinical chemistry analyzer originated from Medigroup Asia Limited, Cambodia Germany).

2.2 Collection, Identification and Extract Preparation of V. vinifera

To conduct the experiment, grapes (*V. vinifera* fruits) were collected from Shahbag, Dhaka. The authentication and taxonomic identification were then carried out. Then fruits were carefully cleaned and dried at the normal room temperature. Next it was placed in oven and kept at 35ºC temperature for 15 days. Next dry fruit was collected and crushed into powder; Following, 70% ethanol and 30% water were used to extract the grapes for 12 days. The extract was shaken moderately and filtered after every three days, the final percentage yield of 2.2% was obtained. The resulting crude residue was carefully collected and preserved for use to perform various pharmacological procedures.

2.3. Experimental Animal Procurement, Nursing and Handling

For the experiment, one hundred (100) healthy male Wistar rats each weighing between 150–200 gm had been purchased from Jahangirnagar University, Dhaka. They were kept at an animal house in the Institute of Nutrition & Food Sciences (INFS) at the University of Dhaka in a well-controlled environment, with relative humidity (RH) of 55±5%, a 12±1 hr light/dark cycle, at a constant temperature of 25±3°C for two weeks for necessary acclimatization before the experiment began. Following they were divided into 10 groups, with 10 rats in each group, based on equal body mass index. All the rats were provided with standard food supplement and purified water. The Institutional Animals Ethics Committee (IEAC) protocols were followed for all experimental procedures. Animals were treated and handled in accordance with the guidelines

of the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT).

Animal grouping and treatment procedures are shown below in **Table 1**:

Group Name	Group Status	Treatment Specimen	Dose administered (mg/kg)	Group Abbreviation
1	Negative Control	Physiological saline	10 mL/kg	C
2	Disease control	CCl_4 + Olive Oil	3 mL/kg	A
3	CCl_4 + Silymarin	Silymarin	3 mL/kg + 80mg/kg	$CCl_4 + SM_{10}$
4	CCl_4+ *V. vinifera*	*V. vinifera*	3 mL/kg + 700mg/kg	$CCl_4 + GP_{500}$
5	CCl_4+ *V. vinifera*	*V. vinifera*	3 mL/kg + 1200mg/kg	$CCl_4 + GP_{1000}$
6	CCl_4+ *V. vinifera*	*V. vinifera*	3mL/kg + 1500mg/kg	$CCl_4 + GP_{1500}$
7	Silymarin	Silymarin	80 mg/kg	SM_{10}
8	*V. vinifera*	*V. vinifera*	700 mg/kg	GP_{500}
9	*V. vinifera*	*V. vinifera*	1200 mg/kg	GP_{1000}
10	*V. vinifera*	*V. vinifera*	1500 mg/kg	GP_{1500}

It appears that this is a description of a scientific experiment performed on rats to induce liver toxicity using carbon tetrachloride. The rats were divided into two groups: group 2–6 which received combination treatments of CCl_4 and silymarin/*V. vinifera*, while groups 7–10 received individual silymarin and *V. vinifera* treatments respectively in their gradually increasing doses. Throughout the duration of six weeks both drugs and the extract were administered orally.

Following treatment, all rats were sacrificed and their blood samples were collected for analysis. The parameters monitored included SGOT, SGPT, ALP (often used to assess liver function and damage), creatinine, urea, TC, HDL, LDL, TG. Overall, this experiment was designed to study the effects of different treatments (silymarin, *V. vinifera*) on liver function and CCl_4-induced hepatocellular injury.

2.4 Experimental Guidelines

The 2013 Declaration of Helsinki's ethical guidelines were followed in the execution of all tests

2.5 Dose Selection

Carbon tetrachloride (CCL_4) is a typical chemical agent used in the laboratory to research a variety of liver problems in both acute and chronic forms. Trichloromethyl free radical (CCL_3), a CCL_4 metabolite produced by CYP2E1 isozymes, reacts with cellular lipids and proteins to form trichloromethyl peroxy radical which attacks lipids on the endoplasmic reticulum membrane causing lipid peroxidation and lobular necrosis. Hepatic damage was caused in all animal groups group by a single oral administration of CCl_4 combined with olive oil as a vehicle in a 1:1 ratio (3mL/kg of rat body weight). Animals with hepatic damage were given their respective treatment

Tests to evaluate the functionality of the liver and kidneys including ALP, AST, SGPT, SGOT and urea, creatinine respectively along with lipid profiles such as HDL, LDL, TG, TC, were analyzed with the help of the Humaluzer-3000

2.6 Statistical Analysis

All our findings (raw data) belonging to several groups according to the different parameters had been recorded and analyzed using MS Excel spreadsheet. Data were subjected to descriptive statistics and the results obtained were represented as (mean ± SD). The “One Way Anova Test’’ of “SPSS 16” software package had been employed for interpreting the inter-group heterogeneity in terms of diverse biological parameters to determine their statistical significance. The events were considered to be statistically significant where ‘p’ value was determined to be less than 0.05 ($p<0.05$).

3. Results and Findings

To determine the hepatoprotective effect of *V. Vinifera* fruit extracts, 100 male Wister rats have been used. They were randomly picked and divided equally into 10 separate groups based on type of medication and the dose administered. The rats remained untreated for the next 1 week, on day 08, the treatment started in groups 2-10 for 6 wks.

Table 3.1: The initial and final body weight of the rats in 10 different groups

Group No.	Group	Initial body weight (gm)	Final body weight (gm)
1	Negative control	114.26 ± 2.22	130.42 ± 2.39

2	CCl_4	118.92 ± 2.61	101.21 ± 4.26
3	CCl_4+SM_{10}	120.32 ± 2.16	96.58 ± 4.13
4	CCl_4+ GP_{500}	113.56 ± 1.94	105.56 ± 3.14
5	CCl_4+ GP_{1000}	122.23 ± 2.25	110.25 ± 4.23
6	CCl_4+ GP_{1500}	116.62 ± 2.28*****	113.56 ± 3.29*****
7	S_{10}	109.56 ± 1.40	102.42 ± 2.82
8	GP_{500}	108.92 ± 2.22	123.56 ± 1.23
9	GP_{1000}	115.56 ± 2.05	124.53 ± 2.31
10	GP_{1500}	111.93 ± 1.33	122.36 ± 1.25

*Values are given as mean ± SEM for 10 groups of rats each *p<0.05. The data of extract-treated groups were compared to that of the CCl_4 control group*

As observed from the data in Table 2, the rats in group 1 (control group) have a visible weight gain due to increase in their age and body mass. There has been a significant and rapid reduction of rat weight in groups 2 and 3 following CCL_4 administration since it exerted damaging metabolic and histopathological effects on body tissue. In groups 4–6 where grapefruit extracts have been administered alongside CCL_4 depict weight loss too, however at lower rate and extent when compared to that of group 2 since the plant extracts may hinder and minimize the tissue-damaging effects of CCL_4. The change in group 6 was statistically significant. Group 7 where drug API silymarin only had been administered also indicates weight loss while in groups 8–10 where grapefruit extract only has been administered in low, medium and high doses respectively show a visible increase in rat body weight.

Liver Function Test

The observed effects of three gradual doses of the 'Grape Extract' (GP) on liver enzymes in the blood samples of CCl_4–administered rats (groups 2–10) had been monitored carefully where changes in hepatic marker enzyme concentrations such as SGPT, SGOT, and ALP indicate hepatocellular damage (CCl_4–induced oxidative stress) in rat liver. The results are recorded below in Table 3.1.

Table 3.2: The plasma concentration of enzymes involving liver function among 10 group of rats

Group No.	Group	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
1	C	32.40 ± 2.94	44.68 ± 3.63	126.29 ± 9.59
2	A	82.20 ± 4.12	94.39 ± 5.51	330.09 ± 21.40
3	CCl_4+SM_{10}	56.92 ± 5.63	73.89 ± 4.14	205.53 ± 17.71
4	CCl_4+ GP_{500}	80.46 ± 4.59	93.99 ± 5.31	291.99 ± 15.05
5	CCl_4+ GP_{1000}	73.51 ± 3.94	85.43 ± 5.28	267.99 ± 19.01
6	CCl_4+ GP_{1500}	69.45 ± 4.84*****	80.81 ± 3.27*****	249.24 ± 9.25*****
7	SM_{10}	34.58 ± 1.89	43.79 ± 2.48	124.35 ± 7.82
8	GP_{500}	33.97 ± 1.97	45.15 ± 4.28	134.18 ± 13.68
9	GP_{1000}	30.48 ± 1.83	43.42 ± 4.02	132.03 ± 11.08
10	GP_{1500}	31.48 ± 1.68	42.57 ± 3.47	127.32 ± 11.38

*Values are given as mean ± SEM for 10 groups of rats each *p<0.05. The data of the extract-treated groups were compared to that of the CCl_4-control group*

In the CCl_4-treated group (Group 2), the enzymes SGPT, SGOT, and ALP were much more active after injection ($p<0.05$) than in the negative control group (Group 1). Administration of GE prevented the rise in SGPT, SGOT, and ALP enzymes level brought on by CCl_4 and led to a recovery towards normalization that was similar to the negative control (Group 1). Groups 4-6 showed gradual decline in all three markers compared to group 2, the changes in group 6 being statistically significant ($p<0.05$). The highest levels of enzymes indicate maximum occurring cellular damage in this experiment. When compared to the positive group of CCl_4-treated alone animals, treatment with three doses of GE substantially decreased the activities of serum SGPT, SGOT, and ALT enzymes. Groups 7-10 showed data similar to that of negative control (group 1) depicting cell injuries to be minimized following GP administration.

Kidney Function Test

The effect of three doses of the Grapefruit Extract (GE) on kidney function markers e.g., serum creatinine and urea in CCl_4-induced rats had been recorded and are shown below in Table 3.2.

Table 3.3: The impact of GE on renal function marker substances in rats of groups 1-10

Group No.	Treatment	Creatinine (mg/dL)	Urea (mg/dL)

1	Negative control	0.62±0.13	31.34±3.42
2	CCl_4	2.99±0.65	92.03±4.70
3	CCl_4 + SM_{10}	1.44±0.33	66.56±5.82
4	CCl_4 + GP_{500}	2.4±0.52*****	90.33±13.37
5	CCL_4+GP_{1000}	1.9±0.38*****	79.97±7.87
6	CCL_4+GP_{1500}	1.3±0.19*****	68.59±6.29
7	SM_{10}	0.5±0.11	32.28±2.40
8	GP_{500}	0.6±0.11	33.98±4.77
9	GP_{1000}	0.5±0.13	31.04±3.27
10	GP_{1500}	0.6±0.13	29.56±2.44

*Values are given as mean ± SEM for 10 groups of rats each *p<0.05. The data of extract-treated groups were compared to that of the CCl_4 control group*

After 06 weeks of treatment, the negative control group (Group 1) had creatinine and urea levels of 0.62 and 31.34 mg/dL, respectively. On one hand, it was evident that CCl_4 treatment (Group 2) led to a rise in the concentrations of creatinine and urea (2.99 and 92.03 mg/dL, respectively) due to nephrocellular damage; while the administration of GE led to a reduction in the amounts of urea, and creatinine. Groups 4-6 showed gradual decrease in both urea and creatinine in comparison to group 2, where the change in group 6 was significant. The changes in creatinine values of groups 4-6 respectively were found to be statistically significant ($p<0.05$) in comparison to that of group 2 while no statistically significant changes in serum urea concentration was recorded. The groups 7-10 showed results akin to the negative control where creatinine concentration lies within the acceptable standard of 0.6-0.8 mg/dL and urea 30-45 mg/dL respectively indicating that the normal renal function remains nearly undisrupted.

Cardiac Profile

The effects of GE on the lipid profile parameters including serum triglyceride, LDL, HDL, cholesterol levels of rats with liver damage caused by CCl_4 are shown in Table 3.3

Table 3.4: Lipid profile parameters in rats of groups 1-10

Group No	Treatment	LDL (mg/dL)	HDL (mg/dL)	Triglyceride	Cholesterol

				(mg/dL)	(mg/dL)
1	Negative control	41.88±2.06	73.74±2.79	53.21±5.49	102.51±4.02
2	CCl_4	75.96±4.91	42.48±4.004	115.76±3.12	168.84±7.38
3	CCl_4+SM_{10}	55.25±3.66	59.40±5.56	82.24±5.43	148.01±4.23
4	CCl_4+GP_{500}	73.28±4.58	43.85±4.55	112.72±3.59	161.97±5.38
5	CCL_4+GP_{1000}	70.89±5.61	46.69±4.78	110.38±3.81*	159.07±5.57*
6	CCL_4+GP_{1500}	62.22±1.66*	52.44±3.20*	106.94±2.64*	154.10±5.56*
7	SM_{10}	41.75±2.05	74.27±3.83	55.49±4.48	104.10±3.82
8	GP_{500}	37.74±1.47	73.21±5.44	52.64±3.13	100.23±3.12
9	GP_{1000}	41.32±2.46	71.95±2.67	54.15±3.18	97.92±4.26
10	GP_{1500}	41.76±1.28	76.02±5.13	53.77±3.69	101.16±2.63

*Values are given as mean ± SEM for 10 groups of rats each *p<0.05. The data of extract-treated groups were compared to that of the CCl_4 control group*

With the exception of HDL, most of the lipid parameters (Triglyceride, Cholesterol, LDL, and HDL) rose following CCl_4 administration. The LDL, HDL, TG and TC values in group 4-6 remained similar to that of group 2 while groups 7-10 showed data similar to the negative control.

The changes in TG and TC values were statistically significant in group 5 while all the four basic lipid profile parameters showed significant changes in group 6 ($p<0.05$). Following therapy with CCl_4, HDL levels dropped, whereas treatment with three doses of GE caused HDL levels to rise.

4. Discussion

Hepatic diseases pose a major global public health concern, till date no suitable remedies or medications being established. Since this vital organ performs a range of crucial roles involving metabolic, regulatory, secretory, synthetic and storage functions, any form of impairment may lead to severe consequences towards liver failure and eventually death. Medicinal plants are a tremendous treasure trove of potentially beneficial, unique and diverse natural compounds that can serve to be a formidable weapon in the fight against a variety of hepatocellular diseases.

The present study looked into the unexplored *in vivo* hepatoprotective and anti-oxidant potentialities of *V. vinifera* fruit extract against toxic chemical CCl_4-induced hepatotoxicity in male Wistar rats. The pathway is known to proceed via formation of highly reactive ROS radicals.

According to the experimental data obtained from our study, exposure to CCl_4 significantly decreased the body weight of rats in the disease control group. This finding suggests that CCl_4-induced hepatic impairment may hinder the body's normal ability to process dietary nutrients, resulting in metabolic imbalance and a severe loss of body weight. Contrarily, rats in the negative control group exhibited an increase in terminal body weight, thus demonstrating a healthy metabolic balance that controlled the normal growth rate and body weight of rats. However, the groups treated solely with the test extract (Groups 8-10) exhibited steady gain in rat body weight, the change being statistically non-significant ($*p>0.05$) when compared to that of the negative control group, inferring that the extract had no detrimental effect on the normal, healthy growth rate of rats. Silymarin-treated groups demonstrated the opposite result, indicating its detrimental effect in terms of tissue damage hence bringing about weight loss. Again, as observed in the data of groups 4-6, plant extracts aided to reverse the CCl_4-induced weight loss in a dose-dependent manner, thus revealing the promising potency of this phytochemical extract in restoring metabolic balance involving normal growth rate and healthy body weight in diseased rats.

A noticeable incongruity was observed between the two control groups (i.e., negative control group and disease control group) in the serum concentration of enzymes (such as SGPT, SGOT and ALP) that serve as the most sensitive indicators of hepatic function. In the negative control and extract-treated groups (group 1, groups 7-10), the serum levels of the enzymes were reported to remain within fairly normal range which showed that the bulk content was confined to liver cells, not leaked or released into the bloodstream via membrane rupture. This clearly indicated healthy, well-functioning liver cells with intact plasma membranes. In contrast, marked elevation was noted in the serum levels of the disease control and CCl_4-group (Groups 2-6), demonstrating the pathological manifestations of CCl_4-induced hepatotoxicity, which resulted in cellular injury, membrane structure disruption, and rupture, resulting in the release of the enzymes (SGPT, SGOT, and ALP) into the bloodstream.

However, the *in-vivo* administration of *V. vinifera* to Groups 4-6 was found to minimize the CCl_4-induced damaging alterations in the levels of the hepatic damage biomarkers (i.e., SGPT, SGOT, ALP), in a dose-dependent manner yielding statistically non-significant ($p>0.05$) outcomes with the standard drug (silymarin). This reinforced the promising hepatoprotective

action of *V. vinifera*. The serum enzyme levels of the 4 remaining non-CCl_4 treated groups demonstrated statistically non-significant ($p>0.05$) deviation from those of the negative control group, signifying the potential safety margin of silymarin and *V. vinifera*. Findings of preserving and maintaining hepatocellular structural integrity similar to our study had been reported by Lorenzo et al (2019), Sharma et al. (2020) and Sherie et al. (2020).

The phytoconstituents present in our test extract contributing towards such outcome may involve bioactive flavonoids (eg myricetin, catechin, epicatechin), phenolic acids, polyphenols (ellagic acid, gallic acid), tannins, stilbene-derivatives (trans-resveratrol) etc. that work via inhibiting the generation and activation cascade of free radical species.

Creatinine and urea are key indicators of renal function. Groups 3-6 showed elevated serum creatinine levels following CCl_4-administration, which is likely to be the result of hepatotoxicity-related physiological alterations exerting an adverse impact on renal perfusion and blood circulation, thereby indicating impaired kidney function. Creatinine level was reported to be normal in non-diseased individuals (negative control, group 1), signifying healthy renal function. The values of creatinine levels recorded from in groups 8-10 among rats treated with three different doses (low, medium and high) of fruit extract were significantly lower than the values obtained from the disease control group, illustrating the dose-dependent efficacy of *V. vinifera* in diminishing the risk of hepatotoxicity-induced renal injury. In the 5 CCl_4-treated groups, urea concentration was observed to be high indicating renal injury to be present.

The serum LDL, HDL, TG and TC values of the negative control group lied within the normal range, denoting regulation and balance in the lipid profiles of healthy rats. However, *in-vivo* of CCl_4-administration significantly affected the serum lipid profiles of the treated rats, resulting in a substantial rise in the levels of serum LDL, TG and TC with a noticeable drop in serum HDL level. These results were fairly similar to those of Singab et al. (2019), Ezzat et al. (2020) who reported the efficacy of *V. vinife*ra in normalizing serum LDH level employing aqueous extract, 80%, and 70% ethanolic extract respectively. Such alterations in serum lipid concentrations of the CCl_4-treated rats can be attributed to a malfunctioning hepatic *de novo* lipogenesis pathway, implying the presence and pathological progression of CCl_4-induced hepatic impairment.

Treatment using *V. vinifera* notably reversed the CCl_4-induced alterations in serum lipid profile in a dose dependent manner, providing non-significant ($p>0.05$) statistical outcomes with the standard medication (silymarin), which proved the potential anti-hepatotoxic action of grapefruit extract. Groups treated solely with the standard (silymarin) or test extract imitated the same trend as the negative control group, demonstrating the safety of silymarin or *V. vinifera* extract.

5. Conclusion and Future Perspectives

Generally, hepatoprotective plants and fruits contain a heterogeneity of phytochemical specimens including coumarins, lignans, essential oils, monoterpenes, terpenoids, glycosides, alkaloids, saponins, carotenoids, pigments, polysaccharides, aromatic organic acids, xanthenes, stilbene-derivatives; phenols, flavonoids and polyphenols being the most powerful free radical scavengers that terminate chain reaction.

V. vinifera fruit extracts possess anti-oxidant, anti-inflammatory and hepatoprotective properties where our experiment identified, evaluated and provided accurate evidence of the *in vivo* hepatoprotective action in male Wister rats. This study may also provide future directions towards more specific study in the isolation, extraction and purification of bioactive compounds from plant sources. Immense potential remains unexplored where pre-clinical and clinical assays may be carried out to determine the safety and efficacy with the aim of introducing novel approaches for combating liver disease as alternatives to the limited therapeutic options. In aggregate, *V. vinifera* compounds may serve to be a safe, promising, clinically-applicable, efficient and profitable natural counteraction when long term or multiple drug therapy is required for providing a potent, long-term, safe hepatoprotective action.

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