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Comparative antioxidant and hepatoprotective potential quercetin and corycavidine from *Hedyotis corymbosa* (L.) Lam. and *Solanum xanthocarpum* Schrad & Wendl.

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Liver disease is the major health issues in current era. Antioxidants play the essential role in hepatoprotection by protecting the hepatic cells against free radicals. Flavonoids and alkaloids are the essential plants bioactive that play the major role in the antioxidant system. In our project we used diamond flower *Hedyotis corymbosa* (L.) Lam. and Yellow berried Nightshade *Solanum xanthocarpum* Schrad & Wendl., and both plants have major phytoconstituent which acts as antioxidants. Thus, the aim of the current study validates the isolation, characterization, and determination of *in vivo* antioxidant and hepatoprotective consequences of corycavidine and quercetin that were isolated from Diamond flower *Hedyotis corymbosa* (L.) Lam. and Yellow berried Nightshade *Solanum xanthocarpum* Schrad & Wendl.. The study intent to isolate and identify the antioxidant and hepatoprotective agent from two different plants and compare their hepatoprotective potential to obtain the most effective liver protective phytoconstituent. Quercetin was isolated from *S. xanthocarpum* by column chromatography employing n-butanol: acetic acid: H_2O (2:2:6) as a solvent system, however, corycavidine was isolated from *H. corymbosa* by column chromatography employing chloroform: methanol: diethylamine (4:1:2.2) as mobile phase. Structural illustrations were confirmed by UV, FT-IR, ^{1}H-NMR, and ^{13}C-NMR, and mass spectroscopy. Both the phytoconstituents, corycavidine and quercetin, were explored for their antioxidant potential by investigating CAT, SOD GSH, and LPO in liver homogenates of experimental rats. Additionally, the *in vivo* hepatoprotective effect was examined against simvastatin (20 mg/kg, *p.o.*), which induced hepatotoxicity in experimental rodents. The liver protective activity was computed by determining distinct biochemical parameters like SGOT, SGPT, ALP, bilirubin, total protein, cholesterol and urea along with hematological parameters and histopathological studies. The results of spectroscopic methods confirmed that the isolated phytochemical constituent from the *H. corymbosa* is corycavidine, a benzylisoquinoline alkaloid, however from *S. xanthocarpum* is quercetin a flavonoid. Both phytoconstituents significantly (P <0.05 P <0.001) and dose-dependently reversed simvastatin induced elevated levels of SGOT, SGPT, cholesterol, urea, total bilirubin and restored the total protein and albumin level in experimental rats. Furthermore, it also signifies the blood parameters at a dose of 50 and 100 mg/kg and restored the body protection system. The histological examination exhibited that corycavidine and quercetin at a dose of 100 mg/kg showed regeneration of hepatocytes around the central vein with nearly normal liver architecture. The results expressed the hepatoprotective outcome of quercetin is preeminent than corycavidine and therefore, scientifically validates its traditional application.

Keywords: Corycavidine, Diamond flower, Flat-top millet grains, Kantakari, Liver disease, *Oldenlandia corymbosa*, Quercetin, Simvastatin,Wild Eggplant, Yellow berried Nightshade

Liver damage, despite considerable advancement in medications, is a censorious health complication[1,2]. It is the vital organ and is accountable for generation of blood anticoagulant factors, synthesis and secretion of bile, and glycogenesis[3,4]. Globally, liver disease is the fifth fatal disease, including alcoholic liver disease, non-alcoholic fatty liver disease, hepatitis, liver cirrhosis, and hepatocellular carcinoma[6-8]. Many plants including algae and fungi are known to possess hepatoprotective potential[9-14]. Kadam *et al.*[15] have demonstrated the protective effect of *Solanum torvum* on monosodium glutamate induced hepatotoxicity and nephrotoxicity.

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Solanum xanthocarpum Schrad & Wendl., commonly called Wild Eggplant or Yellow berried Nightshade, belongs to the genus *Solanum* L., family Solanaceae. The plant contains steroidal alkaloids like solasodine, solamargine solanacarpine, solanacarpidine, carpesterol, diosgenin[16]. The fruits of *S. xanthocarpum* have been reported to contain steroidal alkaloids like solanacarpine, solanacarpidine, olanzapine, solasonine, kaempferol, and solamargine[17]. It also contains some coumarins aesculetin, aesculin, steroids campesterol, diosgenin campesterol, daucosterol, and triterpenes cycloartenol, and cycloartenol[18]. Chaudhury *et al*.[6] have demonstrated anticancer and immunosuppressive potential of hot aqueous extract of whole plant. *Hedyotis corymbosa* (L.) Lam (*Oldenlandia corymbosa*), known as Diamond flower. Flat-top mille grains, is a flowering plant (Fam. Rubiaceae) and is broadly used in Chinese folk medicines for the treatment of cancer, bronchitis, throat infection, hepatitis, and eye infection. It is employed for treating venomous bites and exhibited anthelmintic, diuretic, expectorant, digestive, and stomachic properties[19]. *H. corymbosa* has various phytoconstituents like iridoid glucosides, anthraquinones, triterpenoids, alkaloids like protopine, corycavidine, p-coumaric acid, syringe acid, melilotic acid, p-hydroxybenzoic acid, ferulic acid, and caffeic acid[20]. Corycavidine is a benzo-isoquinoline alkaloid present in different medicinal plants including *H. coryambosa* and *C. meifolia*[21].

In literature, there is no report available on hepatoprotective activity of corycavidine. However, few reports are available on quercetin against ethanol induced liver toxicity[22,23]. Therefore, in the current study, we have made an attempt to isolate and characterize quercetin from the fruits of *S. xanthocarpum* and corycavidine from *H. coryambosa* and compare their hepatoprotective and antioxidant potential against simvastatin induced liver toxicity.

Materials and Methods

Plant collection and authentication

The plant was collected from CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India, and authenticated by Dr. Alok Lehri, Principal Scientist, CSIR-NBRI.

Isolation of phytoconstituent from *H. corymbosa* and *S. xanthocarpum*

The bioactive compound was isolated from dried fruit material of *S. xanthocarpum* by column chromatography method. Ten grams of ethanolic fruit extract was chromatographed over packed stationary phase and then gradient elution was followed using solvents with increasing order of polarity. n-butanol: acetic acid: H_2O (2:2:6) was used as a solvent system. To optimize the solvent system, initially the elution was started with 100% n-butanol and then polarity was increased to get the pure form of quercetin using n-butanol:acetic acid (5:1), n-butanol:acetic acid (3:1), n-butanol:acetic acid (1:1), acetic acid (100%), acetic acid:H_2O (5:1), acetic acid:H_2O (3:1), acetic acid:H_2O (1:1) and water. All the collected fractions were run for TLC with the same solvent system (n-butanol: acetic acid: H_2O; 2:2:6) selected for the column. Based on the TLC profile, the R_f values of the fractions were calculated and compared with quercetin as standards. Finally, quercetin was characterized by UV-visible spectroscopy, Infrared spectroscopy (IR), ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy. Another bioactive compound corycavidine was isolated from *H. corymbosa* by column chromatography. As done earlier, 10 g of ethanolic extract was chromatographed over packed stationary phase, then gradient elution was followed using solvents with increasing order of polarity, chloroform: methanol: diethylamine (4:1:2.2) were used as solvent system. To optimize the solvent system initially the elution was started with 100% chloroform and then polarity was increased to get pure form of corycavidine using chloroform: methanol (5:1), chloroform:methanol (4:1), chloroform:methanol (3:1), chloroform:methanol (2:1), chloroform:methanol (1:1), methanol (100%), methanol:diethylamine (5:1), methanol:diethylamine(3:1), methanol:diethylamine(2:1), methanol:diethylamine(1:1) and diethylamine (100%). All the collected fractions were run for TLC with the same solvent system chloroform:methanol: diethylamine (4:1:2.2) selected for column. Based on the TLC profile, the R_f values of the fractions were calculated and compared with corycavidine as standard. Finally, corycavidine was characterized by UV-visible spectroscopy, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy.

Animals

Wistar rats (150-180 g) and Swiss mice (25-30 g) of either sex were acquired from the animal house of Shri Ram Murti Smarak, College of Engineering and Technology, Bareilly, Uttar Pradesh, India. They were kept in the departmental animal house in the well cross-ventilated room at 22±2°C with light and dark cycles of 12 h for one week before and during the experiments. The experiment was carried out per the

guidelines mentioned in the CPCSEA, and Institutional Animal Ethical Committee, India (Reg. No. 715/02/CPCSEA).

Acute toxicity study of corycavidine and quercetin

For oral acute toxicity study different groups of mice were charged with 50, 100, 300 and 500 mg/kg doses of quercetin and corycavidine as per OECD guideline (423), and mortality was observed for up to 7 days[1].

Experimental scheme

Wistar rats of either sex were divided into seven different groups, each group having 6 rats. Group I rats served as normal control, received distilled water only for 30 days. Group II rats were provided with simvastatin (20 mg/kg, *p.o.*) alone for 30 days orally. Group III & IV animals were treated with quercetin at 50 and 100 mg/kg, and Group V & VI animals were treated with corycavidine at 50 and 100 mg/kg, *p.o.*, respectively for 30 days. Group VII animals were also administered the same as Group II but treated with 20 mg/kg, silymarin (Syl-20) *p.o*., for 30 days. On the 31st day, blood samples were collected and all the animals were sacrificed by cervical dislocation under mild ether anesthesia and liver sample were harvested, rinsed in saline, and stored at −80°C for further biochemical and histological analysis.[15]

Evaluation of liver protective activity

The collected blood was allowed to clot and serum was separated by centrifugation in a refrigerated tabletop centrifuge at 2500 rpm for 15 min and the biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT, U/L), serum glutamic-pyruvic transaminase (SGPT, U/L) alkaline phosphatase (ALP), total bilirubin (mg/dL), total protein, cholesterol (CHL, mg/dL) and urea (mg/dL) were evaluated[24].

Evaluation of antioxidant activity

Antioxidant activity was examined in liver homogenate by investigating CAT, SOD[25,26], GSH and LPO[27,28].

Evaluation of hematological parameters

The fully automated hematology analyzer (XP 100 Hematology Analyzer, Transasia Bio-Medicals Ltd., India) was used to determine red blood cell (RBC) count, hemoglobin (Hb), white blood cell (WBC) count, platelets (PLT) and lymphocytes.

Histopathological examinations

Histopathology was performed utilizing H&E stained sections of the liver at 40 and 100. All the slides were studied under the light microscope for any investigation of histological destruction and protection[29].

Statistical analysis

The statistical comparisons between the groups were made by one-way ANOVA, followed by Student-Newman-Keul's test. The value P <0.05 was considered statistically using Graph Pad Prism 5.01 Software. The values were represented as mean ± SEM for six rats.

Results and Discussion

Isolation of phytoconstituents

Crude flavonoid (54 mg) was obtained from 6 kg powdered material of *S. xanthocarpum* fruit, the R_f value (0.32) of the isolated compound was the same as the standard quercetin that was confirmed by running standard quercetin as a parallel spot that has the same R_f. The melting point of the isolated compound was determined as 316-318°C, which was the same as standard quercetin[30]. Crude alkaloids (45 mg) were obtained from 6 kg powdered materials of *H. corymbosa*, the R_f value (0.32) of the isolated compound was found to be the same as that of the standard corycavidine which was confirmed by running standard corycavidine as a parallel spot on TLC. The melting point of the isolated compound was determined as 216-218°C, which was the same as standard corycavidine[31].

Characterization of the isolated compound

UV confirmed the structure of the isolated compound, λ_{max} was found to be 340.8 nm and IR (KBr, ν, cm^{-1}): 3650 (O-H), 1656 (C-O), 1443 (C=C), 1161 (aromatic ring stretching). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2.493 (s, C7 hydroxyl group), 2.001 (s, C5 hydroxyl group), 3.468 (s, C3 hydroxyl group), 6.376-6.887 (m, aromatic ring), 7.519, 7.673, 9.358-10.782 (m, aromatic ring), 4.652 (s, C3$^/$ hydroxyl group). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 136.17 (C-8), 147.27 (C-6), 176.29 (C-4), 93.84 (C-5), 98.65 (C-7), 103.46 (C-3$^/$), 122.41 (C-4$^/$), 120.48 (C-3). Mass spectrum of the compound showed parent molecular ion [M+] peak at m/z 303.2, which corresponds to the molecular formula $C_{15}H_{10}O_7$. This assessment is overall in good agreement with the structure of quercetin[20].

UV confirmed the structure of isolated compound, λ_{max} was found to be 276.20 nm and IR (KBr, ν, cm^{-1}): 2917 cm^{-1} [CH3(C-H) Asym/Sym Stretch)], 1441.9 cm^{-1} [(C=C-) Aromatic Ring Stretching)], 1738 cm^{-1}

(C=O) and 1099 cm^{-1} (C-N).^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm)**:** 1.15 (s, C11), 2.71 (s, OCH_3), 2.77-3.11 (m, aromatic ring), 6.13-6.20 (m, aromatic ring).^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm)**:** 122.41(C-14), 176.29 (C-2), 120.48 (C-5), 122.41 (C-6), 93.84 (O-CH_3), 98.65 (O-CH_3), 116.05 (C-11), 111.3 (C-4), 112.1 (C-11), 126.2 (C-12), and 103.46 (O-CH_2-O). Mass spectrum of compound showed parent molecular ion [M+] peak at m/z 383.4, which corresponds to the molecular formula $C_{22}H_{25}NO_5$. This assessment is overall in good agreement for the structure of corycavidine.

Acute oral toxicological outcome

The isolated quercetin and corycavidine did not show any symptom of mortality up to a dose of 500 mg/kg *p.o.* for 7 days in mice.

Effect of quercetin and corycavidine on serum hepatic parameters

In human beings, liver ailments are one of the predominant and considerate health system defects worldwide, which are usually credited to viruses, alcohol, and different chemicals. The quest to understand a hepatoprotective agent has to turn out to be a key challenge over the past decades[32]. Some indispensable and nonessential elements from plant origin also have sturdy antioxidant undertaking[33]. Most of the hepatotoxic agents produced free radicals and induce oxidative stress, which acts on hepatic parenchyma cells[34]. These parenchyma cells are the sites for the production of various liver enzymes. Protection in hepatic parenchymal cells by toxins is the major functions of the liver. For this reason, antioxidant enzymes represent the first line of defense in opposition to oxidative stress and harm triggered by way of free radicals[35]. Likewise, simvastatin increases the level of hepatic markers[4], like GOT, GPT, ALP, total bilirubin, cholesterol and urea, while diminished the serum total protein level, which resulted in the dysfunction of the liver[36,37]. Injury of hepatocytes and obstruction in excretory ducts of the liver interferes with the capability of the liver to excrete normal levels of bilirubin. Thus, serum bilirubin is generally used to identify proper liver functioning. Total bilirubin is a combination of nonhepatic (indirect) bilirubin and hepatic (direct) bilirubin[38]. ALP is considered a leading maker of hepatobiliary effects and cholestasis[39]. Administration of quercetin and corycavidine in the study suppressed the increased hepatic serum marker enzymes like GOT, GPT, cholesterol, urea, and complete bilirubin while elevated the complete protein and albumin levels in a dose-dependent manner[40]. However, quercetin exhibits more hepatoprotective effect as compare to corycavidine as per hepatic serum markers are concerned.

Simvastatin (20 mg/kg, *p.o.*), treated rodents demonstrated the noteworthy inclination of liver serum markers like SGOT, SGPT, CHL, bilirubin (BLB), urea level while declination in the TP and ALB levels. The rodents administered with 50 and 100 mg of quercetin demonstrated altogether declination in the SGOT, SGPT, CHL, bilirubin (BLB), urea levels while augmenting TP and ALB in subordinate way. Hepatic damage instigated by simvastatin caused significant changes in marker as SGOT by 141.45%, SGPT by 92.08%, CHL by 117.09%, urea by 49.66%, ALB by 32.5%, BLB by 201.7 and protein by 73.11% compared with control. The % defence in marker of treated rodents at 50 mg/kg of quercetin for SGOT was 24.27 (P <0.001), SGPT 20.50 (P <0.01), CHL 28.52 (P <0.001), urea 20.58 (P <0.001), ALB 18.93 (P <0.001), BLB 49.11 (P <0.001) and protein 676.85 (P <0.001) compared with simvastatin 20 mg/kg, charged rodents while greatest % defence was observed at 100 mg/kg of quercetin and silymarin (20 mg/kg) as SGOT 37.49 (P <0.05), 45.16 (P <0.001), SGPT 36.55 (P <0.001), 46.41 (P <0.001), CHL 39.35 (P <0.001), 41.88 (P <0.001), urea 29.26 (P <0.001), 31.61 (P <0.001), ALB 32.09 (P <0.001), 44.03 (P <0.001), BLB 62.72 (P <0.001), 46.15 (P <0.001) and protein 503.30 (P <0.05), 185.12 (P <0.001) which is practically equivalent to the group treated with silymarin, an intense liver defensive medication utilized as reference standard. The % defence in marker of treated rodents at 50 mg/kg of corycavidine for SGOT was 11.41 (P <0.01), SGPT 8.84 (P <0.01), CHL 5.63 (P <0.001), urea 14.57 (P <0.001), ALB 5.34 (P <0.01), BLB 33.13 (P <0.01) and protein 88.42 (P <0.01) compared with simvastatin 20 mg/kg, charged rodents while greatest % defence was observed at 100 mg/kg of corycavidine and silymarin (20 mg/kg) as SGOT 21.38 (P <0.001), 45.16 (P <0.001), SGPT 26.65 (P <0.001), 46.41 (P <0.001), CHL 16.09 (P <0.001), 41.88 (P <0.001), urea 21.41 (P <0.001), 31.61 (P <0.001), ALB 25.10 (P <0.001), 44.03 (P <0.001), BLB 47.33 (P <0.001), 46.15 (P <0.001) and protein 173.55 (P <0.001), 185.12 (P <0.001) which is practically equivalent to the group

treated with silymarin, an intense liver defensive medication utilized as reference standard. The results are presented in Table 1.

Effect of quercetin and corycavidine on in-vivo antioxidant tests against simvastatin induced liver toxicity

Although both exhibit excellent hepatoprotective properties and have some role in preserving the structural integrity of the hepatocellular membrane, thus preventing enzyme leakage into the blood circulation[41], as well as repairing hepatic tissue damage caused by Simvastatin. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma as well as decreased the severity of hepatic fibrosis and the regeneration of hepatocytes[42]. The results indicated that the treatment of experimental rodents with quercetin and corycavidine cause a significant decrease in the levels of LPO in liver homogenate, which indicates their liver-protective efficacy[43]. Quercetin restored the activity of GSH, CAT, and SOD, and avoiding lipid peroxidation in a more prominent way as compare to corycavidine[44]. Although both delay or inhibit oxidative damage to target molecules, therefore intracellular and extracellular antioxidants protect the tissues from this oxidative damage.

The outcomes in Fig 1, presented clear noteworthy changes in the dimensions of LPO in simvastatin 20 mg/kg, inebriated rodents as 121.31 (P <0.001) compared with control. Treatment with quercetin as well as corycavidine at the doses of 50 and 100 mg/kg essentially kept this hurl in levels and the rate insurance in LPO was 240.93 (P <0.001) and 46.97 (P <0.001) individually for quercetin, however the rate insurance for corycavidine at the dose level of 50 and 100mg/kg, in LPO was 18.13 (P <0.01) and 35.16 (P <0.001) individually. The GSH, SOD, and CAT content had fundamentally expanded in quercetin-treated groups, however simvastatin 20 mg/kg, inebriated groups had demonstrated huge abatement in these parameters contrasted with the control group. The % change of GSH, SOD, and CAT in SIM-20 inebriated gathering were as 58.51 (P <0.001), 63.66 (P <0.001), and 54.15 (P <0.001) individually. The rate insurance in GSH as 61.53 (P <0.001), 102.56 (P <0.001) and SOD 80.22 (P <0.001), 132.23 (P <0.001) while in CAT 44.43 (P <0.001), 92.40 (P <0.001) at the portions dimensions of 50 mg/kg and 100 mg/kg of quercetin dose, individually. Nevertheless, quercetin at 100 mg/kg has demonstrated the most extreme security that was practically equivalent to those of the ordinary control and silymarin. The rate insurance in GSH as 25.64 (P <0.01), 74.35 (P <0.001) and SOD 22.63 (P <0.01), 95.41 (P <0.001) while in CAT 16.29 (P <0.01), 67.35 (P <0.001) at the portions

Table 1 — Effect of quercetin and corycavidine on serum enzymes and biochemical indices against Simvastatin induced liver toxicity in rats

Treatment	SGOT (U/L)	SGPT (U/L)	BLB (mg/dL)	TP (mg/dL)	ALB (mg/dL)	CHL (mg/dL)	Urea (mg/dL)
Control (Gr. I)	32.4±1.2	22.6±1.2	0.56±0.01	4.5±0.02	3.6±0.01	98.21±3.2	5.96±0.2
Sim-20 (Gr. II)	78.23±2.1†	43.41±1.5†	1.69±0.03†	1.21±0.01†	2.43±0.02†	213.21±4.3†	8.92±0.5†
Q-50 (Gr. III)	59.24±4.5**	34.51±3.1***	0.86±0.03***	9.4±0.04***	2.89±0.01***	152.4±2.2***	7.06±0.5***
Q-100 (Gr. IV)	48.9±3.2***	27.54±1.5***	0.63±0.02***	7.3±0.05***	3.21±0.02***	129.3±3.1***	6.31±0.4***
C-50 (Gr. V)	69.3±2.9**	39.57±1.9**	1.13±0.03**	2.28±0.02**	2.56±0.03**	201.2±3.4***	7.62±0.3***
C-100 (Gr. VI)	61.5±3.1***	31.84±1.6***	0.89±0.05***	3.31±0.03***	3.04±0.02***	178.9±2.9***	7.01±0.4***
Syl-20 (Gr. VII)	42.9±2.7***	23.25±2.2***	0.58±0.02***	3.43±0.07***	3.5 ±0.02***	123.9±2.5***	6.1 ±0.2***

[Values are mean ± SEM. of 6 rats in each group, n: non-significant. P values: †<0.001 compared with respective control group (Gr. I); P values: * <0.05, **<0.01, ***<0.001 compared with Gr. II]

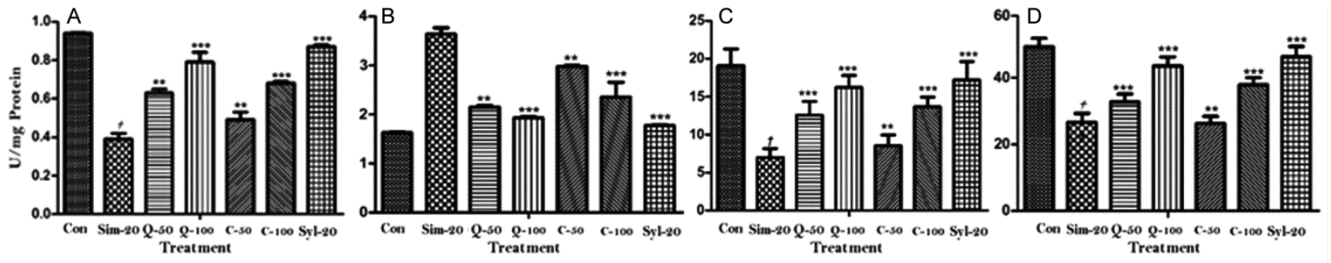

Fig. 1 — Effect of pretreatment with quercetin (50 and 100 mg/kg, body wt.) and corycavidine (50 and 100 mg/kg, body wt.) on *In vivo* antioxidant enzymes [(A) GSH; (B) LPO; (C) SOD; & (D) CAT] against Simvastatin induced liver toxicity. [values are mean ± SEM of 6 rats in each group, n: non-significant. P values: †<0.001 compared with the respective control group (Gr. I). P values: *<0.05, **<0.01, ***<0.001 compared with Gr. II].

dimensions of 50 mg/kg and 100 mg/kg of corycavidine dose, individually. Nevertheless, quercetin at 100 mg/kg has demonstrated the most extreme security that was practically equivalent to those of the ordinary control and silymarin. Corycavidine at 100 mg/kg has demonstrated a greater antioxidant effect as compared to 50 mg/kg of corycavidine that was practically confirmed.

Effects of quercetin and corycavidine on the hematological parameters in rats

Erythrocytes and hemoglobin exhibit the principal function in the transportation of oxygen and carbon dioxide[45]. Inside the RBCs iron is present, which facilitates oxygen transportation to the tissues or cells[46]. Quercetin upsurge the level in the RBCs, while at low dose its non-significant accelerated hemoglobin levels as compared to control, for this reason, these might be indicated in anaemia. Quercetin stimulates the kidney to release the erythropoietin hormone which in turn stimulates the pluripotent stem cell of red bone marrow for erythropoiesis[47,48]. Thrombocytopenia is the most splendid hemostatic imperfection for bleeding in dengue infection[49]. Quercetin and corycavidine notably elevated the platelets count and it may additionally be due to stimulation of thrombopoietin hormone, and has the hemostatic aptitude[50], consequently for this reason quercetin[51] in comparison to corycavidine can be used as a drug of choice for the remedy of dengue fever as well. Leucocytes provide indications on the number of infections of visceral organs. Augmentation of leucocytes arousing the immune system, in retort to noxious environment[52]. Lymphocytes and monocytes are the principal cells of the RES (reticuloendothelial system)[53] and its level increased swiftly with the aid of the pathogenic assault and performs the chief function in body defense mechanisms[54,55]. Increased WBCs and Lymphocytes levels due to quercetin and corycavidine administration might instigate the immune system of the animals. Quercetin and corycavidine at different dose levels offer liver protection, in a dose-dependent manner. It supports our finding s that quercetin protects liver better than corycavidine in simvastatin-induced liver toxicity. Table 2, emphasizes the significant changes in RBCs, Hb, PLT, WBCs, and % lymphocytes count. Simvastatin produced significant changes in blood parameters as RBCs by 31.71%, Hb by 24.59%, PLT by3.23%, and % lymphocytes by 10.74% were declined as compared to the control group. The animals which were treated with 50 and 100 mg/kg of quercetin showed the % increment in RBCs by 49.90 and 65.06%; Hb by 46.73 and 56.52%; PLT by 5.17 and 7.38% while decrease in WBCs by 49.4 and 46.01%; and % lymphocytes by 26.64 and 21.20% as compared to simvastatin treated animals. However, animals which were treated with 50 and 100 mg/kg of corycavidine showed the % increment in RBCs by 20.53 and 53.16%; Hb by 15.21 and 37.82%; PLT by 0.43 and 2.5% while decrease in WBCs by 4.07 and 24.61%; and % lymphocytes by 7.62 and 14.06% as compared to simvastatin treated animals.

Effects of quercetin and corycavidine on body weight and liver weight against simvastatin induced liver toxicity

Furthermore, the liver protective activity of quercetin is quite similar to silymarin, as a reference hepatoprotective agent. Changes in organ weight are considered as a sensitive indicator that assesses the toxic effects between treated and untreated groups of animals[56,57]. The body weight and the liver weight differed appreciably between the Sim-20 treated group and the normal control group. However, therapy with quercetin and corycavidine has diminished the impact of Sim-20 on a rat body weight and liver weight. The results of our findings reveal the significant preventive effect on liver toxicity against simvastatin.

In the toxic group (Sim-20), the body weight was decreased by 4.43% while liver weight was

Table 2 — Effect of quercetin and corycavidine on the hematological parameters against Simvastatin induced liver toxicity in rat

Treatment	RBC ($\times10^{12}$)	Hb (g/dL)	PLT ($\times10^{9}$/L)	WBC ($\times10^{9}$)	Lymphocytes (%)
Control (Gr. I)	7.6±0.1	13.01±0.2	613.2±5.6	8.1±0.12	41.2±2.1
Sim-20 (Gr. II)	5.21±0.3†	9.2±0.4†	598.6±2.6†	5.89±0.4†	34.9±1.9†
Q-50 (Gr. III)	7.81±0.3**	13.5±0.4**	629.6±4.1***	8.8±0.5***	44.2±3.1***
Q-100 (Gr. IV)	8.6±0.2***	14.4±0.3***	642.8±4.2***	8.6±0.2***	42.3±3.2***
C-50 (Gr. V)	6.28±0.1**	10.6±0.2**	601.2±5.2*	6.13±0.4*	37.56±3.8**
C-100 (Gr. VI)	7.98±0.2***	12.68±0.3***	613.6±4.2***	7.34±0.2***	39.81±3.2***
Syl-20 (Gr. VII)	9.26±0.2***	14.2±0.3***	614.5±4.2***	8.4±0.2***	42.58±3.2***

[Values are mean ± SEM. of 6 rats in each group, n: non-significant. P values: †<0.001 compared with respective control group (Gr. I); P values: * <0.05, **<0.01, ***<0.001 compared with Gr. II]

increased by 33.55%. The animal treated with 50 mg/kg of quercetin significantly increased their body weight by 0.86%, whereas the liver weight was decreased by 15.3%. The animal treated with 100 mg/kg of quercetin, significantly increased their body weight by 2.48%, while liver weight was decreased by 25.59%. The animal treated with 50 mg/kg of corycavidine significantly increased their body weight by 0.0.672%, whereas the liver weight was decreased by 5.80%. The animal treated with 100 mg/kg of corycavidine, significantly increased their body weight by 1.14%, while liver weight was decreased by 20.31%. The results are represented in Fig. 2.

Histopathological observations

The hepatic section of normal control (Gr. I) rats in Fig. 3, showed normal hepatic cells with youthful cytoplasm. In the negative control group i.e. simvastatin (20 mg/kg) treated rats (Gr. II), displayed the macro-vesicular and micro vesicular steatosis, cholestasis (Indicated by arrow 4, 5, 2), hypereosinophilic cytoplasm (Indicated by arrow 6), the appearance of ballooning cells with Mallory and councilman bodies (Indicated by arrow 1), lobular injury and bile plug seen in canalicular spaces (Indicated by arrow 3), whereas, animal treated with 50 mg/kg of quercetin (Gr. III) showed with less central vein congestion with less cholestasis with absent of steatosis. The animal treated with 100 mg/kg of quercetin (Gr. IV), showed the absence of Mallory and councilman bodies in the balloon with the revival of hepatocytes and owning supreme hepatoprotection. The animal treated with 50 mg/kg of corycavidine (Gr. V), showed moderate Mallory bodies, loss of hepatic membrane with moderate congestion in the central vein however rodents dosed with 100 mg/kg of corycavidine (Gr. VI), showed

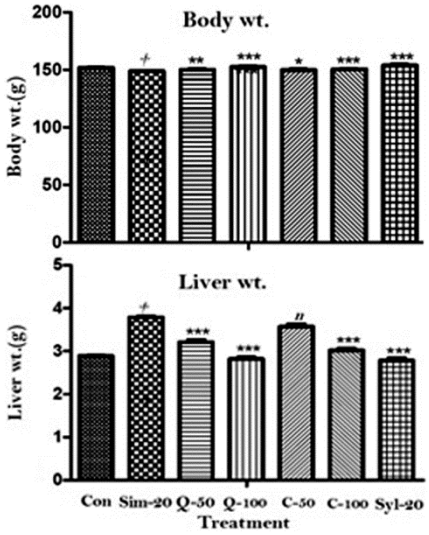

Fig. 2 — Effects of quercetin (50 and 100 mg/kg, body wt.) and corycavidine (50 and 100 mg/kg, body wt.) on body weight and liver weight against simvastatin induced liver toxicity. [values are mean ± SEM of 6 rats in each group, n: non-significant. P values: †<0.001 compared with the respective control group (Gr. I). P values: *<0.05, **<0.01, ***<0.001 compared with Gr. II].

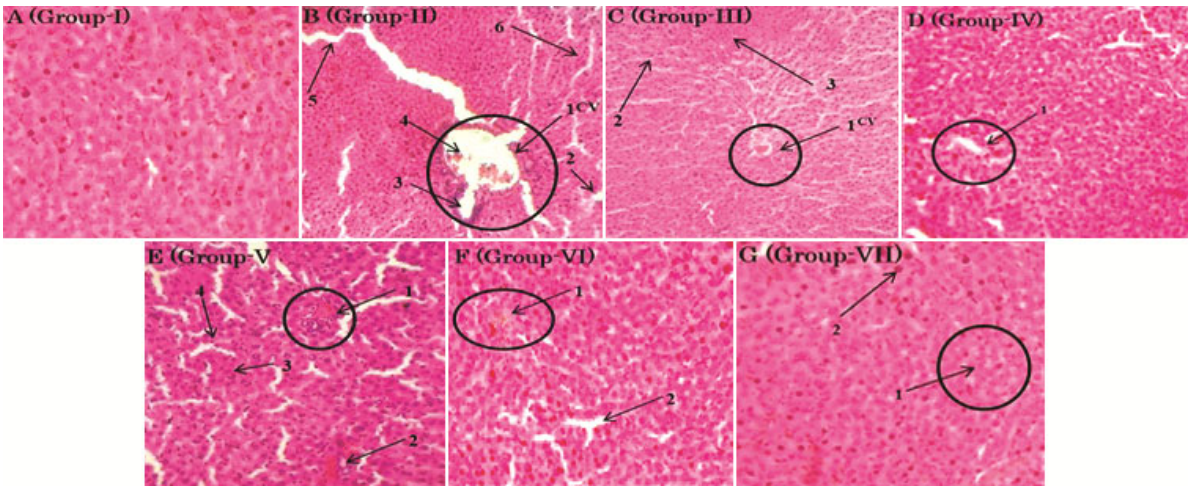

Fig. 3 — Hepatic section of (A) normal control (Gr. I) showing normal hepatic cells with youthful cytoplasm H & E 40X; (B) negative control group i.e. Simvastatin (20 mg/kg) treated rats (Gr. II) displaying the macro-vesicular and micro-vesicular steatosis, cholestasis (Indicated by arrow 4, 5, 2), hypereosinophilic cytoplasm (Indicated by arrow 6), the appearance of ballooning cells with Mallory and councilman bodies (Indicated by arrow 1), lobular injury and bile plug seen in canalicular spaces (Indicated by arrow 3) H & E 100X; (C) Gr. III, rats treated with 50 mg/kg of quercetin H & E 100X; (D) Gr. IV, rats treated with a large dose of quercetin (100 mg/kg) H & E 40X; (E) Gr. V, rats treated with a low dose of corycavidine (50 mg/kg) H & E 40X; (F) Gr. VI, rats treated with the higher dose of corycavidine (100 mg/kg) H & E 40X; and (G) Gr. VII, rats treated with standard treatment silymarin (20 mg/kg) exhibited well-preserved cytoplasm, with near-normal architecture H & E 40X.

the absence of moderate Mallory bodies, regeneration of hepatic membrane with a repaired central vein. The rats treated with Sim-20 (Gr. VI) exhibited well-preserved cytoplasm, with near-normal architecture.

Conclusion

As per the clinical significance of the test drug is concerned, it can be correlated with its exclusive indication with antihyperlipidemic drugs as prophylaxis to prevent liver toxicity. The above results suggest that quercetin might be a better drug of choice as prophylaxis to prevent hepatotoxicity induced by simvastatin as compared to corycavidine. Both phytoconstituents, quercetin and corycavidine, showed significant antioxidant and liver protection, and quercetin offers better hepatic defensive action as compared to corycavidine against simvastatin induced liver toxicity in rats.

Conflict of interest

Authors declare no competing interests.

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