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Open Access Research Article

Evaluation of *in-vitro* antioxidant potential and *in-vivo* hepatoprotective activity of root extract of *Quercus oblongata D. DON*

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ABSTRACT

Objective: The main potential target of the study was to investigate evaluation of *in-vitro* antioxidant potential and *in-vivo* hepatoprotective activity of root extract of *Quercus oblongata D. DON* belonging to family fagaceae. **Material & Methods:** The root sample of plant was extracted by different solvents like n-hexane (NHEQO), Chloroform (CEQO), Ethyl acetate (EAQO) Hydroethanolic (HEEQO) and Ethanolic (EEQO). The antioxidant activity (AA) was determined by the possible four complementary test assay methods namely total phenolic content, total flavonoids content, Inhibition of 2,2 diphenyl -1 picrylhydrazyl (DPPH) radicals and ABTS (2,2'-azinobis (3-ethyl benzthiazoline -6- sulphuric acid) radical scavenging activity or quenching activity, For hepatoprotective experimental study, albino wistar rats (120-180gm) were taken and divided into 6 group, each group content 5 animals , Group I: Received distilled water (5ml/kg. p.o) once daily, and served as normal control. Group II: Received paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally as toxin control. Group III: Received standard drug Silymarin (25 mg/kg. p.o.) + paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally once daily Group IV, V, VI administered Ethanolic extract of *Quercus oblongata* (EEQO)at different doses 100, 200, 300 mg/kg orally + paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; for 21 days. On 21st after 1 hr. of treatment blood was collected from experimental animals by retrorbital puncture for estimation of biochemical parameters and other parameter also evaluated like physical histological changes in livers of rats. **Results:** Experimental finding revealed that Paracetamol produced significant change in physical (increase liver weight) biochemical (increase alkaline phosphate, serum glutamic oxalacetic transaminase, serum glutamic pyuruvic transaminase, total protein, total bilirubin, direct bilirubin and decrease the level of total protein and albumin) histological (damage to hepatocyte) and in liver parameters. Pretreatment with extract significantly minimized physical, biochemical, histological and functional change induced by Paracetamol in liver**. Conclusion:** Experimental data and analysis of different parameter declared that ethanolic extract of *Quercus oblongata* could be useful hepatoprotective agents with antioxidant potential.

Keywords: *Quercus oblongata*, paracetamol, hepatoprotective, alkaline phosphate, serum glutamic oxalacetic transaminase, serum glutamic pyuruvic transaminase, 2,2 diphenyl -1 picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethyl benzthiazoline -6- sulphuric acid (ABTS).

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1. INTRODUCTION

Antioxidant nowadays became the most vital member of majority of prescriptions. Since years back *yin-yang* (antioxidant-oxidation process) was existed concept in Chinese system of healthcare. Antioxidants have been reported to prevent oxidative damage which is generally caused by ROS by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen radical scavengers. Antioxidants are a compound which converts harmful reactive free radical into harmless stable species by donating an electron to the free radical, and protect cells from oxidative damage that leads to ageing and several diseases. However the innate defense may not be enough for severe or continued oxidative stress. Hence, certain amounts of exogenous anti-oxidants are constantly required to maintain an adequate level of anti-oxidants in order to balance the ROS in

human body. Liver is one of the largest glands in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body.[1] It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction, Histologically the liver is composed of hepatocytes which are the major functional unit of the liver and perform a numerous array of metabolic, secretory, and endocrine functions.[2] Hepatocytes are specialized hexagonal epithelial cells that make up about 80% of the volume of the liver. A complex three-dimensional arrangement of hepatocytes is known as hepatic laminae. Between neighboring hepatocytes grooves are present and provide spaces for canaliculi, into which the hepatocytes secrete bile. Bile in appearance is a yellow, brownish, or olive-green secretion of hepatocytes, serves as both an excretory product and a digestive secretion too. [3] Paracetamol induces a number of deleterious metabolic changes in the liver. Its excessive use for a long-time leads to development of steatosis, alcoholic hepatitis and cirrhosis resulting in weight and volume changes.[1] At least 80% of heavy drinkers had been reported to develop steatosis, 10-35% alcoholic hepatitis, and approximately 10% liver cirrhosis.[2] Recent studies in animal models suggest that liver injury in chronic alcoholics is due to oxidative stress that leads to fibrosis and impaired liver functions and increased apoptosis.[4] The present study describes the hepatotoxic effects of Paracetamol in albino rats [5] *Quercus oblongata* Banj oak is the most well-known broadleaf tree found generally in the mid height of Central Himalayas in India, It is likewise found in Myanmar, Pakistan, Nepal, Thailand, and Vietnam.[6] the synonyms of *Quercus oblongata is* best adjusted to territories having mellow and sodden climate This evergreen tree, found in the focal Himalayas between 1300-2600 m above ocean level and speaks to the peak vegetation happens. Nerves of *Quercus oblongata* are potential sources of common colors, gallic acid and some other beneficial secondary metabolites. The forests are mainly dominated by different oak species *viz., Quercus glauca* (Harinj), *Quercus leucotrichophora* (Banj), *Quercus floribunda* (Moru) and *Quercus semecarpifolia* (Kharsu) which form the climax vegetation at different climatic zones. These forests are not only fulfilling the day to day requirements of local inhabitants but also associated with the ecological and hydrological balance and support other species to grow splendidly.[7] *Quercus* is an important genus of temperate regions in Pakistan. Antibacterial, antioxidant and gastro-protective action of quercus and its species has been reported scientifically. [8] Local residents and tribal groups in Hilly region of Uttarakhand and other states uses *Quercus leucotrichophora* A. Camus for treatment of various body disorders. Various ethano-medicinal uses of *Quercus oblongata* are- Fruit powder is used for the treatment of urinary infection. [9] Toothache and piles are treated by bark of *Quercus leucotrichophora* A. Camus. Leaves are used as an astringent and in the treatment of diarrhea while the gum resin is used as stomach ache. [10]

2 MATERIAL & METHODS

Silymarin was obtained from Yarrow chem products, Mumbai, India. The kits for biochemical estimation were purchased from Transasia Bio Medical, HP, India. The solvent and other chemical was used of analytical grade. The root extract of plant *Quercus oblongata* was collected during September to October in 2015 from the wild region of Rudraprayag, Uttarakhand, India, and authenticated by botanist Professor S. K. Srivastava, Botanical Survey of India, Dehradun, Uttarakhand, India with a Specimen number for plant is 115572. The whole plant was shade dried at room temperature and extracted with with ethanol 95% v/v for 24 hrs using hot soxhlet apparatus and extracts were dried at 50°C on water bath and the % yield of 14.85%. Phytochemical screening of whole plant extracted with ethanolic solvents revealed the presence of various secondary plant metabolites (Table 1).

(1) Total Phenolic Content:

UV spectrophotometric method was use to estimate the total phenolic content about 5ml of Folin-ciocalteau's phenol reagent was mixed with 5 ml of sample and 60 ml of water (Spanos et al. 1990). After 5 min. 15ml of 10% Na_2CO_3 solution was mix and the volume was adjusted to 100 ml with deionised water. The whole mixture was incubated for 2 hrs at 23 °C and the absorbance was measured at 517 nm. The total phenol content was expressed as milligrams of Gallic acid equivalent per g of dried sample.[11]

(2) Total flavanoid content:

Total flavonoid content was estimated by method described by (Eom et al). In a glass test tube, the minimum amount of extract 0.3 gm was mixed with exact amount of 0.15 ml of 0.5 M $NaNO_2$ and 0.15 ml of 0.3 M $AlCl_3.6H_2O$ and 3.4 ml of 30% methanol. Then 1ml of 1M NaOH was added after 5 min and mixed thoroughly. Further the absorbance of the solution was measured at 506 nm. Rutin was used as standard. Flavonoid content was expressed as milligrams of rutin equivalents per gm of dried fraction. [12]

(3) DPPH radical scavenging activity:

The 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) was used for determination of free radical scavenging activity of different extract. Different aliquot of each extract ethanolic, methanolic and aqueous extracts as per the method described by (Mitchell, M. F) In 100 ml of methanol dissolve 24 mg DDPH at 20°C making stock solution. The solution used for studying was obtained by dilution of methanol and the absorbance was adjusted to 0.98±0.02 at 517nm. A 3ml aliquot of this solution was mixed with 100 µl of the sample at various concentration (10-500 µg/ml).The mixture was then shaken and kept in dark for incubation at room temperature. Then absorbance was taken at 517 nm. The percentage of DPPH radical scavenging activity is calculated as the following equation.[13]

Scavenging effect (%) = Control absorbance- sample absorbance/control absorbance × 100

(4) ABTS radical scavenging assay:

Free radical scavenging activity of *Q. oblongata* was by determine as per the method described by (Haung et al. 2011) The ABTS (2,2'-azinobis (3-ethyl benzthiazoline - 6- sulphuric acid) cation was measured spectrophotometrically reaction of simple mixing with ABTS (7mm) and potassium persulfate (2.45mm) to make dark green colored solution contain ABTS solution and keeping it overnight in dark. The ABTS radical cation was priorly diluted with 50% methanol and adjusted to an initial absorbance of about 0.70±0.02 at 745 nm and kept in temperature of 30°C. The radical scavenging activity was assessed next day by mixing previously prepared ABTS solution with 300 µl of test solution in micropipette. The decrease in absorbance after mixing test solution with ABTS solution was measured within 1 min.[14].

Scavenging effect % = (control absorbance -sample absorbance)/control absorbance × 100

2.3 Animal:

Wistar albino rats (120-180 gm) of either sex were obtained from, Department of pharmaceutical sciences Bhimtal campus, Kumaun University, Nainital, Uttarakhand. On the animal house, experimental rats were acclimatized for 10 days, under a room temperature of 24±2^{0}c relative humitidity 45-55^{0}c with 12:12 hrs light and dark cycle. The animals had free accesses to food (Ashirivad food industry Mohali, Chandigarh) and water ad lebitum. The animals were habituated to laboratory condition for 48 hrs prior to the experimental protocol to minimize the non specific stress. The institutional animal ethics committee of Department of pharmaceutical sciences Bhimtal campus Kumaun University Nainital, Uttarakhand, India, approved the experimental protocol in accordance with the guideline provided by committee for purpose of control and supervision of experimental on animals (CPCSEA) with the registration no KUDOPS/38/16/03/2016.

2.4 Acute toxicity Test:

Acute toxicity studies, Healthy Wistar albino rats of either sex weighing 120-180 g maintained under standard laboratory conditions were used for acute oral toxicity test according to Organization for Economic Cooperation and Development guidelines 423. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for period of 3 days (OECD, 423). Observations were done daily for changes in skin and fur, eyes, mucus membrane (nasal), respiratory rate, circulatory signs (heart rate), autonomic effect (salivation, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes. The root extract of plant *Q. oblongata* at a dose of 2 gm/kg body weight was given to 6 animals and was continuously observed for 14 days for mortality and general behaviour. No deaths were observed till the end of this study. The plant extract was considered to be safe up to a dose of 2 gm/kg body weight. From these results, test drug dose of 100, 200 &300 mg/kg body weight was chosen for the efficacious studies. [15]

2.5 Experimental design:

The albino wistar rats (120-180gm) were divided into 6 groups, each group contain 5 animals. Group I: Received distilled water (5ml/kg. p.o) once daily, and served as normal control. Group II: Received paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally and Served as toxin control, Group III: Received Standard drug Silymarin (25 mg/kg. p.o.) + paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally once daily. Group IV, V, VI administered EEQO at the doses of 100, 200 and 300 mg/kg orally + paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally repetitively for 21 day.

On 21 day after administration of test as well as standard drug, suspension (640 mg/kg suspended in 1% methyl cellulose; orally within 30 minute, and all animals were anesthetized using Thiopentone sodium 4 mg/kg ip injection. The blood samples were collected from retro orbital puncture and allowed to stand for 30 min at 37^{0}C then serum was separated from it with the help of centrifugation for further estimation of serum biochemical parameters. The animals were sacrificed under mild diethyl ether anesthesia and livers get isolated, washed with ice cold saline and weighed and send for further histopathological examination.

2.6 Histopathological examination:

Histopathology was carried by modified methods of "Luna" (Luna, 1999) [16]. In brief the autopsied livers were washed with normal saline and material were fixed in 10% buffered neutral formalin for 2 days followed by bovine solution for 6 hrs and paraffin embedded livers get sectioned of 5µ thickness by using microtome than processed in absolute alcohol- xylene, served and stand with haematoxyline and eosin blue. The slides were examined under a light microscope for any histological damages/protection.

2.7 Statistical Analysis:

In animal study, the data are expressed as mean ±SD. For statistical analysis data was subjected to analysis of variance (ANOVA) by using Graph Pad Instat. Values are considered statistically significant at P<0.001(n=5).

3. RESULTS

Preliminary Phytochemical Screening:

The distribution of different phytochemical constituents in n-hexane, Chloroform, Ethyl acetate, ethanol and ethanolic extracts of whole *Quercus oblongata* DC and root extract of *Quercus oblongata* D. DON were evaluated qualitatively.

Table 1: Phytochemical screening of *Quercus oblongata*.

Name of Plant		*Quercus oblongata*				
S. No	**Test**	**N-hexane**	**Chloroform**	**Ethyl acetate**	**Hydroethanolic**	**Ethanolic**
1.	**ALKALOIDS**					
	Dragendroff's test	-	-	-	++	++
	Mayer's test	-	-	-	++	++
	Wagner's test	-	-	-	++	++
	Hager's test	-	-	-	-	++
2.	**CARBOHYDRATES**					
	Molisch test	-	-	-	++	++
	Fehling test	-	-	-	++	++
	Benedict test	-	-	-	--	++
3.	**SAPONINS**					
	Haemolysis test	-	-	-	++	-
	Foam test	-	-	-	-	++
4.	**PROTEINS**					
	Biurets test	-	-	-	-	++
	Millon's test	-	-	-	++	++
	Xanthoproteins	-	-	-	-	++
5.	**Phenolic compounds**					
	$FeCl_3$ test	-	-	-	++	++
6.	**Tannins**					
	Lead acetate test	-	-	+	++	++

Antioxidant activity

a) DPPH radical scavenging activity

The ethanolic extract of *Q. oblongata* possessed significant DPPH scavenging activity with an IC_{50} of 3.48µg/ml followed by ethanolic extract when compared with standard which IC_{50} value was found to be of 3.41µg/ml.

Table 2: IC50 values of free radical scavenging effect by DPPH method of various extracts of *Q. oblongata*.

Con	NHEQO	EAEQO	CHEQO	EEQO	HEEQO	STD
10 µg/ml	23.62%	17.15%	27.21%	15.10%	8.12%	36.11%
20 µg/ml	29.12%	26.25%	42.50%	23.39%	11.88%	40.43%
30 µg/ml	32.63%	46.14%	46.28%	30.16%	27.53%	45.11%
50 µg/ml	38.72%	49.15%	47.40%	40.39%	36.26%	52.62%
100 µg/ml	42.29%	50.21%	51.19%	43.60%	39.65%	59.34%
200 µg/ml	47.26%	52.17%	62.18%	52.82%	47.06%	67.70%
300 µg/ml	54.62%	53.43%	67.99%	67.36%	67.48%	74.85%
400 µg/ml	57.21%	62.12%	72.89%	70.30%	67.89%	82.93%
500 µg/ml	61.06%	72.54%	75.22%	77.41%	78.64%	92.56%
IC50µg/ml	**5.21µg/ml**	**4.35 µg/ml**	**4.62µg/ml**	**3.48 µg/ml**	**3.92µg/ml**	**3.41 µg/ml**

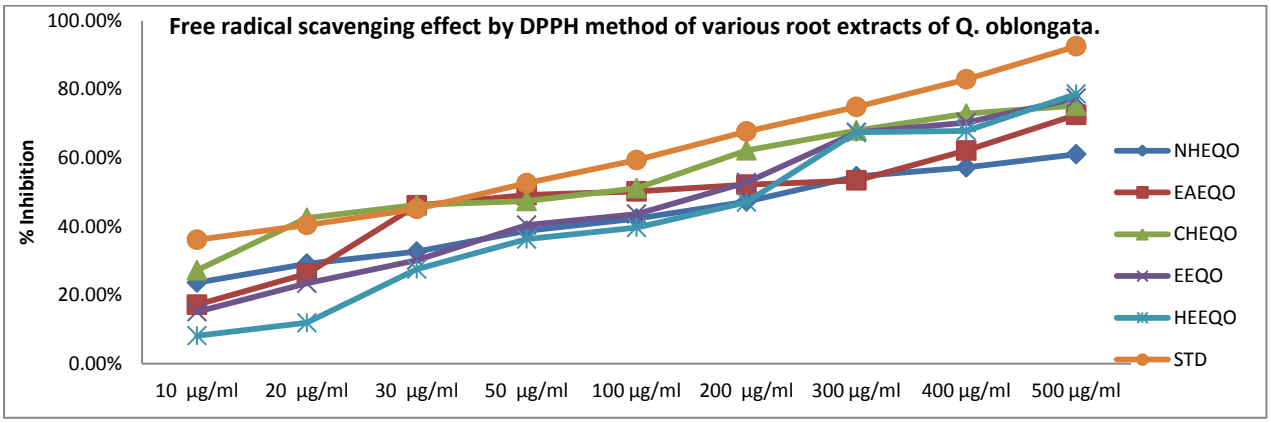

Figure 1: Free radical scavenging effect by DPPH method of various root extracts of *Q. oblongata*.

b) Total phenolic content:

The ethanolic extract of *Q. oblongata* possessed significant phenolic content.

Table 3: Total Phenolic content of different extract of *Q oblongata*.

S.No	Extracts of *Q.oblongata*	Total phenolic content (mg GAE/g of extracts) ± SD
1	Ethanolic extract	43.64±24.2
2	Ethanolic extract	49.24± 32.8
3	Chloroform extract	57.82±30.6
4	Ethyl acetate extract	62.31± 46.7
5	n-hexane extract	64.35±36.2

c) ABTS 2-2'- azinobis) radical scavenging activity:

By reducing the ABTS and potassium persulfate, this assay generates a blue/green ABTS chromophore. All the extracts of Q. oblongata were *neutralized*, ABTS radical in a concentration dependent way (10- 500 µg/ml). The free radical scavenging activity of different extracts on ABTS radicals were in the following order- EEQO> HEQO > CHQO> EAQO > NHQO the IC_{50} value of the standard was found to be 3µg/ml. The results for ABTS free radical scavenging activity have been summarized in order of the highest antioxidant activity in Table no.3

Table 3: IC50 values of free radical scavenging effect by ABTS method of various extracts of *Q. oblongata.*

Conc	NHEQO	EAEQO	CHEQO	EEQO	HEEQO	STD
10 µg/ml	21.59%	28.76%	29.16%	28.26%	25.87%	31.85%
20 µg/ml	33.98%	38.78%	39.57%	34.27%	38.67%	41.68%
30 µg/ml	35.74%	39.27%	45.66%	44.60%	49.92%	50.69%
50 µg/ml	37.53%	40.25%	56.12%	53.91%	57.05%	58.06%
100 µg/ml	48.24%	46.62%	61.54%	65.45%	65.71%	67.56%
200 µg/ml	56.57%	55.04%	63.55%	73.57%	74.94%	75.83%
300 µg/ml	60.13%	56.80%	78.47%	78.72%	76.93%	83.94%
400 µg/ml	65.21%	64.25%	79.16%	80.63%	89.19%	90.09%
500 µg/ml	79.25%	78.78%	82.57%	90.80%	92.40%	95.98%
IC50µg/ml	5.68µg/ml	5.36 µg/ml	5.15µg/ml	4.25 µg/ml	3.45µg/ml	3 µg/ml

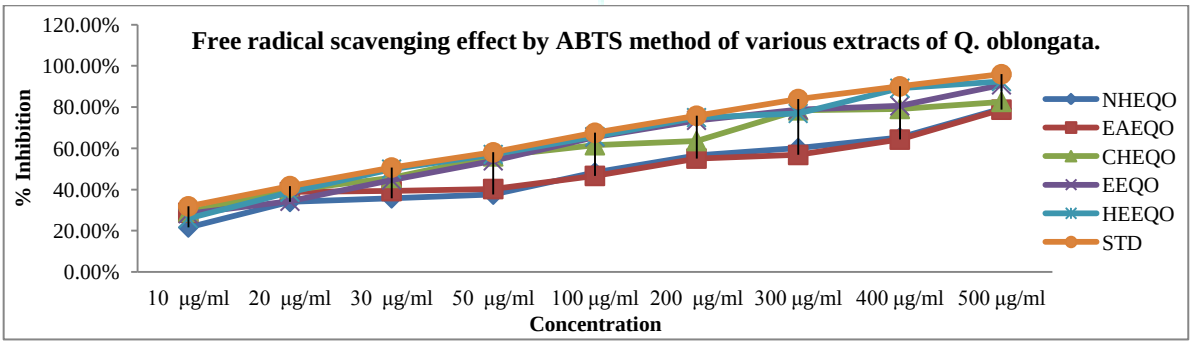

Figure 3 Free radical scavenging effect by ABTS method of various extracts of *Q. oblongata.*

d) Total flavonoid content:

The naturally occurring substance flavonoids have been confirmed to be responsible for the antioxidant activity in plants.

Table 4: Total Flavonoid content of different extract of *Quercus oblongata.*

S. No	Extracts of *Q. oblongata*	Total flavonoids content (mg rutin /g of extracts) ± SD
1	**Ethanolic extract**	**31.27±29.3**
2	**Ethanolic**	**35.42± 29.3**
3	**Chloroform extract**	**49.56±21.8**
4	**Ethyl acetate extract**	**57.56± 25.3**
5	**n-hexane extract**	**62.73±39.2**

3.2 *In-Vivo* Hepatoprotective activity of ethanolic root extract of *Quercus oblongata.*

3.2.1. Body Weight:

The body weight estimation in the control and Paracetamol induced rats were shown in Fig 1. The body weight was reduced in Paracetamol treated rats when compared to normal control rats. Treatment with ethanolic extract of *Quercus oblongata* at the dose of 100, 200, 300 mg/kg p.o and Silymarin at 25mg/kg dose to Paracetamol induced toxic rats cause an increase in the body weight during the experimental period.

Liver Weight

The liver weight in Paracetamol control group was increased significantly ($p<0.01$) in comparison with control group. Pretreatment with ethanolic extract of *Q. oblongata* and Silymarin showed significantly reduction in liver weight as compared to Paracetamol treated rats as shown in Fig.8.

3.2.2. Estimation of biochemical parameter:

The serum levels of SGOT, SGPT, ALP, Total bilirubin, direct bilirubin total protein and albumin were significantly increased in Paracetamol treated group in comparison normal control group, while the level of total protein albumin were reduced significantly**.** Pre-treatment with ethanolic extract of *Q. oblongata* and Silymarin significantly *($p<0.001$) get reduced the elevated serum enzyme such as SGOT, SGPT, ALP, total bilirubin and direct bilirubin as well as increased the level of total protein, albumin when compared with Paracetamol control group. The results indicates that pretreatment with EEQO (100, 200, 300 mg/kg) and Silymarin significantly prevented the biochemical changes may induced by Paracetamol. EEQO produce the greater hepatoprotective effect by normalizing the elevated serum enzymes level in Paracetamol induce liver damage in rats.

Table 5: Effect of ethanolic extract of *Q. oblongata* on SGPT, SGOT, and ALP in Paracetamol induce hepatotoxicity on the 21 day.

Treatment Group	Dose mg/kg	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
Normal control	5 ml/kg	78.68±29.22	96.56±22.19	126.43±16.03
Toxin Control (Paracetamol control)	640 mg/kg suspended in 1% methyl cellulose; orally	322.49±26.36	388.87±45.87	398.75±46.78
Silymarin+ 640 mg/kg suspended in 1% methyl cellulose; orally	25mg/kg	95.17±41.14*	124.72±29.51*	226.02±91.47**
EEQO +640 mg/kg suspended in 1% methyl cellulose; orally	100mg/kg	212.36±44.32#	256.44±53.88*	268.21±13.07*
EEQO +640 mg/kg suspended in 1% methyl cellulose; orally	200mg/kg	124.26±24.12**	161.04±36.81**	219.04±19.04*
EEQO+640 mg/kg suspended in 1% methyl cellulose; orally	300mg/kg	91.35±40.51**	128.96±52.13*	226.19±27.38*

Data are expressed as mean ± SD (n = 5). One-way ANOVA followed by Tukey's post hoc ** P< 0.001 compared with group II.

Table 6: Effect of ethanolic extract of *Q. oblongata* on total bilirubin, direct bilirubin, total protein, and albumin in Paracetamol induce hepatotoxicity on 21 day.

Treatment Group	Dose mg/kg	Total bilirubin mg/dl	Direct bilirubin mg/dl	Total protein (g/dl)	Albumin (g/dl)
Normal control	5ml/kg	0.48±0.09	0.19±0.04	8.16±0.05	4.57±0.03
Toxin Control (Paracetamol control)	640 mg/kg suspended in 1% methyl cellulose; orally	2.30±0.12	0.84±0.081	5.38±0.10	2.39±0.01
Silymarin+ 640 mg/kg suspended in 1% methyl cellulose; orally	25mg/kg	0.68±0.13**	0.26±0.76**	7.81±0.06**	4.45±0.07**
EEQO +640 mg/kg suspended in 1% methyl cellulose; orally	100mg/kg	1.83±0.25*	0.53±0.11*	7.32±0.03*	3.18±0.06*
EEQO +640 mg/kg suspended in 1% methyl cellulose; orally	200mg/kg	1.48±0.18**	0.38±0.12**	7.32±0.01**	3.69±0.10*
EEQO+640 mg/kg suspended in 1% methyl cellulose; orally	300mg/kg	0.89±0.26*	0.29±0.03**	7.56±0.02*	3.81±0.03**

Data are expressed as mean ± SD (n = 5). One-way ANOVA followed by Turkey's post hoc: * P< 0.001 compared with group II.

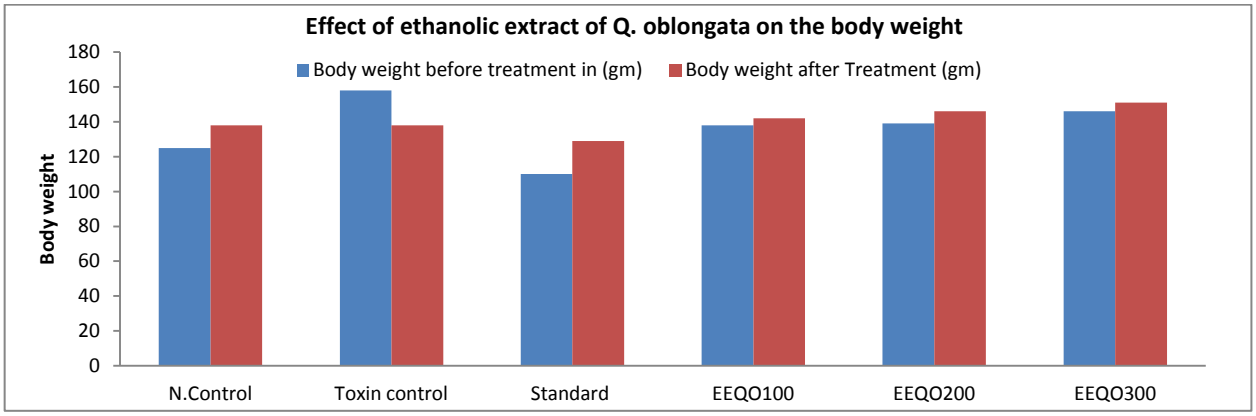

Figure 7: Effect of ethanolic extract of *Q. oblongata* on the body weight (Before and after treatment) in Paracetamol induces hepatotoxicity.

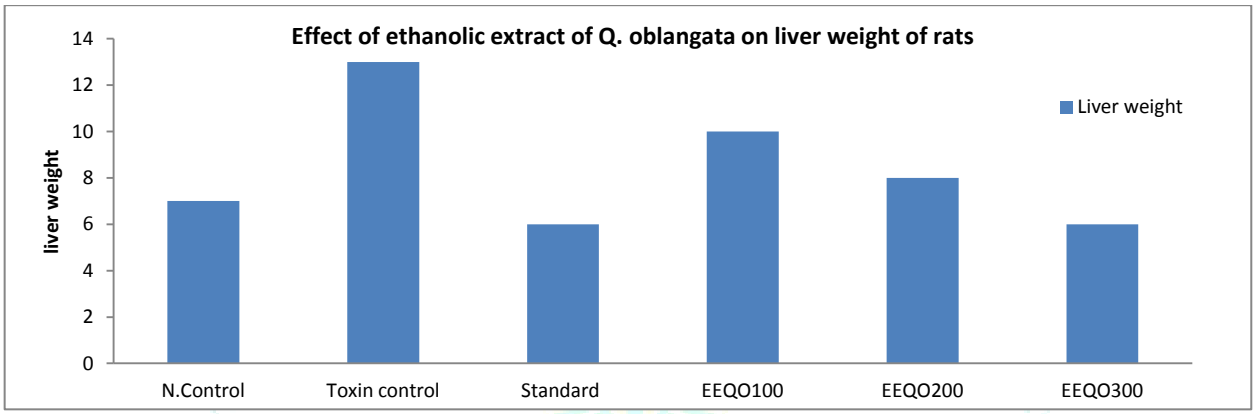

Figure 8: Effect of ethanolic extract of *Q. oblongata* on liver weight in Paracetamol induced hepatotoxicity.

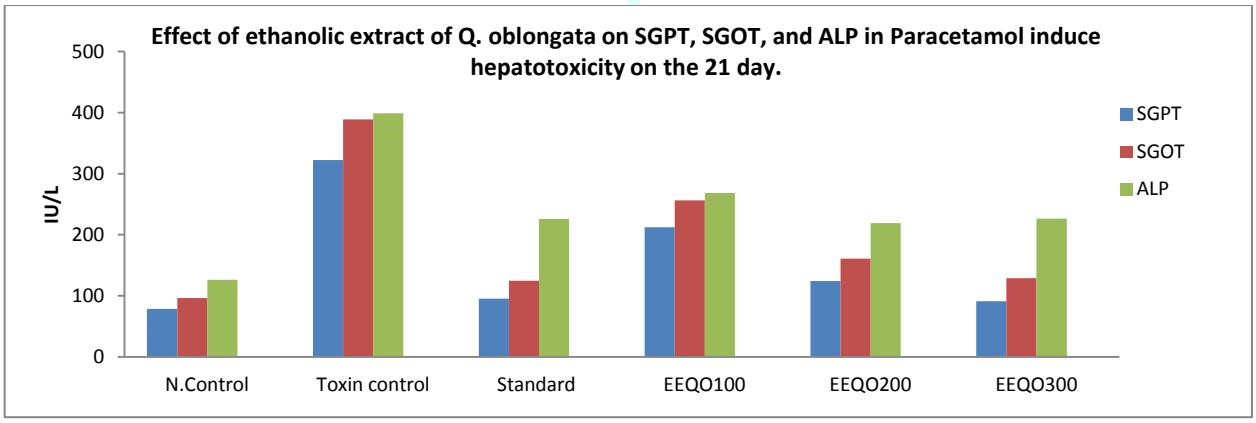

Figure 9: Effect of ethanolic extract of *Q. oblongata* on SGPT, SGOT, and ALP in Paracetamol induce hepatotoxicity on the 21 day.

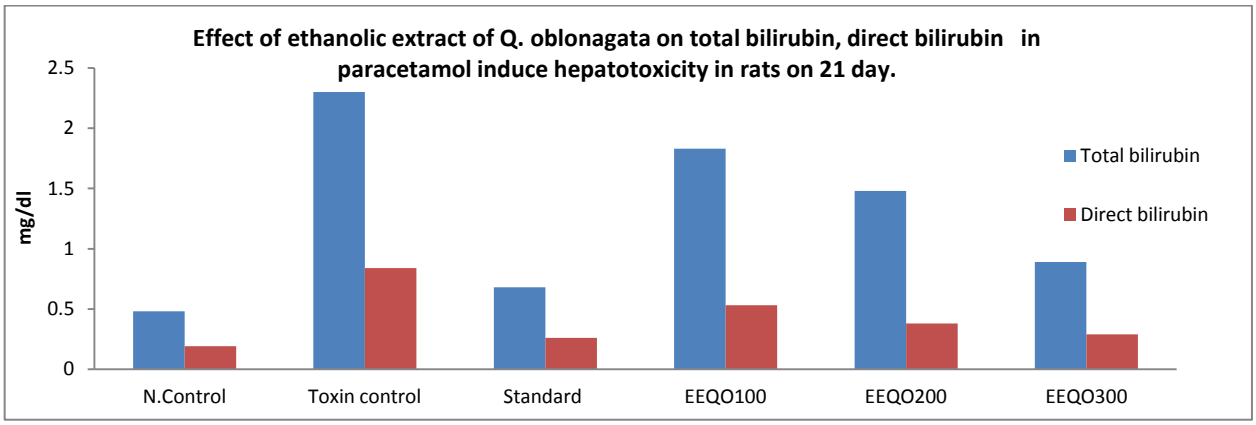

Figure 10: Effect of ethanolic extract of *Q. oblongata* on total bilirubin and direct bilirubin in Paracetamol induce hepatotoxicity on the 21 day.

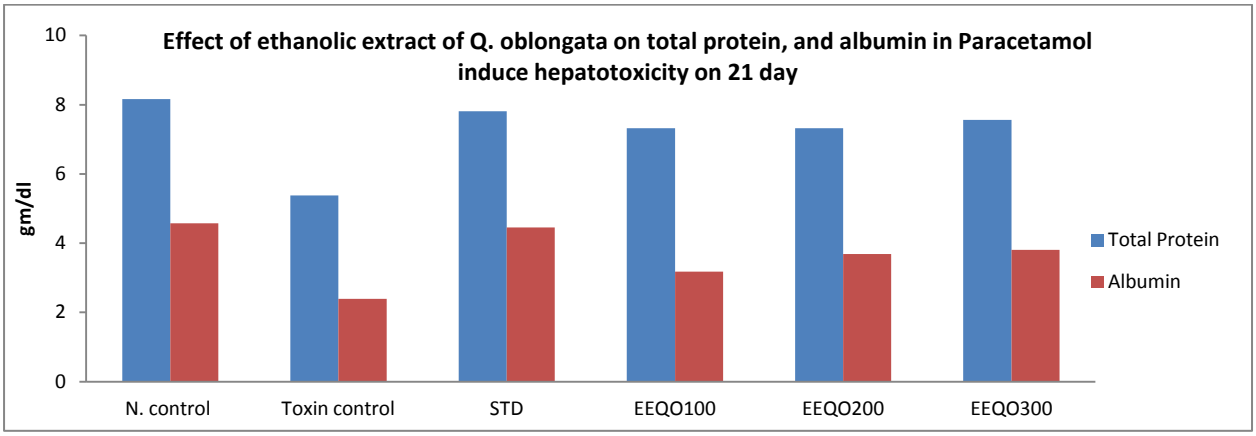

Figure 11: Effect of ethanolic extract of *Q. oblongata* on total protein and albumin in Paracetamol induce hepatotoxicity on the 21 day.

Histopathology:-

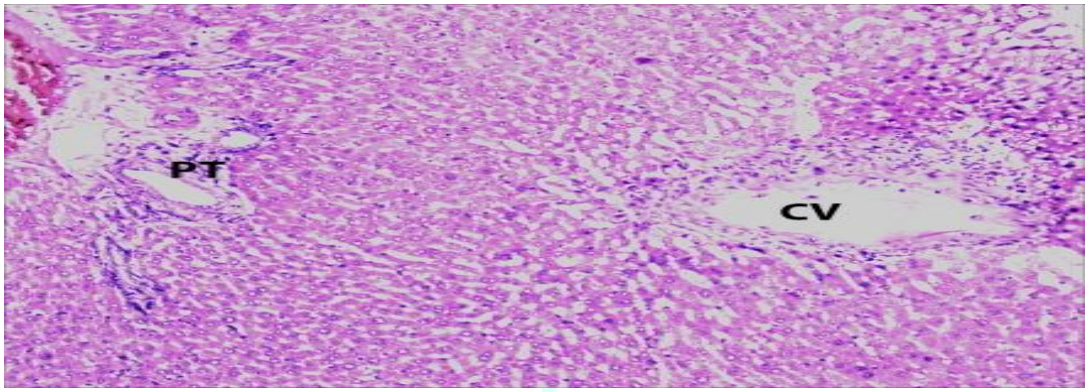

Figure 12: Histopathological assessment of EEQO 100mg/kg

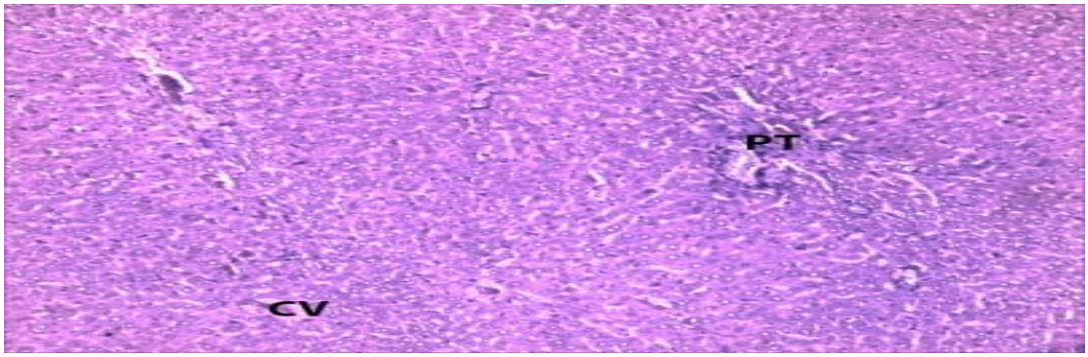

Figure 13: Histopathological assessment of EEQO 200mg/kg

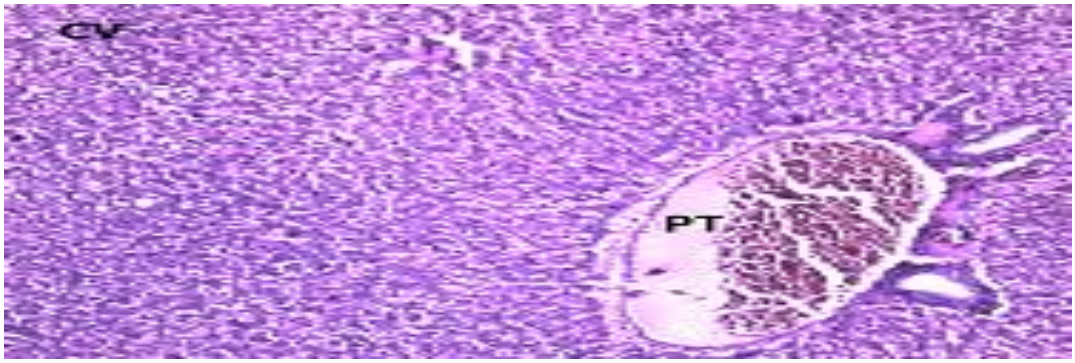

Figure 15: Histopathological assessment of EEQO 300mg/kg

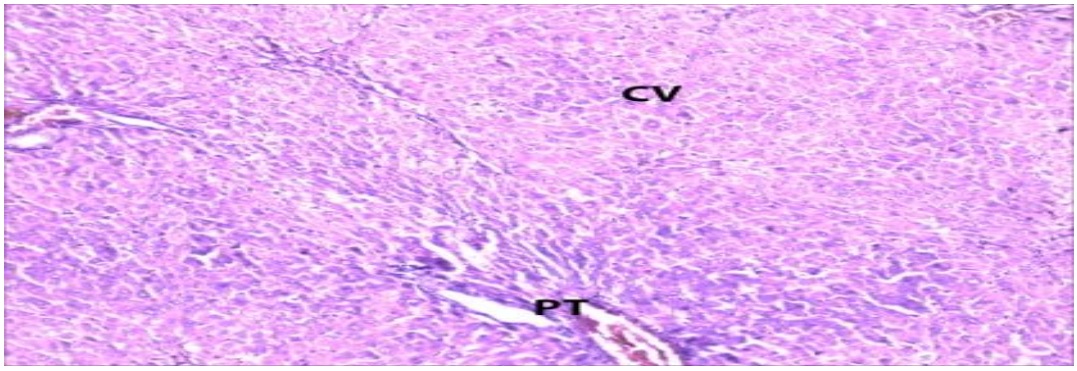

Figure16: Histopathological assessment of Standard drug Silymarin

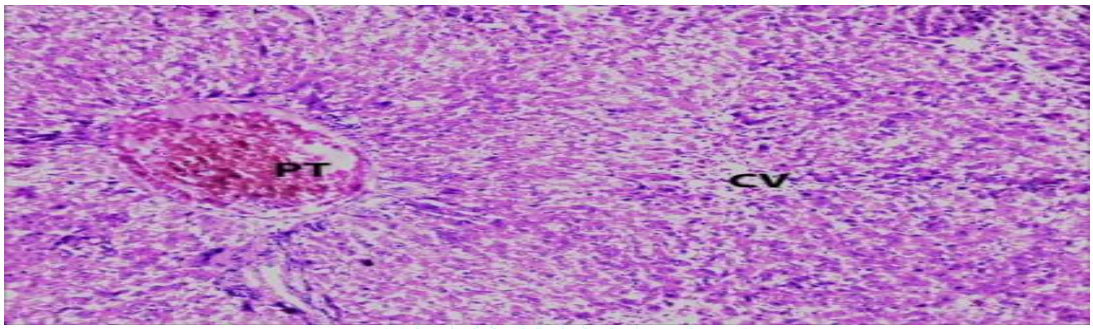

Figure 17: Histopathological assessment of toxic control

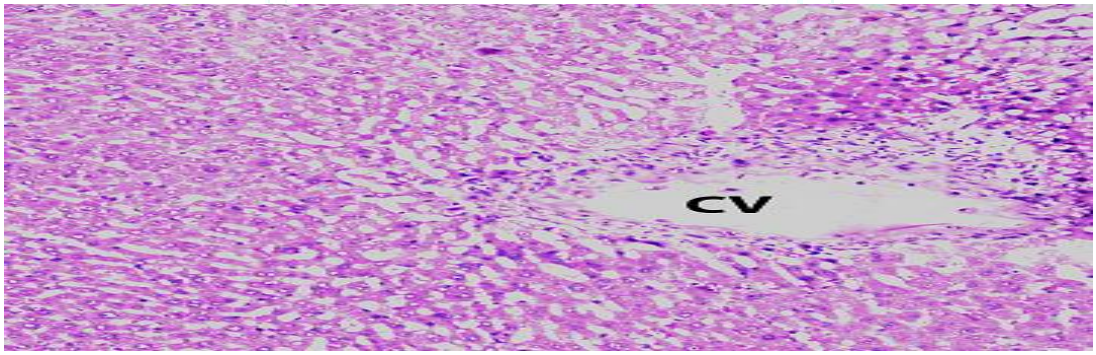

Figure 18: Histopathological assessment of normal control

3.6 Inferences of hepatocellular necrosis/protection

Fig. (12) Section of the liver tissue of animal treated with ethanolic extract of *Q. oblongata* (EEQO) at dose 100 mg /kg treated animal showing .Normal arrangement of hepatocyte around the portal vein hepatic artery shows few necrosis and fatty vacuoles necrosis (H×E 100x).

Fig. (13) Section of the liver tissue of animal treated with ethanolic extract of *Q. oblongata* (EEQO) at dose 200 mg /kg treated animal showing .Normal arrangement of hepatocyte around the portal vein hepatic artery shows few necrosis and fatty vacuoles necrosis (H×E 100x).

Fig. (14) Section of the liver tissue of animal treated with ethanolic extract of *Q. oblongata* (EEQO) at dose 300 mg /kg treated animal showing .Normal arrangement of hepatocyte around the portal vein hepatic artery shows few necrosis and fatty vacuoles necrosis (H×E 100x).

Fig. (15) Section of the liver tissue of animal treated standard drug Silymarin treated animal showing normal histology and portal triad showing normal portal vein, hepatic artery and bile duct (H×E 100x).

Fig. (17) Section of the liver tissue of animal treated with paracetamol showing a central vein necrosis and heavy necrosis in fatty acid vacuoles (H×E 100x).

Fig. (18) Section of the liver tissue of normal control (vehicle) showing normal histology and portal triad showing normal portal vein, hepatic artery and bile duct (H×E 100x).

4. DISCUSSIONS

Quercus leucotrichiphora is best adapted to regions with a mild and moist climate. Quercus leucotrichiphora is an evergreen tree which occurs in the central Himalayas between 1300- 2600 m above sea level and represents the climax vegetation Generally, the average annual rainfall ranges 200 to 250 cm; three forth of this occurs These galls are potential sources of natural dyes, gallic acid and some other secondary metabolites.[17] In

Quercus oblongata Paracetamol induced liver damage in rats successfully treat the wounds on the basis of biochemical parameters EEQO has significant role to minimize hepatotoxicity, in the comparison to standard drug Silymarin the dose of EEQO at the dose of 300 mg/kg has potent hepatoprotective agent however further study to needed to explore this for human being.

5. CONCLUSION

These studies have reflected that Quercus leucotrichiphora contains bioactive compound with the potential of being good hepatoprotective agents and potent antioxidant agents. On the basis of histopathology study report. However further study still needed to be causes on exposure of extract to human beings.

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Conflict of interest: Author has no conflict.

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