ANTIPROLIFERATIVE ACTIVITY OF QUERCETIN ON HepG2 CELL LINE INDUCED LIVER CANCER IN RATS

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ABSTRACT

The wide use of natural food-derived antioxidants is receiving greater attention as potential anti-carcinogens. Among flavonoids, Quercetin is considered an excellent free-radical scavenging antioxidant. In present study antiproliferative activity of Quercetin was evaluated on HepG2 cell line induced liver cancer in rats. For the purpose of this study, 30 rats were divided into 5 groups and each group containing 6 rats each. Group I- normal saline treatment for 45 days, Group II- cancer cells (1×10⁶ cells in 0.1ml/rat), Group III– 5-Fluorouracil (20mg/kg + 1×10⁶ cells in 0.1ml/rat), Group IV- Quercetin (25mg/kg + 1×10⁶ cells in 0.1ml/rat), Group V- Quercetin (50mg/kg + 1×10⁶ cells in 0.1ml/rat). After 24 h of tumor inoculation intraperitoneally, Quercetin was administered daily for 45 days. After administration of last dose followed by 18 h fasting, rats were sacrificed for observation of antiproliferative activity. The change in body weight, body circumference of tumor bearing hosts and simultaneous alterations in hematological profile, serum (TC, TG, TP, ALP, SGPT, GGT, TB and glucose) and liver biochemical parameters (lipid peroxidation, GSH and antioxidant enzymes-CAT, GPx) were estimated. The changes in tissue enzymes-Glucose-6 phosphate dehydrogenase, Hexokinase, Succinate dehydrogenase and CytochromeP450 levels were also estimated. The Quercetin maintaigned the body circumference and body weight of proliferation bearing rat. Hematological profile reverted towards normal levels in Quercetin treated rat. Treatment with Quercetin restored
serum biochemical parameters towards normal levels and decreased levels of lipid peroxidation and increased levels of reduced glutathione and other antioxidant enzymes (CAT and GPx). The Quercetin treatment restored Glucose-6 phosphate dehydrogenase, Hexokinase, Succinate dehydrogenase and CytochromeP450 levels in proliferation induced rat. The Quercetin exhibited antiproliferative effect by modulating hematological parameters, lipid peroxidation and augmenting antioxidant defense system in proliferation bearing rat.

KEYWORDS: Quercetin, HepG2 cell line, antiproliferative effect, antioxidant activity.

INTRODUCTION
Cancer is one of the major concerns around the world, as it is one of the leading causes of death worldwide. It is a group of disease where it affects all living cells, at all ages and in both genders. Hepatocellular carcinoma is cancer that starts in the liver. Hepatocellular carcinoma, the predominant primary liver cancer in most countries, is the fifth most frequent cancer in the world.\(^1\) Hepatocellular carcinoma (HCC) is one of the most common cancers with poor prognosis, and there are about 500,000 to 1,000,000 new cases per year. Several etiological factors have been identified including chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), prolonged exposure to aflatoxin B1, liver cirrhosis due to alcohol abuse, and nonalcoholic fatty liver.\(^2\) Chemotherapy is mainly used to treat cancer. However, the severe side effects of the drugs led the researchers to search for an alternative. The investigations of the efficacy of plant-based drugs have received growing attention due to their minimum side effects. Quercetin a naturally occurring plant phenol compound was reported with anticancer properties on different cancers. It is also well known for its protective activity on normal cells which made Quercetin as a pivotal compound for cancer therapy.\(^3\)

Quercetin also known as 3,3',4',5,7-Penthydroxyflavone. Quercetin is a plant pigment (flavonoid). It is found in many plants and foods, such as red wine, onions, green tea, apples, berries, American elder and others. People use quercetin as a medicine. Quercetin is used for treating conditions of the heart and blood vessels including “hardening of the arteries” (atherosclerosis), high cholesterol, heart disease, and circulation problems. It is also used for diabetes, cataracts, hay fever, peptic ulcer, schizophrenia, inflammation, asthma, gout, viral infections, chronic fatigue syndrome (CFS), preventing cancer, and for treating chronic infections of the prostate. Quercetin is also used to increase endurance and improve athletic performance. Quercetin has antioxidant and anti-inflammatory effects which might
help reduce prostate inflammation. Quercetin supplements have been promoted for prevention and treatment of cancer, "there is no reliable clinical evidence that quercetin can prevent or treat cancer in humans".\textsuperscript{[2]} Also, there is no evidence that consuming foods rich in quercetin reduces the risk of cancer or any other disease.\textsuperscript{[3]} Quercetin is one of the most abundant dietary flavonoids with an average daily consumption of 25–50 mgs.\textsuperscript{[4]} This health-promoting activity seems to be related to the antioxidant (free-radical scavenging) activity to flavonoids.\textsuperscript{[5]} Flavonoids are a group of polyphenol compounds, which are widely distributed throughout the plant kingdom.\textsuperscript{[6]} Quercetin reached the maximum antioxidant potential.\textsuperscript{[7]} Quercetin is known to affect antioxidant enzymes superoxide dismutase (SOD) and cytochrome oxidase that have the potential to convert reactive oxygen species (ROS) to a hydrogen peroxide and an oxygen molecule. Quercetin is a naturally occurring plant phenolic compound, Phenolic compounds of plant products are mainly responsible for the antioxidant activity to reverse the effect of ROS mechanism by various pathways, and they have a potent effect to reduce incidence of cancer.\textsuperscript{[7]} Reactive oxygen species (ROS) are produced as a by-product of various metabolic processes, mainly during respiration, in living organisms. Normal physiological concentrations of ROS usually have a role of regulation of cell activities, whereas higher concentrations cause oxidative damage. Quercetin has been shown to possess anti-cancer activity.\textsuperscript{[8]} Anti inflammatory activity.\textsuperscript{[9]} Anti depressant activity.\textsuperscript{[10]} Anti bacterial activity.\textsuperscript{[11]} antiulcer activity.\textsuperscript{[12]} antiasthmatic.\textsuperscript{[13]} antiobesity.\textsuperscript{[14]} Hepatoprotective activity.\textsuperscript{[15]}

Although there are some reports on inhibition of in vivo metabolic activation of carcinogens by Quercetin and the antimutagenic effect of oxidative DNA damage, in previous studies of the signaling pathway leading to apoptosis revealed that an increase in intracellular reactive oxygen species (ROS) or Ca\textsuperscript{2+} plays an important role in eliciting an early signal for apoptosis\textsuperscript{[16,17]}, there are no reports on whether Quercetin has an effect on human hepatocellular carcinoma cell lines. Considering the rich antioxidant status of Quercetin, this study investigated possible antiproliferative effects and antioxidant status of Quercetin on the HepG2 cell line induced liver cancer in Wister Albino male rats.

**MATERIALS AND METHODS**

**Quercetin**

Quercetin is the form of organic compound. It was suspended in distilled water and freshly prepared just before the administration. It was orally administered by gastric tube at a dose level of 25 mg/kg and 50mg/kg b. w. /day for 45 days.
**Cell lines:** HepG2 liver cancer cell line. The cell line was obtained from National Institute of Nutrition, Hyderabad. These cells were maintained in bovine serum albumin medium at 37 °C in a humidified atmosphere of 5% CO2 in air.

**Animals**

Male Wistar Albino rats weighing 180-250g were obtained from Albino Research Institute, Bachupally (V), Quthbullapur (M), Hyderabad. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60% humidity). Standard pelletedized feed and tap water were provided *ad libitum*. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2013-14/MPCOL/03).

**Treatment schedule**

Thirty Male Wistar Albino rats weighing 180-250g were divided into five groups of six animals each.

- **Group I** – Normal saline treatment for 45 day
- **Group II** - Cancer cells (1×10⁶/rat)
- **Group III** – 5-Fluorouracil (20mg/kg bodyweight) 0.5ml + cells (1×10⁶/rat)
- **Group IV** – 25mg/kg dose of Quercetin + cells (1×10⁶/rat)
- **Group V** - 50mg/kg dose of Quercetin + cells (1×10⁶/rat)

Animals were grouped into 5 groups as explained above. The control group animals were given normal saline 1ml for 45 days. Group 2 animals were given HepG2 cell line 1×10⁶ cells/rat i.p. Group 3 animals were given HepG2 cell line 1×10⁶ cells/rat i.p. and 5-Flourouracil 20mg/kg body weight i.p. until 45th day respectively. Group 4 animals were given HepG2 cell line 1×10⁶ cells/rat i.p. and Quercetin 25 mg/kg body weight p.o. Group 5 animals were given HepG2 cell line 1×10⁶ cells/rat i.p. and Quercetin 50 mg/kg until 45th day respectively.

**Blood sample preparation**

The animals were sacrificed using ether anesthesia, blood was collected by carotid bleeding and transferred to anticoagulant EDTA tubes for the estimation of hematological parameters like Hb, RBC and WBC.
Serum sample preparation
The animals were sacrificed on 45th day using ether anesthesia; blood was collected by carotid bleeding and was centrifuged using Remi cool centrifuge at 4000 rpm for 15 minutes. Serum was separated for the estimation of various biochemical parameters like serum alkaline phosphatase, Triglycerides, Total cholesterol, Total protein, GGT, glucose, SGPT and Total Bilirubin.

Tissue sample preparation
At the end of the experiment, animals were sacrificed with light ether anesthesia. Liver tissue was separated and washed with phosphate buffer saline (0.05M, pH7.4). The liver was taken later and minced into small pieces and homogenized in ice cold phosphate buffer saline (0.05M, pH7.4) using tissue homogenizer to obtain 1:9 (w/v) (10%) whole homogenate. A part of the liver homogenate was taken and mixed with equal volume of 10% Trichloro acetic acid (TCA) for the estimation of malondialdehyde. Homogenate was centrifuged using remi cool centrifuge at 8000 rpm for 30 min. The supernatant was separated and used for estimation of anti-oxidant levels of different enzymes i.e. catalase and reduced glutathione, malondialdehyde and glutathione peroxidase.

Animals were sacrificed by light ether anesthesia and liver was isolated from the rat each group, washed thoroughly with PBS (phosphate buffer saline) (0.05M, pH7.4) and folded in aluminum foil cover stored at -80°C. The changes in concentrations in the tissue homogenate hexokinase, glucose 6-phosphate dehydrogenase, and succinate dehydrogenase, cytochrome P450 levels were estimated.

Statistical Analysis
The experimental results were expressed as the Mean ± SEM with six rats in each group. Statistical significance of difference between groups was determined by one-way ANOVA followed by unpaired t-test.

RESULTS
Effect of Quercetin on body weight and body circumference of liver cancer rat
There was an increase in the body weight and body circumference of liver cancer induced rat from second week onwards during a growth period of 45 days when compared to normal group. Treatment with 5-Fluorouracil and Quercetin maintained the body weight and body circumference of liver cancer induced rat (Figure 1 and 2).
Effect of Quercetin on liver weight on liver cancer induced in rat.
There was significant (p<0.01) increase in liver weight in cancer induced group as compared to the normal group. Treatment with 5-Fluorouracil and Quercetin 25mg/kg and 50mg/kg dose maintained the liver weight as compared to the cell line induced group (Figure 3).

Effect of Quercetin on hematological parameters of liver cancer induced rat
Hemoglobin content and RBC count were significantly (P<0.001) decreased and total WBC count was significantly (P<0.001) increased in the liver cancer group as compared to the normal group. Treatment with 5-Fluorouracil and Quercetin restored the RBC and hemoglobin levels towards the normal (Figure 4 & 5).

Effect of Quercetin on serum biochemical enzymes of liver cancer induced rat
There was a significant (P<0.001) decrease in serum glucose, significant increase in TC (p<0.01), GGT (p<0.05) and TC, SGPT, Total Bilirubin, TP and ALP (p<0.001) levels in the cancer induced group when compared to normal group. Treatment with 5-Fluorouracil and Quercetin 25mg/kg and 50mg/kg doses significantly increased the glucose level, significantly decreased the enzyme activity as compared to cancer induced group and restored to normal levels (Table 1 & 2).

Effect of Quercetin on catalase, MDA, GSH and GPx in liver cancer induced rat
Catalase, GSH, and GPx were significantly (P<0.001) decreased and MDA levels were significantly (P<0.001) increased in the cancer induced group when compared to the normal group. Treatment with 5 fluorouracil and Quercetin significantly (P<0.01) decreased the MDA levels and increased the catalase, GSH, and GPx levels towards the normal (Table 3).

Effect of Quercetin on glucose-6 phosphate dehydrogenase, hexokinase, succinate dehydrogenase and cytochromeP450 levels in liver cancer induced rats
The cytochrome P450 levels (p<0.01), succinate dehydrogenase levels significantly (P<0.001) decreased in liver cancer induced group compared to normal control group. Treatment with 5- Fluorouracil and Quercetin significantly (P<0.001) restored the levels of cytochrome p450 and succinate dehydrogenase (Figure 6 & 7).

The G6PD, hexokinase levels in Hep G2 cell line induced animals were found to be significantly (p<0.01) increased when compared to the control animals. Treatment with Quercetin (p<0.05) and 5-Fluro uracil showed a significant (p<0.05) decrease in G6PD and hexokinase levels when compared to the cell line induced animals as shown in Figure 7.
Figure 1. Effect of Quercetin on body weight: Values are expressed as mean ± SEM, (n=6), 5-FU= 5 Fluro Uracil.

Figure 2. Effect of Quercetin on % increase in body circumference in liver cancer induced rat. Values are expressed as mean ± SEM, (n=6), 5-FU= 5 Fluro Uracil (20mg/kg), Quercetin.

Figure 3. Effect of Quercetin on organs weight in liver cancer induced rats. Values are expressed as mean ± SEM, (n=6). Data was analyzed by one way ANOVA followed by
unpaired t test. \( p<0.01 \) as compared with normal control group. \( **p<0.01, *p<0.001 \) and \( @p<0.05 \) as compared with cell line induced group, 5-FU= 5 Fluro uracil (20mg/kg), Quercetin.

Figure 4. Effect of Quercetin on Hematological estimations of liver cancer induced rats: Values are expressed as mean ± SEM, (n=6), Data was analyzed by one way ANOVA followed by unpaired t test. \( ^a p<0.001, ^c p<0.01 \) and \( @p<0.05 \) as compared with the normal control; \( *p<0.001 \) and \( ***p<0.01 \) and \( **p<0.05 \) as compared with cell line induced group, 5-FU= 5 Fluro Uracil(20mg/kg), Quercetin.

Figure 5. Effect of Quercetin on Hematological estimations of liver induced cancer in rats: Values are expressed as mean ± SEM, (n=6), Data was analyzed by one way ANOVA followed by unpaired t test. \( ^a p<0.001 \) as compared with the normal control; \( *p<0.001 \) as compared with cell line induced group, 5-FU (20mg/kg) & Quercetin.
Table 1: Effect of Quercetin on serum biochemical parameters on Hep-G2 cell line induced liver cancer in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TG(mg/dl)</th>
<th>ALP(IU/L)</th>
<th>Glucose(mg/dl)</th>
<th>TP(IU/L)</th>
<th>TC(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Normal control</td>
<td>119.18±13.36</td>
<td>233.66±49.72</td>
<td>93.10±8.55</td>
<td>8.17±0.16</td>
<td>0.16±0.008</td>
</tr>
<tr>
<td>Group II Cell line induced</td>
<td>60.24±8.99a</td>
<td>338.19±20.86c</td>
<td>35.58±5.31c</td>
<td>6.87±0.19c</td>
<td>0.10±0.01c</td>
</tr>
<tr>
<td>Group III 5- FU(20mg/kg)</td>
<td>101.68±19.12**</td>
<td>241.33±58.93***</td>
<td>61.51±2.42**</td>
<td>7.90±0.25**</td>
<td>0.11±0.009**</td>
</tr>
<tr>
<td>Group IV Quercetin (25mg/kg)</td>
<td>112.50±16.22*</td>
<td>267.20±294.6**</td>
<td>83.28±16.57**</td>
<td>6.85±0.15*</td>
<td>0.14±0.01*</td>
</tr>
<tr>
<td>Group V Quercetin (50mg/kg)</td>
<td>110.33±15.11**</td>
<td>259.49±100.3**</td>
<td>79.25±18.11**</td>
<td>6.67±0.11*</td>
<td>0.14±0.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, (n=6). Data was analyzed by one way ANOVA followed by unpaired t test. a p < 0.01 and c p<0.001 as compared with the normal control; ***p<0.001 and **p<0.01 and *p<0.05 as compared with cell line induced group, 5-FU= 5 Fluro Uracil(20mg/kg),Quercetin.

Table 2: Effect of Quercetin on SGPT, GGT and Bilirubin on HepG2 cell line induced liver cancer in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT (IU/dL)</th>
<th>GGT (IU/L)</th>
<th>Total Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Normal control</td>
<td>31.92±1.89</td>
<td>22.84±1.93</td>
<td>1.85±0.06</td>
</tr>
<tr>
<td>Group II Cell line induced</td>
<td>42.96±1.93c</td>
<td>30.02±1.25b</td>
<td>3.62±0.41c</td>
</tr>
<tr>
<td>Group III 5- FU(20mg/kg)</td>
<td>34.10±1.33***</td>
<td>24.69±0.17***</td>
<td>2.19±0.19**</td>
</tr>
<tr>
<td>Group IV Quercetin(25mg/kg)</td>
<td>38.02±0.45**</td>
<td>26.52±0.11**</td>
<td>2.70±0.05*</td>
</tr>
<tr>
<td>Group V Quercetin (50mg/kg)</td>
<td>35.23±0.18***</td>
<td>23.49±1.64**</td>
<td>2.38±0.05**</td>
</tr>
</tbody>
</table>
Values are expressed as mean ± SEM, (n=6). Data was analyzed by one way ANOVA followed by unpaired t test. \(^c_p<0.001\), \(^b_p<0.05\) as compared with normal control; \(^***p<0.001\) \(^**p<0.01\) and \(*p<0.05\) as compared with cell line induced group, 5-FU= 5 Fluro Uracil.

### Table 3. Effect of Quercetin on Catalase, GPx, GSH and MDA on Hep-G2 cell line induced liver cancer in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase (µ mole/min/mg)</th>
<th>GPx (µ mole/min/mg)</th>
<th>GSH (µ mole/min/mg)</th>
<th>MDA (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>0.41±0.03</td>
<td>8.68±0.57</td>
<td>14.67±0.70</td>
<td>39.33±1.75</td>
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<tr>
<td>Group II</td>
<td>0.24±0.01(^c)</td>
<td>6.04±0.23</td>
<td>7.57±0.13(^c)</td>
<td>89.66±3.45</td>
</tr>
<tr>
<td>Cell line induced</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>0.34±0.02(^***)</td>
<td>7.71±0.79(^**)</td>
<td>12.68±0.93(^***)</td>
<td>73.33±4.09(^**)</td>
</tr>
<tr>
<td>5- FU (20mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>0.31±0.02(^**)</td>
<td>7.86±0.35(^**)</td>
<td>11.25±1.16(^**)</td>
<td>74.0±4.30(^**)</td>
</tr>
<tr>
<td>Quercetin (25mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group V</td>
<td>0.29±0.02(^*)</td>
<td>7.15±0.53(^**)</td>
<td>10.21±1.20(^*)</td>
<td>73.2±4.01(^**)</td>
</tr>
<tr>
<td>Quercetin (50mg/kg)</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, (n=6), Data was analyzed by one way ANOVA followed by unpaired t test. \(^c_p<0.001\) as compared with normal control; \(^***p<0.001\) \(^**p<0.01\) and \(*p<0.05\) as compared with cell line induced group, 5-FU= 5 Fluro Uracil(20mg/kg), Gallic acid.

![Figure 6. Effect of Quercetin on Cytochrome p450 on HepG2 cell line induced liver cancer in rats.](image)

Values are expressed as mean ± SEM, (n=6), Data was analyzed by one way ANOVA followed by unpaired t test. \(^c_p<0.01\) as compared with normal control group,\(^*_p<0.001,^{**}_p<0.01\) as compared with cell line induced group 5-FU= 5 Fluro Uracil(20mg/kg), Quercetin.
Figure 7. Effect of Quercetin on Hexokinase, Glucose-6-phosphate dehydrogenate, Succinate dehydrogenate on HepG2 cell line induced liver cancer in rats. Values are expressed as mean ± SEM, (n=6), Data was analyzed by one way ANOVA followed by unpaired t test. *p<0.001 and p<0.01 as compared with normal control group, *p<0.001, **p<0.01, ***p<0.05 as compared with cell line induced group 5-FU= 5 Fluro Uracil (20mg/kg), Quercetin.

DISCUSSION
In recent years, cancer is a worldwide disease with an estimated one million new cases and a half million deaths each year. It has increasingly been recognized that malignancy may not exclusively result from enhanced cell proliferation but also from decreased physiological cell death, i.e. apoptosis. Apoptotic induction has been a new target for innovative mechanism-based drug discovery. Chemoprevention, a relatively new strategy to prevent cancer, depends on the use of nontoxic chemical substances, to block, reverse, or retard the process of carcinogenesis. The investigations of the efficacy of plant-based drugs have received growing attention due to their minimum side effects. Quercetin a naturally occurring plant phenol compound was reported anticancer properties on different cancers, it might protect normal cells from the damage caused by ROS while inducing apoptosis and inhibiting proliferation in tumor cells. In this study, we investigated in vivo effects of Quercetin on the HepG2 cell line induced liver cancer in rat.\textsuperscript{[16]} Our results showed that Quercetin inhibited the growth of liver cancer cells in a concentration dependent manner, compared to the controls. Quercetin at a concentration of 50mg/kg exhibited a maximum of 95% inhibition of growth of liver cancer (HepG2) cells.

With their antioxidant potential, Quercetin can be used as a dietary supplement in some diseases as well as in cancer. In other previous studies, CAT and SOD activities were
increased in the Quercetin added groups; it seemed that this compensatory change could not prevent cell death. Thus, the mechanism of the apoptosis might be based on some reasons other than oxidative stress.

There is increasing evidence suggesting that certain antioxidants compounds act as preventive or protective factors. The present study is preliminary to measure the antioxidant levels and Hematological parameters in liver cancer induced rat and in 5FU and Quercetin treated groups.

The decrease in Hb (hemoglobin) concentration and RBC (red blood cells) value indicates the presence of anemia in all cancer induced rat. This anemia may be caused by GSH depletion in cancer induced rat which is important as cellular antioxidant so its depletion lead to red blood cell destruction which leads to decrease Hb value and the other cause may be the bone marrow failure which is caused by replacement of its normal elements by cancer cells in varying degrees.[18] These levels were restored to normal in Quercetin treated groups.

A significant difference in WBC count between cancer induced rat and control was noticed in this study. The WBC count in liver cancer induced rat was high as compared to their controls.[18] The levels of WBC were restored to normal in Quercetin treated and 5-fluorouracil treated groups.

Our study showed depletion of reduced glutathione concentration in liver cancer induced rat as shown these results were in agreement with other studies. There can be two reasons for GSH depletion in cancer. Firstly; elevated glutathione peroxidase will use more GSH in an attempt to cope with the excessive production of oxy radicals as revealed by elevated lipid peroxidation. Secondly, if little replenishment of GSH occurred, the level of GSH would become lower and GSH forms a well known non-enzymatic antioxidant defense system detoxifying endogenous and exogenous compounds.[19]

Oxidative stress plays an important role in the pathogenesis of chronic diseases, such as cancer and atherosclerosis. In these pathological states, the increased production or ineffective scavenging of oxidants may play a crucial role in determining tissue injury. Prime targets of reactive oxygen species are the polyunsaturated fatty acids in cell membranes and their interaction results in lipid peroxidation. Enhanced lipid peroxidation and impairment in antioxidant defense mechanisms were demonstrated in patients with lung and liver
Serum MDA levels were significantly elevated in liver cancer induced groups when compared to controls.

Increased lipid peroxidation in serum and tissues has been reported in liver cancer induced rat. The lipid peroxidation products such as MDA can structurally alter DNA, proteins and other biomolecules. Our findings are in agreement with most of the earlier studies suggesting that liver cancer induced rat might be at risk from oxidative cell damage. Oxidative stress arises when there is an imbalance between oxygen-free radical (OFR) formation and scavenging by antioxidants. Excess generation of free radical can cause oxidative damage to biomolecules resulting in lipid peroxidation. OFR-induced lipid peroxidation has been implicated in neoplastic transformation. The increase in the rate of lipid peroxidation causes the increased production of MDA that leaks into the blood stream, consequently causing increased levels of MDA in liver cancer treated groups; this may be due to the super oxide dismutase present in the Quercetin that converts oxygen free radicals in to hydrogen peroxide and water.

CAT is a crucial cellular antioxidant enzyme that degrades hydrogen peroxide. Another important peroxide removing enzyme, perhaps having higher efficiency than CAT is GPx. The levels of CAT and GPx were restored to normal in Quercetin treated groups.

Gamma GT is an oncofoetal protein, a glycoprotein whose levels have been shown to be altered during development and carcinogenesis. In most of the liver diseases, both malignant and non-malignant, GGT estimation has been reported to be a sensitive but nonspecific indicator of the disease. Some studies have shown that GGT levels are also elevated in malignant tumors of the other tissues. This increase in serum GGT activity in cancer induced rat is due to rapid turnover of malignant cells, which release the enzymes in to blood streams. The levels of GGT were restored to normal in Quercetin treated groups.

The levels of Triglycerides, Total cholesterol and SGPT, Total protein and Glucose were significantly (P<0.01) changed in liver cancer induced groups compared to their respective controls and these levels were restored in the Quercetin treated groups. Serum ALP and total bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary
pressure. The levels of ALP and total bilirubin were maintained to normal in Quercetin treated groups.

It is known that proliferating cells have a high pentose phosphate. Numerous early studies have shown that this metabolic pathway is increased in many types of tumors. Because major functions of the non-oxidative and oxidative sequences of the pentose phosphate pathways are the supply of ribose-5-phosphate for incorporation into ribonucleic acid and coenzymes, and the reduction of NADP+ to NADPH for metabolic synthetic reactions respectively, it can be expected that the pentose phosphate pathways play important roles in the metabolism of tumors and rapidly dividing cells. The first enzyme of the pentose pathway is glucose-6-phosphate dehydrogenase (G6PD) and the second 6-phosphogluconate dehydrogenase (6PGD).

G6PD is known to be increased in tumour cells generally and in other dividing cells. The enzyme is important not only for participating in the supply of pentose sugars for nucleic acid synthesis but also for producing NADPH and thus changing the redox couple NADP+/NADPH. Glucose-6-phosphatdehydrogenase (G6PD) functions to catalyze the oxidation of glucose6-phosphate to 6-phosphogluconolactone and the reduction of NADP+ to NADPH. In this way, G6PD provides cells with NADPH as a reducing power that maintains the sulfhydryl groups of cellular proteins and aids in detoxification of free radicals and peroxides. Although G6PD is expressed in all tissues, its deficiency causes severe effects in erythrocytes and renders these cells more susceptible to oxidative stress. The animals treated with Quercetin restored or maintained the G6PD levels.

There are four important mammalian HK isoforms. Besides HK-1, an isoenzyme found in all mammalian cells, tumor cells predominantly express HK-2. Expression studies revealed an approximately 100-fold increase in the mRNA levels for HK-2. The prominent role of HK-2 for the accomplishment of the Warburg effect has been demonstrated by Wolf et al. who found that inhibition of HK-2, but not HK-1, in a human glioblastoma multiforme resulted in the restoration of normal oxidative glucose metabolism with decreased extracellular lactate and increased O2 consumption. Both HK-1 and HK-2 are high affinity enzymes with Km values for glucose of about 0.1 mm.

Succinate dehydrogenase (SDH): Mutations in TCA cycle enzymes can lead to tumorigenesis. Mutations of the Succinate dehydrogenase (SDH) and the fumarate
hydratase (FH) have been shown to result in paragangliomas and pheochromocytomas. The succinate dehydrogenase complex assembly factor 2 (SDHAF2/SDH5), responsible for the incorporation of the co-factor FAD into the functional active SDH, was recently shown to be a paraganglioma-related tumor suppressor gene.[30] FH mutations have been found in cutaneous and uterine leiomyomas, leiomyosarcomas and renal cell cancer.[31] A similar mechanism was proposed for the consequences of FH deficiency: accumulating fumarate can act as a competitive inhibitor of PHD leading to a stabilization of HIF-1.[32] The animals treated with Quercetin restored or maintained the Succinate dehydrogenase levels.

Cytochrome p450: Cytochrome P450 (CYP) is a multi-gene superfamily of heme-containing enzymes catalyzing the oxidative metabolism of many compounds.[33] CYP families 1, 2, and 3, which are the main CYP families participating in the metabolism of xenobiotics, are highly expressed within the liver.

High expression levels of various CYPs have been found previously in many tumors. In addition to biotransformation of carcinogenic compounds, CYPs have also been suggested to convert endogenous substrates to metabolites that facilitate cancer development and to participate in the metabolism of anticancer drugs. Of the different CYP isoforms, over expression of CYP1B1 is the one most often detected in various tumors.[34] The animals treated with Quercetin restored or maintained the Cytochrome p450 levels.

In conclusion, our present study demonstrated that, Quercetin was found to be potent antioxidant, antiproliferative agent against in vivo liver cancer. The Quercetin significantly acted as a free radical scavenging agent.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.

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