



RESEARCH ARTICLE



Received on: 05-11-2014  
Accepted on: 30-11-2014  
Published on: 15-12-2014

**Ogundajo A.T**  
Department of Biochemistry, Federal  
University of Technology, Akure, Ondo  
State, Nigeria,  
Email: adetap@yahoo.com



QR Code for Mobile users

Conflict of Interest: None Declared !

DOI: 10.15272/ajbps.v4i38.629

## Quercetin Potentiates Hepatoprotective and Antioxidant Response to Intraperitoneal, Intravenous, Subcutaneous and Oral Administration in Wistar Rats

Ogundajo A.T<sup>1,2\*</sup>, Imoru J.O<sup>3</sup> Asaolu F.M<sup>4</sup>

<sup>1</sup>Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria

<sup>2</sup> Department of Chemical Pathology, Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State, Nigeria.

<sup>3</sup>Department of Physiology, University of Ibadan, Ibadan, Oyo State, Nigeria.

<sup>4</sup>Department of Biochemistry, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria.

### Abstract

This study investigated the hepatoprotective and antioxidant response to intraperitoneal, intravenous, subcutaneous and oral treatment with quercetin in adult male Wistar rat. The animals were divided randomly into the four groups according to the routes of administration. Quercetin was allowed to react with selected hepatic isoenzymes (aspartate amino transferase, alanine amino transferase) via administration of quercetin at a dose of 50 mg/kg (once/day) for 5 days through different routes (oral, intravenous, intraperitoneal, subcutaneous) to adult male Wistar rats. The plasma hepatic enzyme activities and non-enzymatic antioxidant indices (total phenol, flavonoids, and vitamin C) were quantified spectrophotometrically. *Ex vivo* study showed that the enzymatic activity of AST and ALT was significantly decreased ( $p < 0.001$ ) irrespective of the routes of administration when compared with the standard (control). The plasma non-enzymatic antioxidant indices like phenol, flavonoid except vitamin C were all significantly increased ( $p < 0.001$ ) irrespective of the routes of administration when compared with the standard (control). Parenteral routes of administration showed significant hepatoprotective and antioxidant response compared to the oral route of administration. Quercetin has more capacity to initiate tissue repair, or minimize cellular damage or injury that could lead to hyperactivity of the hepatic enzymes, and enhance the body antioxidant defence mechanism through the parenteral routes of administration.

### Cite this article as:

Ogundajo A.T, Imoru J.O Asaolu F.M. Quercetin Potentiates Hepatoprotective And Antioxidant Response to Intraperitoneal, Intravenous, Subcutaneous and Oral Administration In Wistar Rats. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (38); 2014

## INTRODUCTION

Quercetin, a naturally occurring flavonoid, has been shown to exert multiple pharmacological effects and to be an anticancer agent or a supplementary anticancer agent. Flavonoids are found in many plants. Various pharmacological activities of flavonoids have been studied extensively <sup>(1,2)</sup>. It has been shown that quercetin (3, 3', 4', 5, 7- pentahydroxyflavone) inhibits the growth of cancer cell lines <sup>(3)</sup> and of acute lymphoid and myeloid leukemia cells <sup>(4)</sup>.

The antiproliferative activity of this compound could be mediated by type II EBSt <sup>(5)</sup> and PKC <sup>(6)</sup>. It has been reported that quercetin markedly inhibits the growth of human gastric and colon cancer cells by inhibiting cell cycle progression at the G<sub>1</sub>-S boundary [IZ, 131, and that quercetin arrests the cell cycle in late G<sub>1</sub>, prior to the beginning of the S phase in T-cells <sup>(7)</sup> and at the G<sub>1</sub>/M phase in human MDA-MB468 breast carcinoma cells.

Also, researches have shown that quercetin effectively inhibits the metal-induced lipid peroxidation reactions in rat liver microsomes, catalyses the formation of the hydroxyl-radical from H<sub>2</sub>O<sub>2</sub> in the presence of Fe<sup>3+</sup>-EDTA at pH 7.4, and promotes the bleomycine dependent destruction of DNA in the presence of Fe<sup>3+</sup> <sup>(8)</sup>. Thus, quercetin exhibits pro-oxidant or antioxidant properties, this being determined by the conditions of the experiment and by the properties of the substrate being oxidized. Quercetin as well as myricetin and delphinidin were shown to promote the destruction of the erythrocyte membranes by the superoxide-anion <sup>(9)</sup>. Cytotoxicity of some flavonoids including quercetin on the human leukemia cells HL-60 is thought to be due to its pro-oxidant action <sup>(10)</sup>.

Until recently, quercetin has been marketed in the United States primarily as a dietary supplement <sup>(11)</sup>, with recommended daily doses of supplemental quercetin of 200–1200 mg <sup>(12)</sup>. Based on the specified use-levels of quercetin in foods such as breakfast cereals, chewing gum, fats and oils, frozen dairy desserts and mixes, grain products and pastas, hard and soft candies, milk and plant protein products, beverages and beverage bases, and processed fruits and fruit juices, it was calculated that under a worst-case scenario of estimating intake, a heavy-end consumer of quercetin (90th percentile) would not be exposed to more than 4.70 mg quercetin/kg body weight/day (226 mg quercetin/ person/day) from the intended addition of quercetin to foods. In Japan, quercetin is permitted as a food additive under the list of Existing Food Additives <sup>(13)</sup>.

Okamoto provided an extensive overview of the safety data available for quercetin<sup>(14)</sup>. Presently, the bioavailability and anti-oxidant properties of quercetin appear to be two areas of intense research, specifically

the validation of quercetin as a potent anti-oxidant in vivo. But realizing its potential for pro-oxidant activity following oxidation is also of prime interest in an effort to determine its clinical applicability and acceptability for use in food. This work investigated hepatoprotective and antioxidant response to intraperitoneal, intravenous, subcutaneous and oral administration of quercetin in order to evaluate the bioavailability through the different routes.

## MATERIALS AND METHODS

### Chemicals,

Reagent kits for ALT, AST, were obtained from Randox laboratory, USA. Quercetin was obtained from Sigma-Aldrich (Germany). All other chemicals used were of analytical grade and obtained from FLUKA, BDH (Germany) and other standard commercial suppliers.

### Animals,

Male adult Wistar rats (200–250 g) obtained from the animal facility of the Department of Biochemistry of the Federal University of Technology, Akure, Nigeria were used in this study. Animals were maintained with food and water ad libitum and under a 12-h light/12-h dark cycle. The “principle of laboratory animal care” (National Institute of Health-NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised, 1985). The Ethical Committee of the Faculty Postgraduate Committee, Faculty of Science, Federal University of Technology, Akure approved the research work.

### Administration of quercetin to experimental animal,

The animals were divided randomly into the following groups: group 1 (IP, intraperitoneal); group 2 (IV, intravenous); group 3(OR, oral) and group 4(SC, subcutaneous). Groups 1, 2, 3 and 4 were administered quercetin at a dose of 50 mg/kg (once/day) for 5 days. Quercetin was pre-dissolved in ethanol. A standard (Control) for comparison was prepared to compare with the tests groups.

### Tissue preparation for experiment,

The animals were sacrificed 24 hours after the last administration by cervical dislocation before dissection for tissue collection. The blood of each animal was collected by cardiac puncture to sample bottle containing lithium heparin as anticoagulant. The blood collected was centrifuged at 4,800rpm using bench centrifuge carefully balanced for 5 minutes, and the supernatant (plasma) was collected, labeled and frozen prior to analyses.

### Assay of enzyme activity,

#### Aspartate Aminotransferase,

α-ketoglutarate reacts with L-aspartate in the presence of aspartate aminotransferase (AST) to form L-glutamate and oxaloacetate. The indicator reaction

utilizes the oxaloacetate for a kinetic determination of NADH consumption (15). The samples (0.2ml) and 2ml of the reagent were pipette into labeled test tubes and were mixed thoroughly. The initial absorbance was taken at 1 min was later read at 2 and 3 minutes at 540 nm using Randox AST standard to standardized. All determinations were performed in triplicate.

#### Alanine Aminotransferase,

a-ketoglutarate reacts with L-alanine in the presence of alanine aminotransferase (ALT) to form L-glutamate and pyruvate. The indicator reaction utilizes the pyruvate for a kinetic determination of NADH consumption (15). The samples (0.2ml) and 2ml of the reagent were pipetted into labeled test tubes and were mixed thoroughly. The initial absorbance was taken at 1 min after which it was read again at 2 and 3 minutes at 540nm using Randox ALT standard to standardize. All determinations were performed in triplicate.

#### Antioxidant defence systems,

##### Vitamin C content:

Vitamin C is estimated by the method of Omaye *et al* (16). Vitamin C is oxidized by copper to form dihydroascorbic acid and diketogluconic acid. When treated with 2,4, dinitrophenyl hydrazie, it reacts to form the derivative bis 2,4, dinitrophenyl hydrazine. This compound in strong sulphuric acid undergoes a rearrangement to form a product with an absorption band that is measured at 520 nm. 1.0 ml of plasma made up to 3.0 ml with 5% TCA and then, treated with 0.2 ml of DTC and incubated for 3 hrs at 37°C. Then 1.5 ml of icecold 65% sulphuric acid was added, mixed and kept at room temperature for an additional 30 minutes. Blank contained only 3.0 ml of TCA. Standards in the range of 10-50pgms were treated in the same way. The intensity of the color was measured at 520 nm. Results were expressed as micro gm of ascorbic acid/ mg protein. All determinations were performed in triplicate.

##### Total phenol estimation,

The content of total phenolic compounds in protein free plasma was determined by Folin-Ciocalteu method (17). For the preparation of calibratio curve, 1 ml aliquots of 0.024, 0.075, 0.105 and 0.3 mg/ml ethanolic gallic acid solutions were mixed with 5 ml Folin-Ciocalteu reagent (diluted ten-fold) and 4 ml (75g/l) sodium carbonate. The absorption was read after 30 min at 20°C at 765 nm and the calibration curve was drawn. One ml of protein free plasma was mixed with the same reagents as described above, and after 1 h the absorption was measured. All determinations were performed in triplicate.

##### Flavonoid estimation,

The content of flavonoid was determined using quercetin as reference compound. Briefly, 500µl of protein free plasma of treated animals was mixed with

50µl of Aluminum tri chloride and equal volume of potassium acetate. The absorbance was read at 415nm after 30mins of incubation at room temperature. Standard quercetin solutions were prepared from 0.01g quercetin dissolved in 20ml of distilled water. All analyses were carried out in triplicate. The amount of flavonoid in plasma was expressed as quercetin equivalent.

##### Statistical analysis,

Results are expressed as mean  $\pm$  SEM. Statistical difference was determined by one-way analysis of variance (ANOVA) followed by a post hoc test (Student Newman-Keuls Test (SNK)). Difference between means was considered statistically significant with  $p < 0.05$ . Computer software Graph pad PRISM® version 3.00 was used for the analysis.

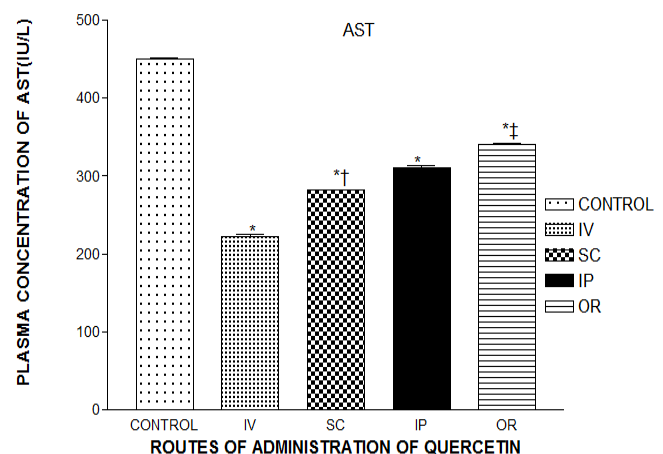
## RESULTS

### Hepatoprotective enzymatic response to quercetin administration,

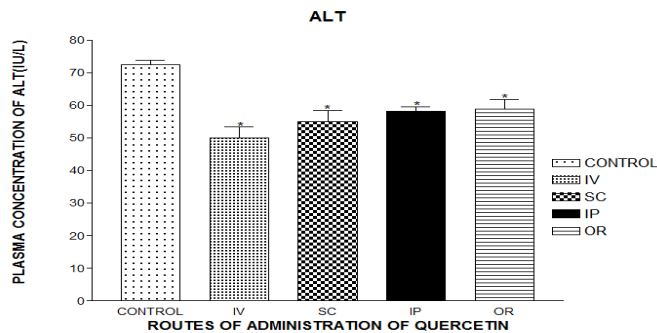
Quercetin administered through different routes of administration caused significant decrease in plasma activity of both AST ( $P < 0.001$ ) and ALT ( $P < 0.001$ ) compared to standard (Control). It was however noted, that parenteral administration caused significant decrease ( $P < 0.001$ ) in the activity of these enzyme compared to oral administration (Figs. 1 and 2).

### Antioxidant response to quercetin administration,

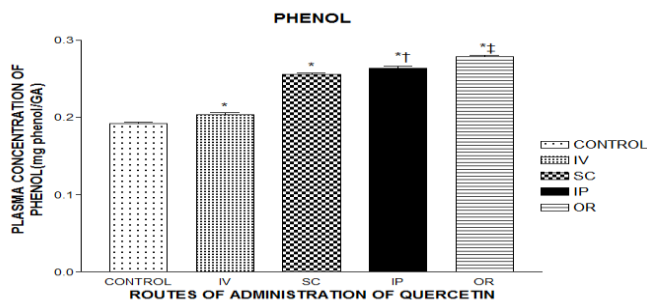
There was significant increase ( $P < 0.001$ ) in plasma antioxidant factors (Phenol and flavonoid) when quercetin was administered though the different routes compared to standard (Control), except for vitamin C which showed a significant decrease (0.001) compared to standard (Control). Parenteral routes of administration showed significant increase in plasma antioxidant factors (i.e. phenol and flavonoid) compared to the oral route (figs. 3, 4 and 5).



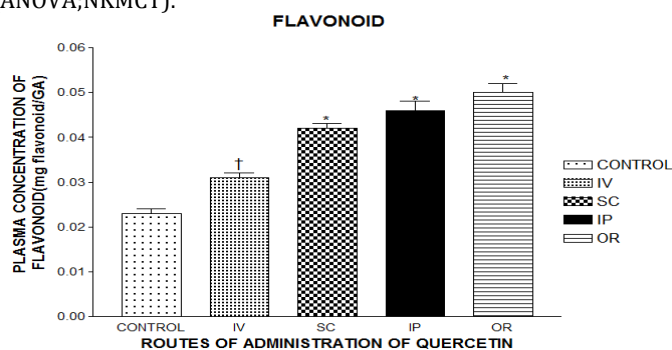
**Figure 1:** Effect of quercetin on rat plasma AST level. Each bar is expressed as Mean  $\pm$  SEM of level of total phenol by routes of administration; (n=5). \* =  $p < 0.001$  compared with control, and \*† =  $p < 0.001$  compared with intraperitoneal route of administration (ANOVA; NKMCT).



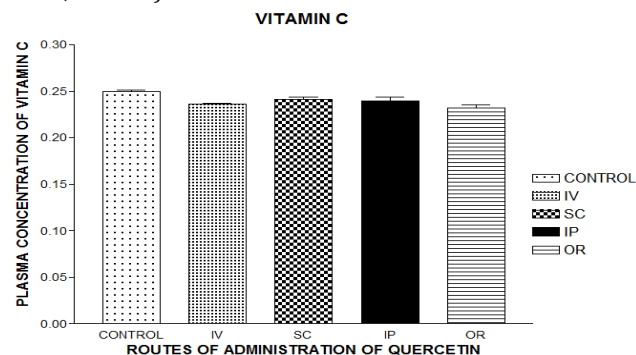
**Figure 2:** Effect of quercetin on rat plasma Vitamin C level. Each bar is expressed as Mean  $\pm$  SEM of level of t by routes of administration; (n=5). \* =  $p < 0.001$  compared with control. (ANOVA; NKMCT).



**Figure 3:** Effect of quercetin on rat plasma total phenol level. Each bar is expressed as Mean  $\pm$  SEM of level of total phenol by routes of administration; (n=5). \* =  $p < 0.001$  compared with control, and  $\dagger = p < 0.001$  compared with intraperitoneal route of administration (ANOVA; NKMCT).



**Figure 4:** Effect of quercetin on rat plasma flavonoid level. Each bar is expressed as Mean  $\pm$  SEM of level of flavonoid by routes of administration (n=5). \* =  $p < 0.001$  compared with control, and  $\dagger = p > 0.05$  compared with IV, SC, OR and IP route of administration (ANOVA; NKMCT).



**Figure 5:** Effect of quercetin on rat plasma Vitamin C level. Each bar is expressed as Mean  $\pm$  SEM of level of t by routes of administration; (n=5). \* =  $p < 0.001$  compared with control. (ANOVA; NKMCT).

## DISCUSSION

This study showed that administration of quercetin to rats through various routes inhibits the activities of AST and ALT in the plasma ( $p < 0.001$ ) with highest inhibition in intravenous route for both enzymes when compared with standard control which support the report that credited quercetin as an enzyme inhibitor<sup>(18-20)</sup> (Figs. 1 and 2). The above suggest that quercetin may exhibit numerous biological and pharmacological effects which may include anti-carcinogenic<sup>(21-23)</sup>. Ability of quercetin to inhibit hepatic enzyme activities (AST/ALT) which usually increased in hepatic degeneration confers a hepatoprotective property on quercetin.

The plasma concentration of both flavonoid and phenol when quercetin was administered via various routes to rats was found to be significantly increased ( $p < 0.001$ ) irrespective of the routes when compared with standard control (Figs. 3 and 4) which support other findings that quercetin is a good source of antioxidant such as flavonoid and phenol which may be responsible for its protective action against breast, lung, liver, ovarian and colon cancer<sup>(24)</sup>, and other health related benefit such as antiviral, and anti-inflammatory activities<sup>(25, 26)</sup>

Phenolic and flavonoid content of quercetin are believed to account for a major portion of its antioxidant activity. Some studies have demonstrated a linear correlation between content of total phenolic compounds and antioxidant<sup>(27)</sup>. Plasma concentration of vitamin C on the other hand showed no significant changes when compared with the standard control irrespective of routes of administration of quercetin (Fig. 5), this may suggest non effect of quercetin on the physiological vitamin C content being a dietary non enzymatic antioxidant.

The results however, showed that administration of quercetin through the parenteral routes caused significant changes in the hepatoprotective and antioxidant response compared to the oral route. This could be said to be due to poor absorption of quercetin in the small intestinal when administered through the oral route<sup>(28)</sup>

As antioxidants, polyphenols such as quercetin may improve cell survival; as pro-oxidants, they may induce apoptosis and prevent tumor growth<sup>(28)</sup>. However, the biological effects of polyphenols may extend well beyond the modulation of oxidative stress.

In conclusion, Quercetin has more capacity to initiate tissue repair, or minimize cellular damage or injury that could lead to hyperactivity of the hepatic enzymes, and enhance the body antioxidant defense mechanism through the parenteral routes of administration'.

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