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In vitro and *In vivo* Effect of Quercetin on Hepatocyte Transmembrane N^+, K^+- ATPase Activity in Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author ATO designed the study, wrote the protocol, carried out all laboratories work and wrote the first draft of the manuscript. Author JOI managed the literature searches, performed the statistical analysis and wrote the final draft of the manuscript. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This study investigated *In vitro* and *In vivo* effect of quercetin on transmembrane hepatocyte Na^+, K^+- ATPase activity in normal Wistar rats.
Study Design: One factor experimental design.
Place and Duration of Study: Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria, between April and September 2010.
Methodology: *In vitro* effect of quercetin on transmembrane hepatocyte Na^+, K^+-ATPase activity was investigated through enzyme assay by measuring the released inorganic phosphate (Pi) through the method of Fiske and Subbarow, while in *In vivo* experiment, three (3) main groups of male Wistar rats weighing 200-250 g (n=5) were randomly selected. Group 1 (IP; Intraperitoneal), group 2 (IV; Intravenous) and 3 (OR; Oral) group. All three groups were administered with quercetin 50 mg/kg body weight (once/day) for 5 days through respective routes. The control for

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each group received equal volume of vehicle through respective routes of administration. Hepatocyte Na^+, K^+-ATPase activity for each group was assayed after days of quercetin administration.

Results: *In vitro* results indicated that quercetin, with IC_{50} of 5.75 µm showed high inhibitory activity on Na^+, K^+-ATPase, while *in vivo* result showed inhibitory activity on hepatic Na+ K+-ATPase irrespective of the routes of administration. Quercetin through the oral route showed significant ($F_{5, 12}$= 265.8; P=0.05) inhibitory effect on hepatic Na^+ K^+-ATPase compared to control. Administration of quercetin though the parenteral routes (intraperitoneal and intravenous) showed significant ($F_{5, 12}$= 265.8; P<0.001) inhibitory effect compared to respective control. The parenteral routes of administration of quercetin have significant ($F_{5, 12}$= 265.8; P<0.001) inhibitory effect on hepatic Na^+ K^+-ATPase compared to the oral route

Conclusion: Quercetin showed high inhibitory effect *In vitro* on hepatocyte Na^+, K^+- ATPase activity in normal rats. *In vivo* administration of quercetin showed significant inhibitory effect on hepatocyte Na^+, K^+- ATPase activity in normal rats, with the parenteral routes of administration exerting significant inhibitory effect compared to the oral route.

Keywords: Quercetin; hepatocyte Na^+; K^+-ATPase; In vitro; In vivo.

1. INTRODUCTION

Ubiquitous transmembrane enzyme, Na^+, K^+-ATPase, also Known as sodium pump transports Na^+ and K^+ across the plasma membrane by hydrolyzing ATP [1]. Like the H^+, K^+-ATPase, Na^+, K^+-ATPase belongs to the P-type ATPases. It is composed of majorly two subunits α and β. The catalytic α subunit is a large membrane protein approximately between 110,000–113,000 Da, and it contains the binding sites for ATP, sodium, potassium, Phosphate and cardiac glycoside [2,3], while the β subunit with approximately 35,000 to 55,000 Da is assumed to be necessary for the activity of the complex [4]. Of the four (4) α isoforms that have been identified in mammals with tissue–specific pattern of expression, the α1 isoform is however ubiquitous ad has been identified in hepatocytes in the basolateral and bile canalicular membranes [5,6].

The Na^+, K^+-ATPase plays an important role in the control of the ionic intracellular milieu, this process is needed for the maintenance of the characteristic intracellular concentration of sodium and potassium, and to determine the transmembrane electrochemical gradients for these ions. It is also needed for regulation of metabolism, proliferation, differentiation and most importantly cell volume. It is a common belief that the electrochemical sodium gradient provides the driving force for secondary active sodium-coupled transport of a variety of solutes, including certain amino acids and bile, and also drives the activity of many other transporters like Na^+, H^+ exchanger and Na^+-K^+2Cl^- cotransporter involved in the regulation of intracellular pH and cell volume respectively [7]. Knowing that apoptotic cells lose K^+, and gain Na^+ in a process that involves the Na^+, K^+-ATPase underpins the significance of this ubiquitous membrane protein in both physiologic and pathologic states [8,9]. It has been hypothesized that variations in hepatocyte ATP might be transduced into neural signaling by modulating Na^+, K^+-ATPase activity, in that, decrease in hepatocyte ATP which reduce the activity of Na^+, K^+-ATPase, thus depolarizing the cell which in turn would affect hepatic neuronal activity through close coupling or release of a neuromodulator. This is evidenced by the observation that feeding behaviour in rats is stimulated by intraperitoneal or hepatic portal administration of ouabain, an inhibitor of the Na^+, K^+-ATPase [10-12].

Quercetin (3,3'4',5,7-pentahydroxyflavone) is one of several naturally-occurring dietary flavonoids compounds, ubiquitously found in fruits, vegetables, herbs, tea, wine, etc. Quercetin belongs to the group of flavonoids, and generally, flavonoids are characterized by a phenyl benzo (γ) pyrone-derived structure consisting of two benzene rings (A and B) linked by a heterocyclic pyran or pyrone ring [13]. In plants, the flavonol aglycone is most commonly present conjugated at the 3- position of the unsaturated C-ring with a sugar moiety, forming O-β-glycosides such as quercitrin or rutin [14]. Quercetin can be obtained from plants via extraction of the quercetin glycosides followed by hydrolysis to release the aglycone and subsequent purification. Flavonols exhibit numerous biological and pharmacological effects, including anti-oxidant, chelation, anti-carcinogenic, cardioprotective, bacteriostatic, and secretory properties [13]. The biological

action of quercetin is connected with its antioxidant properties which are mainly due to: (1) Its ability to scavenge free radicals and reactive oxygen species (ROS) (superoxide anion, hydroxyl-radical) [15], (2) To form complexes with metal ions, thus preventing oxidation of the metals with oxygen yielding ROS (Haber–Weiss and Fenton reactions) [16,17]; and (3) Reduction of Fe^{3+} to Fe^{2+} [18]. These mechanisms explain the inhibition of the lipid peroxidation reactions by quercetin [19-20] and its ability to prevent deoxyribose degradation [18]. The mechanism(s) contributing to the health beneficial effects of quercetin involve the quenching of free radicals, elevating antioxidant status, and membrane modulating effects [21]. Quercetin has shown a potent effect at membrane level modulating the activity of membrane transport processes of erythrocyte's Na^+, K^+-ATPase and Na^+, H^+ antiport in diabetic patients [22].

Our previous researches showed that quercetin elevated antioxidant status and modulated Na^+, K^+-ATPase in rat brain [23], and also potentiated hepatoprotective and antioxidant response to intraperitoneal, intravenous, subcutaneous and oral administration in Wistar rats [24]. Meanwhile, a recent work by Kairane et al. [25] showed that genistein, an exogenous antioxidant like quercetin, has an inhibitory effect on Na^+, K^+-ATPase in normal brain, but improve the activity of this pump in Alzheimer's disease brain. Since our prior works showed inhibitory effect of quercetin on cerebral Na^+, K^+-ATPase, but antioxidant effect in both the brain and liver in rats, we have taken to investigating the effect of quercetin on hepatic Na^+, K^+-ATPase in normal rats.

2. MATERIALS AND METHODS

2.1 Chemicals

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.

2.2 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, 5). The Ethical Committee of the Faculty Postgraduate Committee, Faculty of Science, Federal University of Technology, approved the research work.

2.3 *In vitro* Effect of Quercetin on Na^+, K^+-ATPase Activity

Immediately after the animals were euthanized, the liver was quickly removed and the homogenate was prepared in 0.05 M Tris–HCl, pH 7.4. The homogenate was centrifuged at 4,000 rpm at 4°C for 7 min and the supernatant was used for the assay of Na^+, K^+-ATPase. The reaction mixture for Mg^{2+}-dependent- Na^+, K^+-ATPase activity assay contained 3 mM $MgCl_2$, 125 mm NaCl, 20 mm KCl, 200 mM sodium azide and 50 mm Tris–HCl, pH 7.4 and 100–120 µg of protein, in a final volume of 500 µl. The reaction was initiated by addition of ATP to a final concentration of 3.0 mm. Controls were carried out under the same conditions with the addition of 0.1 mM ouabain [26,27]. Na^+, K^+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphorous (Pi) was measured by the method of Fiske and Subbarow [28].

2.4 *In vivo* Experiment

The animals were divided randomly into the following groups (n=5 per group): group 1 (IP, intraperitoneal); group 2 (IV, intravenous); group, and 3 (OR, oral) group. Groups 1, 2 and 3 were administered quercetin at a dose of 50 mg/kg (once/day) for 5 days. Quercetin was pre-dissolved in ethanol. Control for each group received equal volume of vehicle through respective route of administration.

2.4.1 Tissue Preparation for in- vivo Experiment

Animals were anesthetized with ether and euthanized by decapitation and whole liver were quickly removed, placed on ice and homogenized in cold 50 mMTris–HCl pH 7.4. The homogenate was centrifuged at 4,000 g for 10 min to yield the low-speed supernatant (S2). For all analyses, protein content was determined

by the method of Lowry et al. [29], using bovine serum albumin as the standard.

2.4.1.1 *In-vivo effect of quercetin on Na^+, K^+-ATPase activity*

The assay was carried out as described earlier for in-vitro assay. Released inorganic phosphorous (Pi) was measured by the method of Fiske and Subbarow [28].

2.5 Statistical Analysis

The results of the *In vivo* experiment are expressed as mean±SEM and statistical difference was determined by Student Newman Keul's Test after a one-way analysis of variance (ANOVA) was done. The level of significant was set at 5% ($P < 0.05$) for all treatment carried out. For the *in vitro* experiment, IC_{50} was calculated with the aid of Adobe® Page Maker® 7.0 from graphs plotted with the aid of Graph pad PRISM® version 3.00.

3. RESULTS AND DISCUSSION

3.1 *In vitro* Effect of Quercetin on Na^+, K^+-ATPase Activity

The *In vitro* result in this study showed that quercetin, like ouabain a potent inhibitor of Na^+, K^+-ATPase, has an inhibitory effect on the hepatocyte Na^+, K^+-ATPase activity (see Fig. 1). While the IC_{50} for ouabain was 3.63 µm, the IC_{50} for quercetin was 5.75 µm. These indicate, as noticed in our previous research [23], that quercetin showed inhibitory activity in both normal liver and brain Na^+, K^+-ATPase. However, using Rukunga and Simon's classification of activity of some natural products [30], both ouabain and quercetin (with IC_{50} 3.63 µm and 5.75 µm respectively) have high inhibitory activity on hepatic Na^+, K^+-ATPase activity. Both ouabain and quercetin showed a concentration-dependent inhibitory activity on hepatic Na^+, K^+-ATPase activity. Genistein, an exogenous antioxidant like quercetin, has been shown to have inhibitory effect on Na^+, K^+-ATPase activity in normal brain, but improved the activity of Na^+, K^+-ATPase in Alzheimer disease (AD) brain [25]. The speculation to genistein affecting Na^+, K^+-ATPase activity in control and AD brains in opposite directions was said to be that Na^+, K^+-ATPase is redox sensitive. That whereas shifts both into a more reduced and oxidized states cause the suppression of Na^+, K^+-ATPase activity in control brain, the antioxidant activity of genistein may change redox balance back towards the reduced state and thus helping to improve Na^+, K^+-ATPase activity in the AD brain. We have shown in our previous work [24] that quercetin showed hepatoprotective and antioxidant effects in normal rat liver. Thus quercetin, an antioxidant like genistein, showing inhibitory effect on normal hepatocyte Na^+, K^+-ATPase might achieved this, like genistein, by shifting both into a more reduced and oxidized state to cause inhibitory activity on normal hepatocyte Na^+, K^+-ATPase. We used normal hepatocyte Na^+, K^+-ATPase in this study, a further work will be necessary to investigate the effect of quercetin on Na^+, K^+-ATPase in a diseased liver.

3.2 *In vivo* Effect of Quercetin on Na^+, K^+-ATPase Activity

This study also investigated the effect of *In vivo* (oral, intraperitoneal and intravenous) administration of quercetin on hepatic Na+ K+-ATPase activity. The results indicated that quercetin showed inhibitory activity on hepatic Na^+ K^+-ATPase *In vivo* irrespective of the routes of administration. Quercetin through the oral route showed significant ($F_{5, 12}$= 265.8; P=0.05) inhibitory effect on hepatic Na^+ K^+-ATPase activity compared to control. Administration of quercetin though the parenteral routes (intraperitoneal and intravenous) showed significant ($F_{5, 12}$= 265.8; P<0.001) inhibitory effect compared to respective control. The parenteral routes of quercetin administration have significant ($F_{5, 12}$= 265.8; P<0.001) inhibitory effect on hepatic Na^+ K^+-ATPase activity compared to the oral route (see Fig. 2). The pattern of inhibition exhibited in this study between the oral and parenteral routes, has also been seen in the hepatoprotective and antioxidant effects of quercetin in normal rats [24]. This has been attributed to poor absorption of quercetin in the small intestine when administered through the oral route [30-32].

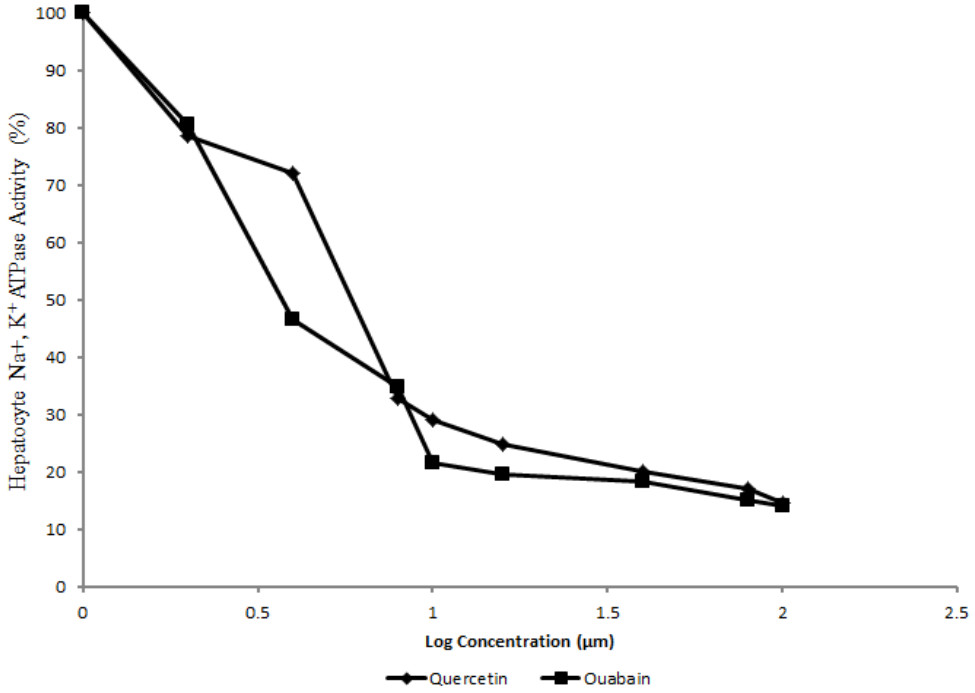

Fig. 1. Na^+, K^+-ATPase inhibitory activity of ouabain compared with quercetin in rat liver

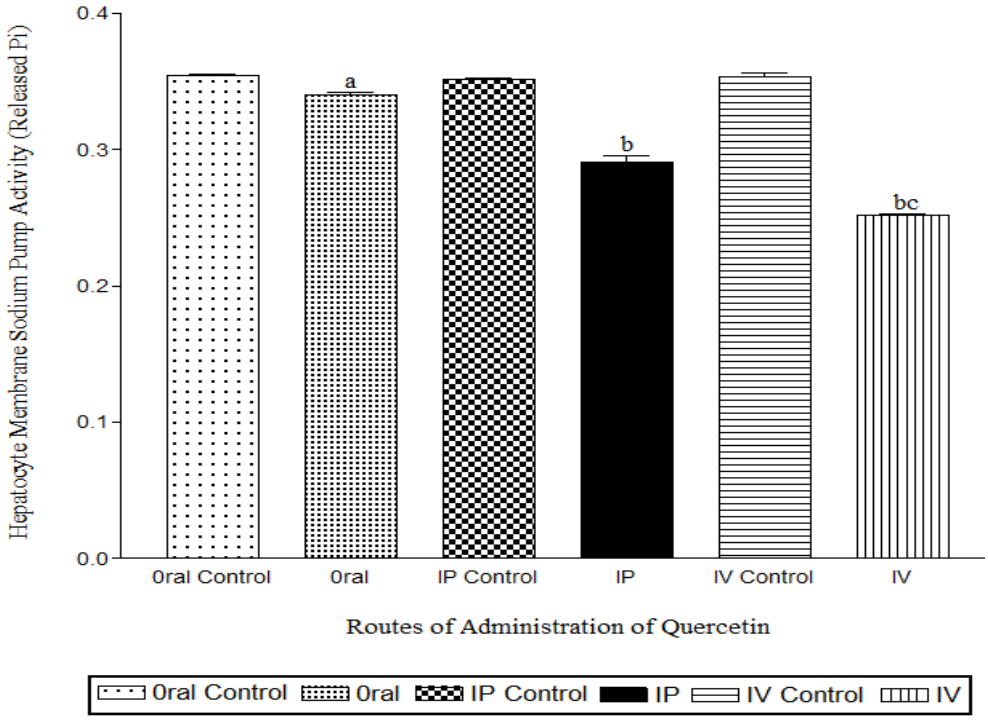

Fig. 2. Effect of quercetin on hepatocyte Na+, K+- ATPase activity; comparing different routes of administration

Each bar is expressed as Mean±SEM of released inorganic phosphate (Pi) (n=5 per group).[a] P=0.05 compared to oral control; [b] P<0.001 compared to oral and [c] P<0.001 compared to intraperitoneal

4. CONCLUSION

Quercetin showed high inhibitory effect *In vitro* on hepatocyte Na^+, K^+- ATPase activity in normal rats. *In vivo* administration of quercetin showed significant inhibitory effect on hepatocyte Na^+, K^+- ATPase activity in normal rats, with the parenteral routes of administration exerting significant inhibitory effect compared to the oral route.

ETHICAL APPROVAL

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, 5). The Postgraduate Ethical Committee of the School of Science, Federal University of Technology, approved the research work.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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