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Quercetin elevates antioxidant status and modulates ouabain-sensitive transmembrane sodium pump in rat brain

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Abstract
This study investigated *in vivo* antioxidant effect of quercetin in normal rat brain and *in vitro* effect of quercetin on cerebral sodium pump. Brain antioxidant factors (phenol, flavonoid, TBARS, Vitamin C and GSH) of normal male Wistar rats were quantified spectrophotometrically, while *in vitro* effect of quercetin on cerebral sodium pump was investigated through enzyme assay by measuring the of phosphate (Pi). The results showed that *in vivo* administration of quercetin significantly ($P<0.001$) increased brain's phenol and flavonoid contents and significantly ($P<0.001$) decreased TBARS irrespective of the routes of administration. Intraperitoneal route caused significant ($P<0.001$) antioxidant effect compared to oral route of administration. *In vitro* investigation showed quercetin (IC_{50} 73.68 µm) like ouabain (IC_{50} 12.21 µm) as a moderate inhibitor of cerebral sodium pump. Its inhibitory effect can be modulated when the incubating medium was altered. Quercetin elevates cerebral antioxidant factors and modulates cerebral sodium pump in normal rats.

Keywords: Quercetin, Ouabain, Routes of Administration, Antioxidant Effect, Cerebral Sodium Pump Inhibitor.

1. Introduction
The Na^+, K^+-ATPase is also known as the sodium pump, is a ubiquitous transmembrane enzyme that transports Na^+ and K^+ across the plasma membrane by hydrolyzing ATP [1]. The energy of ATP hydrolysis is used to maintain Na^+ and K^+ gradients across the cell membrane. The Na^+ gradient plays an essential role in providing the energy for the activity of Na^+-dependent transporters as well as in specialized cellular functions such as muscle contraction or propagation of action potentials in excitable tissues. Together with the Na^+ gradient, the K^+ gradient essentially is involved in preserving the cell volume and membrane potential, and its maintenance of crucial importance for normal electrical signaling and to prevent potential pathologies [2]. New concept is now evolving that Na^+, K^+-ATPase can function as a signal transducer independently of its function as an ion pump [3].

The Na^+, K^+-ATPase belongs to the family of P-type ATPase that transits between E_1 and E_2 conformational states during pumping cycles. It is a heterometric protein composed of α catalytic subunit that binds sodium and potassium ions, ATP and cardiac glycosides, β and γ (FXYD) subunits that can modulate substrate affinity. Different genes code for multiple α, β and γ isoforms. Four α isoforms have been identified, and all except α4 are expressed in the brain4. Modulation of Na^+, K^+-ATPase activity directly affects neurotransmitter signaling, neural activity, as well as whole animal behaviour. Na^+, K^+-ATPase inhibition by ouabain is known to decrease norepinephrine [5], dopamine, and serotonin (5-HT) uptake [6], and increase acetylcholine release [7]. Administration of ouabain or related Na^+, K^+-ATPase inhibitors alters neuronal firing [8] and impairs spatial and other forms of learning [9]. Norepinephrine has been reported in *in vivo* and *in vitro* studies to increase brain Na^+, K^+-ATPase activity [10]. Difference missense mutations in the ATP1A3 gene encoding the α3 subunit of Na^+/K^+-ATPase (NKA α3) have been seen to have caused two movement disorders: Alternating Hemiplegia of Childhood (AHC), characterized by episodes of transient hemiplegia/hemiparesis, dystonia and choreoathetosis [11], and Rapid Onset Dystoni-Parkinsonism (RDP), characterized by abrupt onset of dystonia with parkinsonism after a stressful event, typically in the late adolescent or early adulthood [12]. Analysis of the α2 isoform gene knocked out mice, showed that the animal died shortly after birth, but did not display obvious gross morphological defects in any tissue, including the brain. However, lack of motor activity was significant, but muscle contractility was not found to be critically impaired [13].

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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 [14]. 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 [15]. 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 [14]. 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) [16], (2) to form complexes with metal ions, thus preventing oxidation of the metals with oxygen yielding ROS (Haber–Weiss and Fenton reactions) [17, 18]; and (3) reduction of Fe^{3+} to Fe^{2+} [19]. These mechanisms explain the inhibition of the lipid peroxidation reactions by quercetin [20-21] and its ability to prevent deoxyribose degradation [19]. The mechanism(s) contributing to the health beneficial effects of quercetin involve the quenching of free radicals, elevating antioxidant status, and membrane modulating effects [22]. 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 [23].

In vitro studies have shown that quercetin has strong antioxidant properties. But researchers are not sure if *in vivo* administration of quercetin has the same effects [24]. This present study was carried out to investigate the effect of quercetin on brain membrane sodium pump, and its effects on the brain antioxidant status using different routes of administration.

2. Materials and Methods
2.1 Chemicals
Adenosine triphosphate (ATP), ouabain, cysteine, glutathione were 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, 1985). The Ethical Committee of the Faculty Postgraduate Committee, Faculty of Science, Federal University of Technology, approved the research work.

2.3 *In vivo* Effect of Quercetin on Rat Brain Antioxidant Status:
Male adult Wistar rats (200-250 g) were randomly divided

into the following groups: OR group (oral) and IP group (Intraperitoneal). These groups were administered with quercetin at a dose of 50 mg/kg once daily for five (5) days. Quercetin was pre-dissolved in ethanol. A standard for comparison was prepared to compare with the test groups. *In vivo* effects of quercetin on rat brain antioxidant status was determined through the different methods explained below.

2.3.1 Determination of Total Phenol using Folin-ciocalteau's Method
200 µl protein free supernatant of brain tissue homogenate with equal volume of water were pipette into a test tube. Folin-ciocalteau's reagent (2.5 ml) and 2 ml of 87.5% Na_2Co_3 were subsequently added and the absorbance was measured at 725 nm after incubating at 45 ^{0}C for 20 minutes [25]. Gallic acid was used as the standard and total phenol was calculated as mg phenol/GAE.

2.3.2 Determination of Total Flavonoid Content
500 µl of protein free tissue homogenate was mixed with $AlCl_3$ followed by 50 µl of potassium acetate. The absorbance was read at 415 nm, after 30 minutes of incubation at room temperature. Standard tannic acid dissolved in 20 ml of distilled water. All determinations were carried out in triplicate. The amount of flavonoid in brain tissue homogenate was expressed as tannic acid equivalent (TAE). The standard calibration curve was prepared by preparing tannic acid solution at concentration range of 10-100 µg/ml.

$$\text{Total flavonoid content} = \frac{\text{calibration factor x } A_{test}}{\text{Con. x dilution factor}}$$

2.3.3 Determination of Vitamin C Level
An aliquot of the protein free tissue homogenate (1ml) was incubated for 3 h at 38 ^{0}C then 1ml H_2SO_4 65% (v/v) was added. The reaction product was determined using a colour reagent containing 4.5 mg/ml dinitrophenylhydrazine and $CuSO_4$ (0.075 mg/ml) at 520 nm. The content of ascorbic acid is related to tissue amount (µmol ascorbic acid per gram wet tissue).

2.3.4 Determination of the Level of GSH
0.1M Phosphate buffer (850 µl), protein-free brain tissue homogenate (100 µl) were pipetted into a tube, mixed and 50µl of DTNB added. The absorbance of the mixture assay was measured at 420 nm immediately. A standard curve was prepared to cover the range of 10-100 µM [26]. The level of GSH was calculated as follows:

$$\mu M\ GSH = \frac{\text{calibration factor x } A_{test}}{\text{Conc. x dilution factor}}$$

2.3.5 Determination of *In-vivo* Lipid Peroxidation
The level of thiobarbituric acid reactive species (TBARS) production in the brain tissue homogenate in the presence of quercetin was determined as described by [27]. This is based on the reaction of chromogenic substrate and malondialdehyde. The production of TBARS was determined by expecting that the buffer of colour reaction have pH of 3.4. The colour reaction was developed by adding 3 µl of 8.1% SDS to the brain tissue homogenate, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 500 µl 0.8% thiobarbituric acid (TBA). The mixture was incubated at 95 ^{0}C for 1 h. TBARS produced were measured at 532 nm and the

absorbance was compared to that of a standard curve obtained using malondialdehyde (MDA).

2.3.6 *In vitro* Study of the Effect of Quercetin on Na$^+$/K$^+$-ATPase Activity

Animals were anesthetized with ether and euthanized by decapitation, the whole brain was quickly removed and placed on ice and the homogenate was prepared in 0.05 M Tris–HCl, pH 7.4. The homogenate was centrifuged at 4,000 rpm at 4 ^{0}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 100–180 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. Na$^+$/K$^+$-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphorous (Pi) was measured by the method of [28]. To check whether pre-incubation of homogenates without a cationic component of the assay medium will affect the interaction of quercetin with the Na$^+$/ K$^+$-ATPase activity, quercetin and enzyme were incubated at 37 ^{0}C for 10 min, with the selective exclusion of each Mg^{2+}, Na$^+$, K$^+$ in the pre-incubating medium. All the experiments were conducted at least three times and similar results were obtained. Protein was measured by the method of [29], using bovine serum albumin as standard. In order to establish a possible involvement of thiol oxidation in the interaction of quercetin with the enzyme, we either pre-incubated or post-incubated quercetin with the enzyme with the selective exclusion of either each of the cations or nucleotide necessary for the functioning of the sodium pump. For all enzyme assays, incubation times and protein concentration were chosen to ensure the linearity of the reactions. All samples were run in triplicate. Controls with the addition of the enzyme preparation after mixing with trichloroacetic acid (TCA) were used to correct for non-enzymatic hydrolysis of substrates. Enzyme activity was expressed as nmol of phosphate (P$_i$) released min^{-1} mg protein^{-1}.

2.4 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 significance 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
3.1 *In vivo* Effect of Quercetin on Rat Cerebral Antioxidant Status
### 3.1.1 Effect on Level of Cerebral Phenol
Quercetin administered both through the oral and intraperitoneal routes caused significant increase in cerebral phenol level ($F_{2, 12} = 528.6$; $P < 0.001$) compared to standard control, while increase in cerebral phenol level caused by administration of quercetin through intraperitoneal route was significant ($F_{2, 12} = 528.6$; $P < 0.001$) compared to oral route (Figure 1).
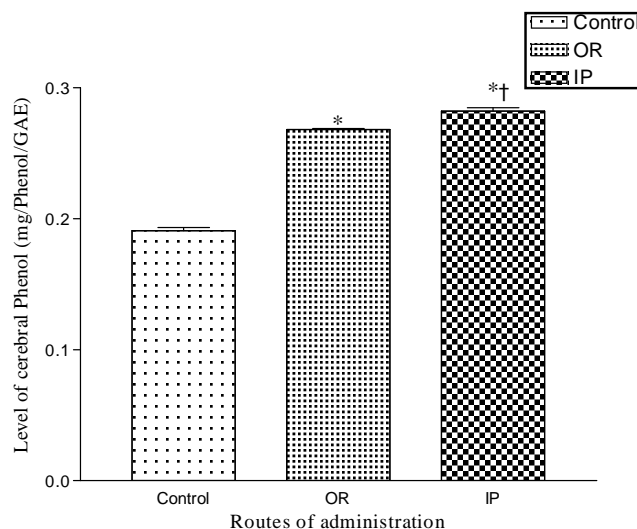
Fig 1: Effect of quercetin on level of total phenol in rat brain. Each bar is expressed as Mean ± SEM of level of total phenol by routes of administration; (n=5). * = p < 0.001 compared with control, and † = p <0.001 compared with oral route of administration (ANOVA; SNK).
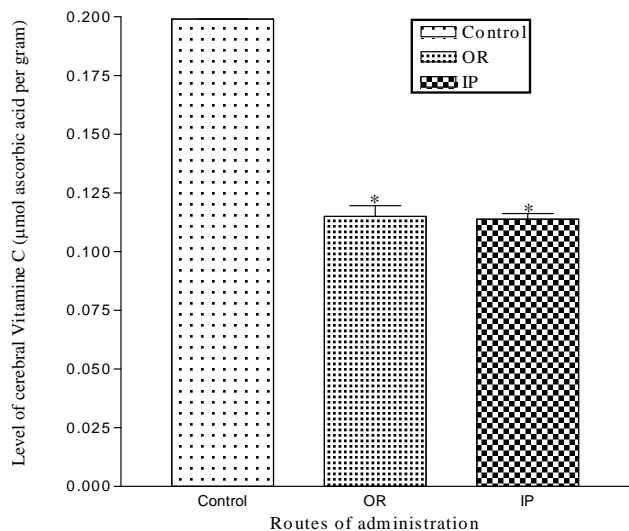
3.1.2 Effect on Level of Cerebral Flavonoid
Administration through the intraperitoneal route caused a significant increase ($F_{2, 12} = 106.8$; $P < 0.001$) in cerebral flavonoid compared to standard control and oral route of administration, while the increase observed in the oral route of administration as compared to the standard control was not significant (Figure 2).
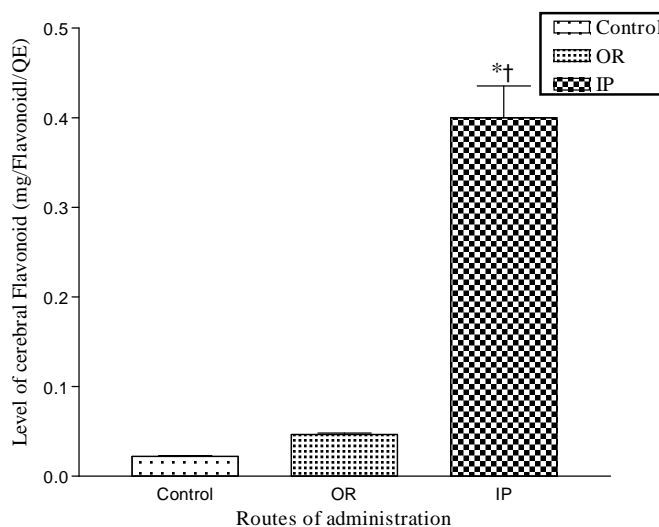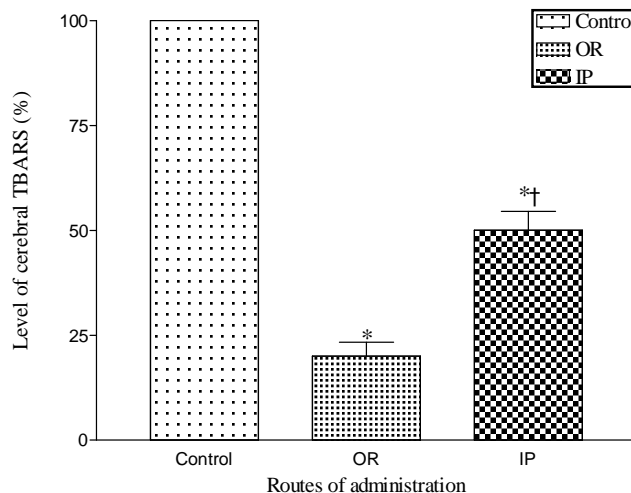

Fig 2: Effect of quercetin on level of total flavonoid in rat brain. Each bar is expressed as Mean ± SEM of level of total phenol by routes of administration; (n=5). * = p < 0.001 compared with control, and † = p < 0.001 compared with oral route of administration (ANOVA; SNK).

3.1.3 Effect on Level of Cerebral Vitamin C
Oral and intraperitoneal administration of quercetin caused a significant decrease ($F_{2, 12} = 270$; $P < 0.001$) in cerebral Vitamin C compared to standard control, while there was no significant change between the administration through oral and intraperitoneal routes (Figure 3).
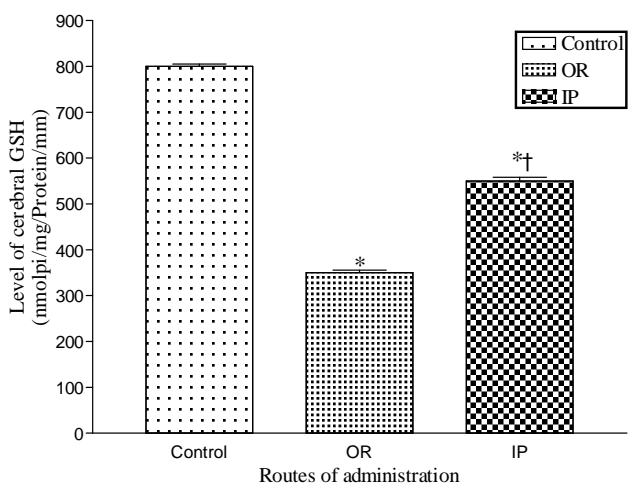
Fig 3: Effect of quercetin on level of vitamin C in rat brain. Each bar is expressed as Mean ± SEM of level of total phenol by routes of administration; (n=5). * = p < 0.001 compared with control (ANOVA; SNK).

3.1.4 Effect on Level of Cerebral GSH

Administration through both the oral and intraperitoneal route caused a significant decrease ($F_{2, 12}$ = 1216; P < 0.001) in cerebral GSH compared to standard control, while administration through oral route caused a significant decrease ($F_{2, 12}$ = 1216; P < 0.001) compared to administration through intraperitoneal route (Figure 4).

Fig 4: Effect of quercetin on level of GSH in rat brain. Each bar is expressed as Mean ± SEM of level of total phenol by routes of administration; (n=5). * = p < 0.001 compared with control, and † = p < 0.001 compared with oral route of administration (ANOVA; SNK).

3.1.5 Effect on Level of Cerebral TBARS

Quercetin administered through both the oral and intraperitoneal route caused a significant decrease ($F_{2, 12}$ = 153.3; P < 0.001) in cerebral TBARS compared to standard control, while administration through oral route caused a significant decrease ($F_{2, 12}$ = 153.3; P < 0.001) compared to administration through intraperitoneal route (Figure 5).


Fig 5: Effect of quercetin on level of TBARS in rat brain. Each bar is expressed as Mean ± SEM of level of total phenol by routes of administration; (n=5). * = p < 0.001 compared with control, and † = p < 0.001 compared with oral route of administration (ANOVA; SNK).

### 3.2 *In vitro* Study of the Effect of Quercetin on Na+/K+-ATPase Activity

The concentration of ouabain that inhibited 50% activity (IC_{50}) of the rat brain sodium pump was found to be 12.21 μm, while the IC_{50} for quercetin was 73.68 μm (Figure 6). Comparing the concentration of quercetin that inhibited 50% activity (IC_{50}) of rat brain sodium pump when the incubating conditions were complete, to when the incubating medium separately lacked individual cations; while the IC_{50} for quercetin was 73.68 μm, the IC_{50} for when the medium lacked sodium ion was 46.32 μm. The IC_{50} for when the incubating medium separately lacked other cations was infinitesimal (see Figure 7). Figure 8 showed the IC_{50} of quercetin, when the incubating conditions were complete (46. 32 μm), when there was pre-incubation with cysteine (33.26 μm) and when there was post-incubation with cysteine (24.42 μm). Figure 9 compared the sodium pump inhibitory activity of quercetin when the incubating conditions were complete (46.32 μm), with when all the cations were excluded and when all the pre-incubating conditions were excluded. While the IC_{50} of when all cations were excluded was 61.47 μm, the IC_{50} when pre-incubating conditions were excluded was infinitesimal.
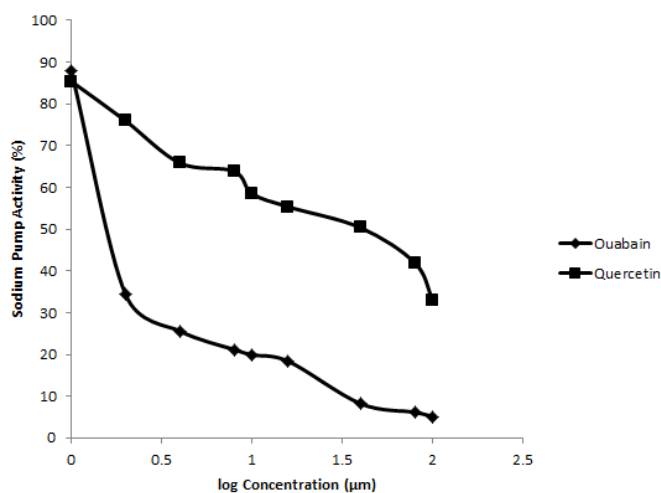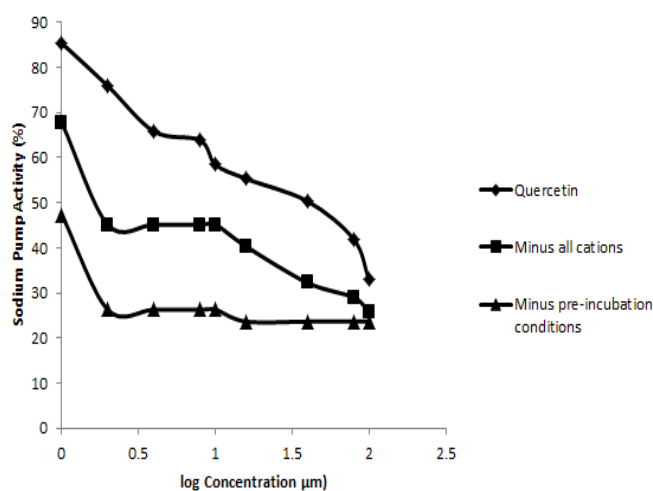

Fig 6: Sodium pump inhibitory activity of ouabain compared with quercetin in rat brain.
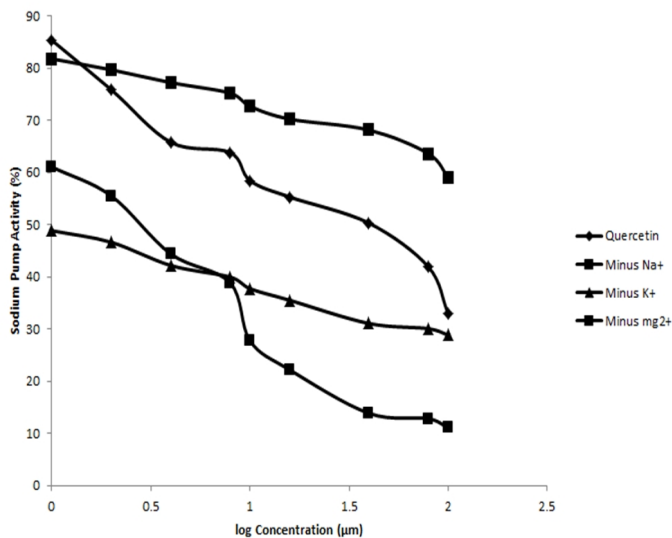
Fig 7: Sodium pump inhibitory activity of quercetin when the incubating medium separately lacked cations.
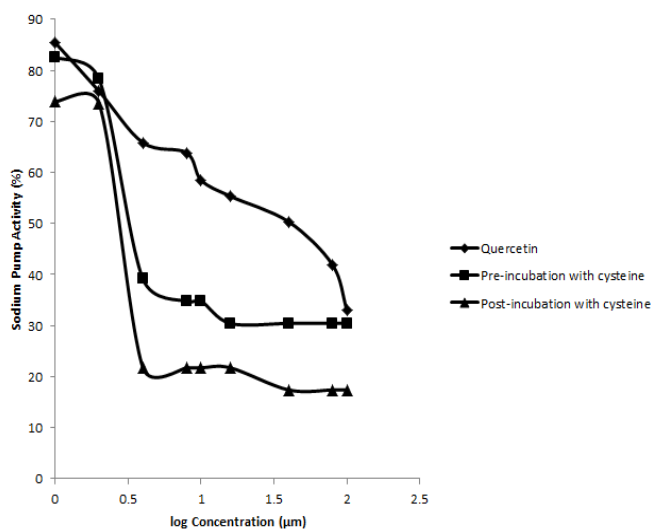
Fig 8: Sodium pump inhibitory activity of quercetin when the incubating medium was pre- and post- incubated with cysteine.

Fig 9: Sodium pump inhibitory activity of quercetin when incubating medium lacked all cations and all pre-incubating conditions.

4. Discussion

This study investigated the effect of *in vivo* (oral and intraperitoneal) administration of quercetin on the antioxidant factors in normal rat brain, and also *in vitro* effect of quercetin on cerebral sodium pump in normal rats. The results showed that *in vivo* administration of quercetin elevated the antioxidant factors in normal rat brain. This was seen in the significant increase in brain phenol and flavonoid contents, and significant decrease in TBARS irrespective of the routes of administration. The results however, showed that administration of quercetin through the intraperitoneal route caused a significant increase in the brain antioxidant factors compared with 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 [30-32]. The reduction observed in cerebral Vitamin C and GHS contents (although both are antioxidant factors) could be because these two do not belong to the polyphenolic groups like phenols and flavonoids [33]. The antioxidant activity of quercetin has been said to be due to its ability to scavenge free radicals and reactive oxygen species (ROS) [16] among others. Since our study investigated cerebral antioxidant activity of quercetin in normal rats, further study is recommended to investigate its antioxidant activity in animal models of cerebral pathologies.

In the *in vitro* investigation, this present study also showed that quercetin, like ouabain, has inhibitory activity on cerebral sodium pump (fig. 6). Using Rukunga and Simons's[34] classification of inhibitory activity of some natural products, both ouabain and quercetin (with IC_{50} of 12.21 μm and 73.68 μm respectively) can be said to have moderate inhibitory activity. Like ouabain, quercetin has concentration-dependent inhibitory activity on the cerebral sodium pump. Figure 7-9 showed the modulating effects of quercetin on the cerebral sodium pump. As said of the effect of ouabain on sodium pump in a potassium-free medium [35], this study showed (figs. 8 and 9) that exposure of the cerebral sodium pump to quercetin in a medium free of any of the cations or the incubating conditions further boost the inhibition of the cerebral sodium pump. Furthermore, in an attempt to find the possible involvement of thiol-oxidation in the inhibitory activity of quercetin on sodium pump, the inhibitory effect of quercetin on sodium pump was investigated with, or without cysteine, a potential thiol containing amino acid. The intention was to investigate whether quercetin was reacting with the sulfhydryl (–SH) group on the alpha–sub unit of the sodium pump and an attempt to relief the inhibition by incubating the medium with cysteine which also contain –SH group. Interestingly, cysteine could not relief the inhibitory effect of quercetin on sodium pump, suggesting that quercetin may not interact with the –SH groups at the alpha-subunit of the enzyme. The inhibitory effect of quercetin on sodium pump could be said to be as a result of this flavonoid acting as a competitive inhibitor of ATP binding to the enzyme [36, 37]. Quercetin thus acts as sodium pump inhibitor at micromolar concentration, may be found to be useful in brain's pathophysiological conditions where enhanced activity of the sodium pump has been observed.

5. Conclusions

We conclude that quercetin has *in vivo* antioxidant activity in the brain by elevating the brain's antioxidant factors, and also *in vitro* inhibitory activity on the cerebral sodium pump in normal rats.

6. References

1. Kaplan JH. Biochemistry of Na,K-ATPase. Ann Rev Biochem 2002; 71:511-535.
2. Pascal B, Gilles C, Florianne M, Marc U, Jean-Danies H, Haim G and Kathi G. FXYD7 is a brain-specific regulator of Na,K-ATPase α1-β isozyme. The EMBO Journal. 2002;21(13):3264-3273.
3. Jorgensen PL, Hakansson, KO and Karlish SJ. Structure and mechanism of Na,K-ATPase: Functional site and their interations. Annu Rev Physiol 2003; 65:817-849.
4. Dostanic-Larson I, Van-Huysse JW, Lorenz JN and Lingrel JB. The highly conserved cardiac glycoside binding site of Na,K-ATPase plays a role in blood pressure regulation. Proc Natl Acad Sci USA 2005; 102:15845-15850.
5. Vatta M, Pena C, Fernandez BE, Rodrigues AG. Endobain E, a brain Na$^+$, K$^+$-ATPase inhibitor, decreases norepinephrine uptake in rat hypothalamus. Life Sci 2004; 76:369-365.
6. Steffens M, Feuerstein TJ. Receptor-independent depression of DA and 5-HT uptake by cannabinoids in rat neocortex involvement of Na$^+$/K$^+$- ATPase. Neurochem Int 2004; 44:529-538.
7. Blasi JM, Cena V, Gonzalez-Garcia C, Marsal J and Solsona, C. Ouabain induces acetylcholine release from pure cholinergic synaptosomes independently of extracellular calcium concentration. Neurochem Res 1988; 13:1035-1041.
8. Vaillend C, Mason SE, Cuttle MF, Alger BE. Mechanism of neuronal hyperexcitability caused by partial inhibition of Na+-K+-ATPase in the rat CA1 hippocampal region. J Neurophysiol 2002; 88:2963-2978.
9. Zhan H, Tada T, Nakazato F, Tanaka Y, Hongo K. Special learning transiently disturbed by intraventricular administration of ouabain. Neurol Res 2004; 26:35-40.
10. Swann AC. Stimulation of brain Na+, K+-ATPase by repeated stimulation. Brain Res 1983; 260:338-341.
11. Kirshenbaum GS, Dawson N, Mullins JG, Johnson TH, Drinkhill MJ, Edwards IJ *et al.* Alternating hemiplegia of childhood-related neural and behavioural phenotypes in Na(+), K(+)-ATPase alpha3 missense mutant mice. PloSone 2013; 8:e60141.
12. Brashear A, Butler IJ, Ozelius LJ, Kramer PI, Farlow MR, Breakefield XO, Dobyns WB. Rapid-onset dystonia-parkinsonism: a report of clinical, biochemical and genetic studies in two families. Adv Neuro 1998; 78:335-339.
13. Moseley AE, Williams MT, Schaefer TL, Bohanan CS, Neumann JC, Behbehani MM *et al.* Defiency in Na,K-ATPase α isoform alters spatial learning, motor activity, and anxiety in mice. J Neurosci 2007; 27(3):616-626.
14. Harwood M, Danielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams GM, Lines TC. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. Food and Chemical Toxicology 2007; 45:2179-2205
15. Merck. Quercetin. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, Edn 13, Merck & Co., Inc., Whitehouse Station, New Jersey 2001, 1438.
16. Jovanovic SV, Steenken S, Tosic M, Majanovic A, Simic MG. Flavonoids as antioxidants. J Am Chem Soc 1994; 116:4846–4851.
17. Morel I, Lescoat G, Cillard P, Cillard J. Role of flavonoids and iron chelation in antioxidant action. Methods Enzymol 1994; 234:437–443.
18. Mira L, Fernandez MT, Santos M, Rocha R, Florencio MH, Jennings KR, Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free Radical Res 2002; 3:1199–1208.
19. Omololu PA, Rocha JBT, Kade IJ. Attachment of rhamnosyl glucoside on quercetin confers protein iron-chelating ability on its antioxidant properties. Experimental and Toxicologic Pathology 2011; 63(3):249-255.
20. Hayek T, Fuhrman B, Vaya J, Rosenblat M, Belinky P, Coleman R *et al.* Reduced progression of atherosclerosis in apolipoprotein E- deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is association with reduced susceptibility of LDL to oxidation and aggregation. Arterioscler Thromb Vasc Biol 1997; 17:2744-2752.
21. Zhao Y, Gao Z, Li H, Xu H. Hemin/nitrite/H$_2$O$_2$ induces brain homogenate oxidation and nitration: effects of some flavonoids. Biochim Biophys Acta 2004; 1675:105–112.
22. Renugadevi J, Prabu SM. Quercetin protect against oxidative stress related renal dysfunction by cadmium in rats. Experimental and Toxicologic Pathology 2010; 62:471-481.
23. Mishra N, Rizvi SI. Quercetin Modulates Na+/K+ ATPase and Sodium Hydrogen Exchanger in Type 2 Diabetic Erythrocytes. Cell Mol Biol 2012; 58(1):148-152.
24. Gakhramanov F. Effect of natural antioxidants on antioxidant activity and lipid peroxidation in eye tissue of rabbits with chemical burn. Bull Exp Biol Med 2005; 140:289-291.
25. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalten reagent. Methods in Enzymology 1999; 299:152-178.
26. Ellman GL. Tissue sulfhydryl group. Achieve of Biochemistry and Biophysics 1959; 82:70-77.
27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Journal Analytical Biochemistry 1979; 95:351-358.
28. Fiske CH, Subbarow TJ. The colorimetric determination of phosphorus. Journal of Biology and Chemistry 1925; 66:375-378.
29. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-Phenol reagent. Journal of Biology and Chemistry 1951; 193:265-275.
30. Brown JP. A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. Mutat Res 1980; 75:243-277.
31. Tamura G, Gold C, Ferro-Luzzi A, Ames BN. Fecalase: A model for activation of dietary glycosides to mutagens by intestinal flora. Roc Natl Acad Sci USA 1980; 77:451-455.
32. Bokkenheuser VD, Shackleton CHL, Winter J. Hydrolysis of dietary flavonoid glycosides by strains of intestinal Bacteroides from humans. Biochem J 1987; 248:953-956.
33. Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr 2005; 81(suppl):243S-55S.

34. Rukunga G, Simons AJ. The Potential pf Plants as a Source of Antimalarial Agents-A Review. Africa Herbal Antimalarial Meeting CDE and ICRAF, Nairobi, 2006, 9.

35. Gutman Y, Boonyaviroj P. Mechanism of inhibition of catecholamine release from adrenal medulla by diphenylhydantoin and by low concentration of ouabain ($10^{-10}\mu$) Naunyn-Schmidebergs Arch Pharmac 1977; 296-296.

36. Shoshan V, Shahak Y, Shavit N. Quercetin interaction with the chloroplast ATPase complex. Biochem Biophys Acta 1980; 591:421-433.

37. Shoshan V, MacLennan DH. Quercetin interation with the ($Ca^{2+}+Mg^{2+}$) ATPase of sarcoplasmic reticulum. J Biol Chem 1981; 256:887-892.