INFLUENCE OF ACIDOSIS ON ANTIOXIDANT POTENTIAL OF SESAMIN ON H_2O_2 INDUCED LIPID PEROXIDATION IN DIFFERENT ORGANS OF RAT

ODUMBONI, A.A., EKEOCHA, A.H. AND OYATOKUN, E.G.

ABSTRACT - Sesamin is a lignin isolated from the bark of fagara plants and from sesame oil. It has been used as dietary fat-reduction supplement, although no controlled studies on this application have been performed. Its major metabolite is enterolactone, which has an elimination half-life of less than 6 hours. Sesamin is a minor component of sesame oil, on average comprising only 0.14% 0f the oil by mass. It has a molar mass of 354.35 g/mol and a chemical formula of $C_{20}H_{18}O_6$. The oil has wide medical and pharmaceutical application. Sesamin has been found to protect the liver from oxidative damage. It is naturally antibacterial for common skin pathogens such as *Staphylococcus* and *Streptococcus* as well as common skin fungi such as athlete's foot fungus. It is anti-viral and anti-inflammatory. This research study was designed to access the influence of acidosis on the antioxidant potential of sesamin on H_2O_2 induced lipid peroxidation in different rat organs (brain, spinal, liver and kidney). Eighty adult albino rats were obtained from an acclimatized colony. The animals were used according to standard guidelines of the committee on care and use of experimental resources. It was conducted at the department of Biochemistry laboratory, Federal University of Technology, Akure, Nigeria. Their tissues were subjected to different pH: 4.4; 5.4; 6.4 and 7.4. The result showed that at physiological pH (pH 7.4), sesamin did not exact any observable inhibitory effect on H_2O_2 induced lipid peroxidation. Similarly, increase acidiosis did not improve the antioxidative potency of sesamin. Therefore, it appears under in vivo situations involving diabetes in which sesamin has been reported to exert considerable antioxidant effect; its metabolic product may have contributed to its observed pharmacology effect in vivo.

Keywords: Acidiosis, Antioxidant, Hydrogen Peroxide, lipid peroxidation and sesamin

1 INRODUCTION

Sesame (*Sesamum indicum*) is a flowering plant in the genus *sesamum*. Numerous wild relatives occur in Africa and a smaller number in India (Ogasawara *et al*., 1988). It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds, which grow in pods (Frederic, 2004). Sesame seed is one of the oldest oilseed crops known, domesticated well over 3000 years ago. The sesame seed contains carbohydrates, fat, protein, vitamins and trace elements. Its chemical composition include; lignans, sesamolin, sesamin, pinoresinol and lariciresinol (Kuo *et al.,* 2011). Sesamin is the phytochemical used for the purpose of this research project. It is a lignan isolated and purified from sesame oil. It has been used as fat-reduction supplement, although no controlled studies on this application have been performed (Kuo *et al.,* 2011). This lignan has various toxicological effects which includes formation of free radicals which leads to oxidative stress. Toxic levels of sesamin inhibits Δ^5-desaturase activity, resulting in accumulation of dihomo-gamma-linolenic acid (DGLA), which displaces arachidonic acid (AA) and consequently decreases the formation of proimflammatory 2-series prostaglandins (Sugano and Akimoto, 1993).

Free radicals are atoms are atoms or molecules containing unpaired electrons. Various free radicals include superoxide, hydrogen peroxide, and peroxynitrite.

Oxidative stress is the condition that occurs when the steady-state balance of pro-oxidants to antioxidants is shifted in the direction of the former, creating the

potential for organic damage (Singh *et al.*, 1995). Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species generated, hydroxyl radical and hydrogen peroxide (Valko *et al.*, 2007). Some reactive species act as cellular messengers in redox signalling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signalling. Sources of free radicals include; cigarette smoking, air pollution, radiation, UV light (such as sunlight), excessive alcohol/drug use (Singh *et al*., 1995).

Iron and hydrogen peroxide are capable of oxidizing a wide range of substrates and causing biological damage. The reaction known as Fenton-reaction is complex and capable of generating both hydroxyl radicals and higher oxidation states of the iron (Winterbourn, 2008). The Fenton chemistry and other metal-catalyzed free radical chain reactions are initiated by the inadvertent by-products of aerobic respiration, such as hydrogen peroxide and superoxide (Rubbo *et al.*, 1996). Fenton's reagent is a solution of hydrogen peroxide and an iron catalyst that is used to oxidize contaminants or waste waters. Iron (II) is oxidized by hydrogen peroxide to iron (III), forming a hydroxyl radical and a hydroxide ion in the process. Iron (III) is then reduced back to iron (II) by another molecule of hydrogen peroxide, forming a hydroperoxyl radical and a proton (Bromme, 2004).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^*$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + HOO^*$$

The free radicals generated by this process then engage in secondary reactions. For example, the hydroxyl is a powerful, non-selective oxidant. Oxidation of an organic compound by Fenton's reagent is rapid and exothermic and results in the oxidation of contaminants to primarily carbon dioxide and water.

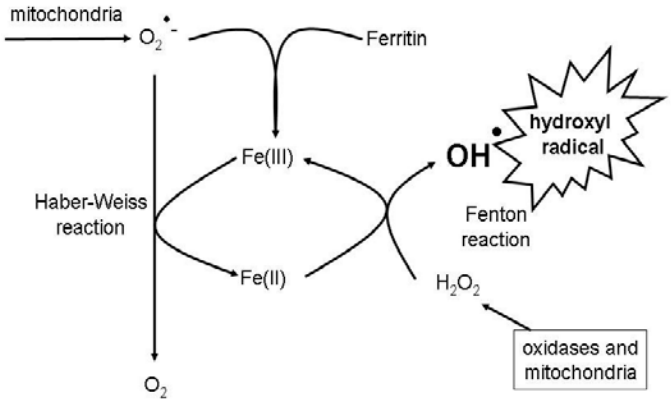

Fig 1: Basic reactions in Fenton chemistry (Bromme, 2004)

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges (-CH_2-) that possess especially reactive hydrogens (Muller *et al*., 2007). The end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde and 4-hydrononenal, the second one being known as "second messenger of free radicals" and major bioactive marker of lipid peroxidation, due to its numerous biological activities resembling activities of reactive oxygen species. However, if the process is not terminated fast enough, there will be damage to the cell membrane, which consists mainly of lipids (Muller *et al*., 2007). Phototherapy may cause hemolysis by rupturing red blood cells cell membrane in this way. In addition, end-products of lipid peroxidation may be carcinogenic and mutagenic (Marnett, 1999).

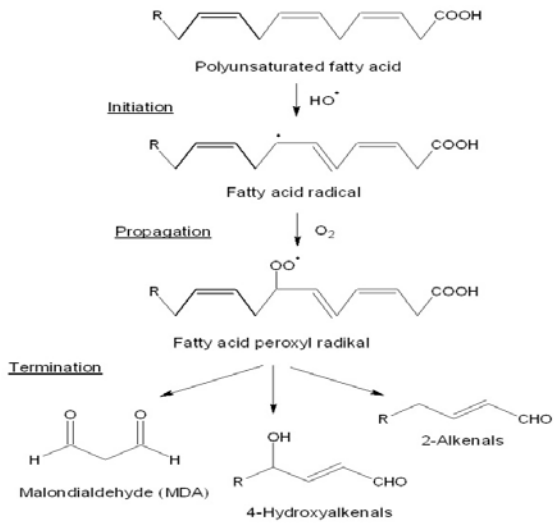

Fig 2: Mechanism of lipid peroxidation (Muller *et al*., 2007).

1.1 JUSTIFICATION

Oxidative stress has been the major cause of various neurodegenerative diseases such as; Asperger syndrome, Parkinson's disease, Lafora disease, Alzheimer's disease etc. The plight to reduce oxidative stress has brought the advent of both natural and synthetic compounds with antioxidant activities. Sesamin has been proven to be a potent antioxidant but the influence of acidosis on its antioxidant potential on H_2O_2-induced lipid peroxidation in rat organs are yet to be fully elucidated hence this study.

2 AIMS AND OBJECTIVES THIS RESEARCH STUDY

The main aim of this research project is to elucidate for the influence of acidosis on antioxidant potential of sesamin on hydrogen peroxide-induced lipid peroxidation in different organs of rats.

3 MATERIALS AND METHODOLOGY

3.1 MATERIALS

3.1.1 CHEMICALS

50µM Tris Hcl, sodium dodecyl sulphate (SDS), thiobarbituric acid (TBA), quercetin (standard antioxidant), sesamin (compound of interest), Tris salt and other reagents were of analytical grade and obtained from standard chemical suppliers.

3.2 ANIMALS

Eighty adult albino rats were obtained from an acclimatized colony. The animals were used according to standard guidelines of the committee on care and use of experimental resources. It was conducted at the department of Biochemistry laboratory, Federal University of Technology, Akure, Nigeria.

3.3 METHODS

3.3.1 TISSUE PREPARATION

The tissues (liver, kidney, brain, spinal) were quickly removed and immediately placed on ice; the tissues were weighed, placed in pestle and mortar for homogenization. The pestle and mortar was placed and buried in ice, the ratio of tissue to buffer was 1:10, w/v. Tris-HCl (50mM) (pH 7.4) was used for homogenization. The homogenate was centrifuged at the speed of 4000rpm for 10 minutes to yield pellets that were discarded and a low speed supernatant was collected and used for all assays

3.4 TBARS ASSAY (THIOBARBITURIC ACID REACTIVE SPECIES ASSAY)

An aliquot of 100µl of tissue was homogenated with hydrogen peroxide (H_2O_2) (10µl/100ml H_2O) as pro-oxidant. Fresh organs were incubated for 1hour at 37^{O}C. Production of thiobarbituric acid reactive species was determined as described by Ohkawa *et al.,* (1979) with slight modification by the addition of 200µl of 8.1% sodium dodecyl sulphate (SDS), followed by sequential addition of 500µl acetate buffer (pH 3.4) and 500µl of 0.8% of TBA (pH 5-6). Colour of the reaction was developed by heating the reaction system at 100oC for 30 minutes and the absorbance was read at 532nm.

4 RESULTS

4.1 EFFECTS OF ACIDOSIS ON THE ANTIOXIDANT ACTIVITY OF SESAMIN ON H_2O_2-INDUCED LIPID PEROXIDATION IN RAT LIVER

Sesamin inhibited the formation of thiobarbituric acid reactive species at pH 7.4 as shown in figure 3 but its antioxidant activity declined as pH decreases as shown in figures 4, 5, 6.

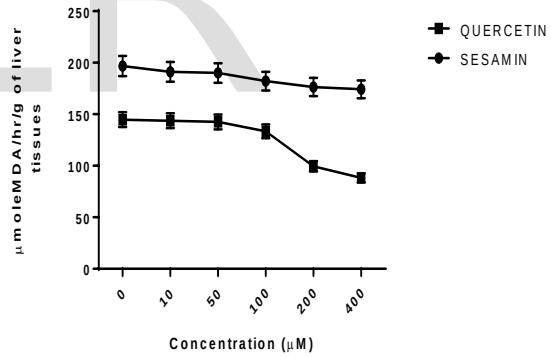

Fig 3: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat liver at pH 7.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

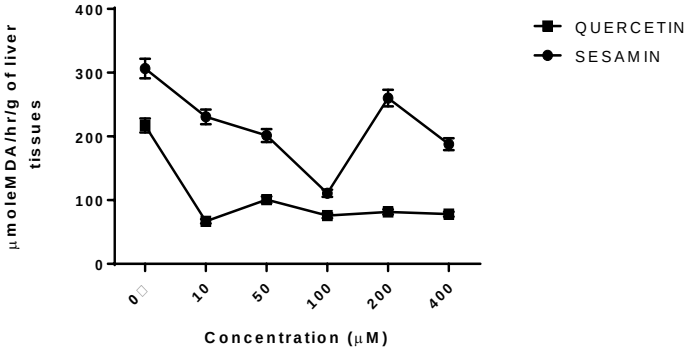

Fig 4: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat liver at pH 6.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

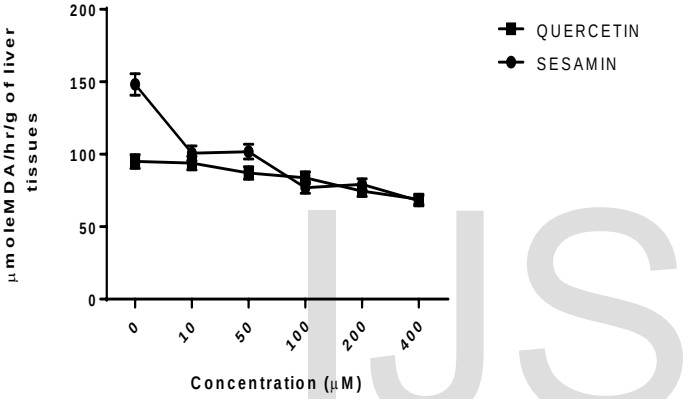

Fig 5: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat liver at pH 5.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

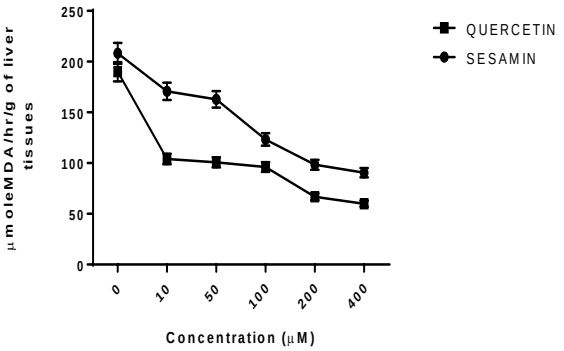

Fig 6: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat liver at pH 4.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

4.2 EFFECTS OF ACIDOSIS ON THE ANTIOXIDANT ACTIVITY OF SESAMIN ON H_2O_2-INDUCED LIPID PEROXIDATION IN RAT KIDNEY.

Sesamin inhibited the formation of thiobarbituric acid reactive species irrespective of the pH as shown in figures 7, 8, 9, 10.

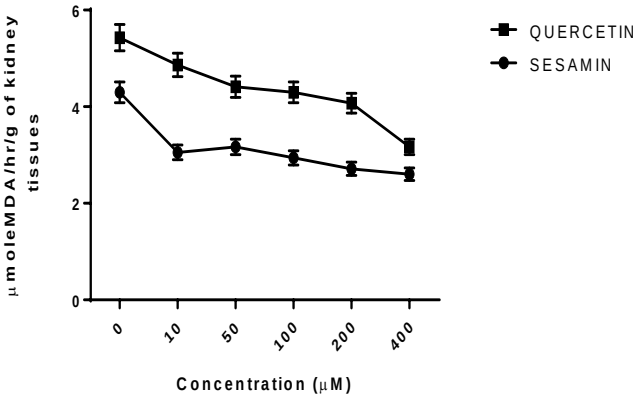

Fig 7: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat kidney at pH 7.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

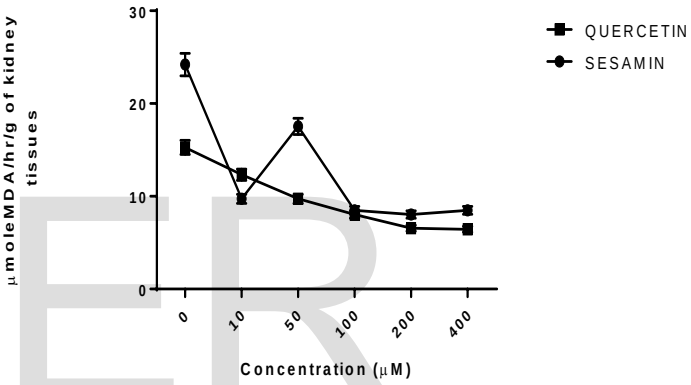

Fig 8: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat kidney at pH 6.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

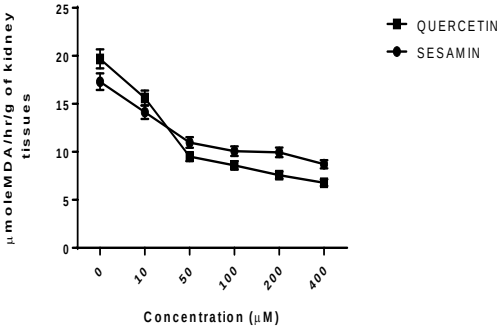

Fig 9: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat kidney at pH 5.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

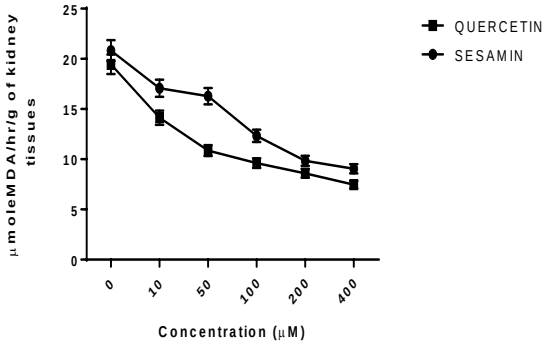

Fig 10: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat kidney at pH 4.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

4.3 EFFECT OF ACIDOSIS ON THE ANTIOXIDANT ACTIVITY OF SESAMIN ON H_2O_2-INDUCED LIPID PEROXIDATION IN RAT BRAIN

Sesamin inhibited the formation of thiobarbituric acid reactive species at pH at 7.4, 6.4, 5.4 as shown in figures 11, 12, 13 but showed reduced inhibition potency as the pH becomes more acidic as shown in figure 14

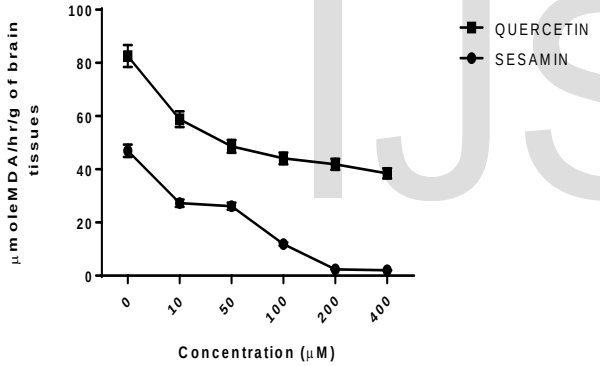

Fig 11: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat brain at pH 7.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

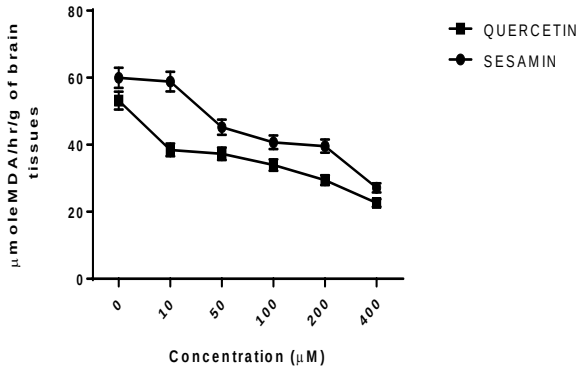

Fig 12: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat brain at pH 6.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

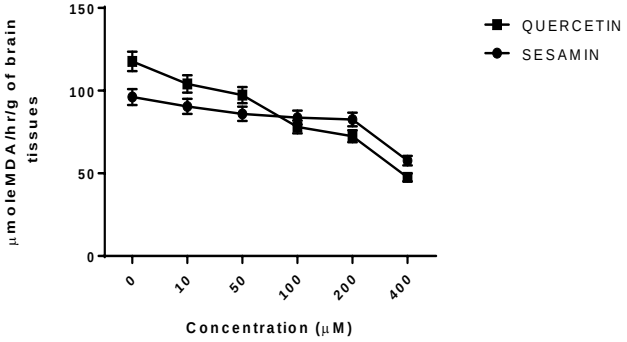

Fig 13: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat brain at pH 5.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

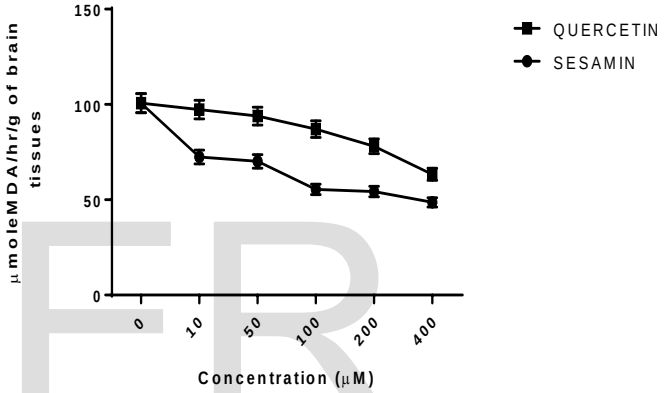

Fig 14: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat brain at pH 4.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

4.4 EFFECT OF ACIDOSIS ON THE ANTIOXIDANT ACTIVITY OF SESAMIN ON H_2O_2-INDUCED LIPID PEROXIDATION IN RAT SPINAL

Sesamin inhibited the formation of thiobarbituric acid reactive species at pH 7.4, 6.4 as shown in figures 15, 16, but showed reduced inhibition potential as the pH becomes more acidic as shown in figures 17, and 18.

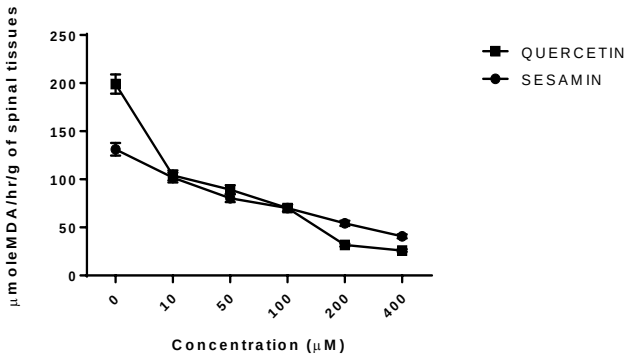

Fig 15: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat spinal at pH 7.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

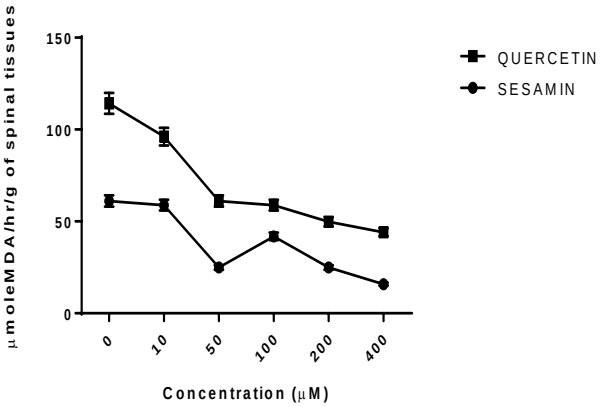

Fig 16: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat spinal at pH 6.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

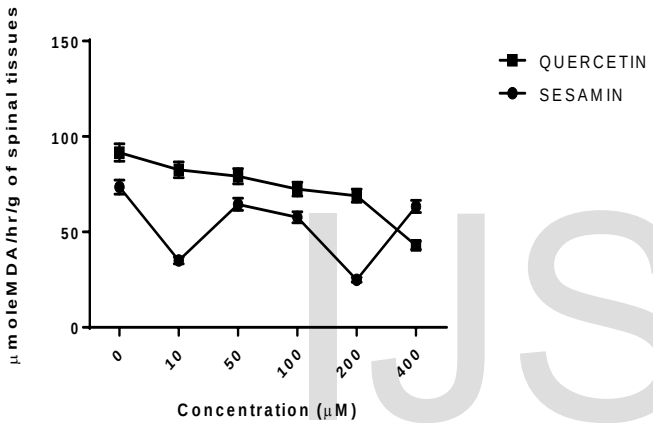

Fig 17: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat spinal at pH 5.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

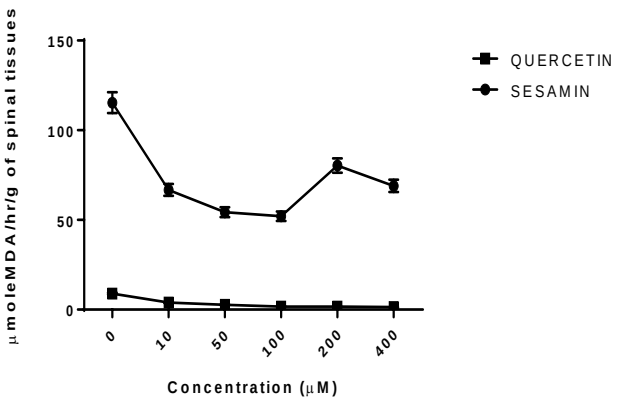

Fig 18: Effect of sesamin and quercetin on H_2O_2-induced lipid peroxidation in rat spinal at pH 4.4. Data shows means and SEM values averages from 2 to 3 independent experiments.

5 DISCUSSION

Several reports have shown that compounds having antioxidant properties against oxidative stress could consequently serve as a potential candidate in the management of degenerative diseases (Bjelakovic *et al*., 2004). The biological function of reactive oxygen species more importantly superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2) is capable of destroying molecules of biochemical classes in different organs or tissues of the biological systems and this can be linked to different pathologies (Winterbourn, 2008). Hydrogen peroxide (H_2O_2) is a non-radical with limited reactivity but relatively long half-life. It is the most effective species for cellular injury, its relatively long half-life enables H_2O_2 generated during oxidative stress to diffuse easily into target sites therefore causing damage to proteins, nucleic acid and cell membranes and this have been associated with cancer, aging, and several chronic neurodegenerative diseases, hydrogen peroxide gives a Fenton reaction in the presence of Fe^{2+} which may be responsible for most of its damaging effects by generating more reactive oxygen species (Jiao *et al*., 2006). The prevention of chain reaction initiation steps by scavenging various reactive species such as free radicals is considered to be an important antioxidant mode of action. Quercetin has anti-tumor potential as it is able to inhibit growth of various cancer cells. This nutrient often occurs in plants as glycosides, such as rutin (quercetin rutinoside) in tea. Quercetin as a standard antioxidant is known to mob up free radicals and this was noticed in the slope of the various graphs with respect to the tissues and pH (Leclerq *et al.,* 1979). High concentrations of quercetin lead low concentrations of reactive species due to its scavenging properties and consequently low absorbance values. However, in biological systems sesamin has numerous functions, including a central role in coordinating the antioxidany defence network (Jiang *et al.,* 2010). After oxidant challenge, GSH is transformed within the cell to GSSG (oxidized form of glutathione). When the rate of oxidation is low, much of the GSSG thus produced may be enzymatically reduced by GSSG reductase activity to GSH. However, within more severe oxidative stress, the rate of GSSG reduction cannot match the rate of its formation, which may result in the accumulation of intracellular GSSG (Stanner *et al*., 2004).
Results obtained from H_2O_2-induced lipid peroxidation in different organs of rats are as follows.

In figure 3, the scavenging properties of quercetin were observed slightly at pH 7.4. On the other hand, sesamin only showed slight inhibition on malondialdehyde formation. The graph shows that sesamin was able to mop up every available reactive species produced by

H_2O_2 only to an extent. Figures 4, 5, 6 showed that both quercetin and sesamin inhibited the production of malondialdehyde on H_2O_2-induced lipid peroxidation in the liver even as the pH becomes acidic but the potency of inhibition was higher in quercetin than that of sesamin.

In figure 7, sesamin and quercetin inhibited the formation of thiobarbituric acid reactive species at pH 7.4. In figures 8, 9, 10, both sesamin and quercetin were able to inhibit the production of malondialdehyde on H_2O_2-induced lipid peroxidation in the kidney.

In figures 11, 12, 13, sesamin inhibited the formation of thiobarbituric acid reactive species at pH at 7.4, 6.4, 5.4, but showed reduced inhibition potency as the pH becomes more acidic in the brain as shown in figure 14. Quercetin inhibited the production of malondialdehyde on H_2O_2-induced lipid peroxidation in the brain irrespective of the pH.

Sesamin inhibited the formation of thiobarbituric acid reactive species at pH 7.4, 6.4 as shown in figures 15, 16 but showed reduced inhibition potential as the pH reduces as shown in figures 17, 18 in the spinal. As shown in figures 15, 16, 17 and 18 quercetin exerted good scavenging properties irrespective of the pH.

6 CONCLUSION

Conclusively, the results obtained showed that sesamin exerted inhibitory effect on H_2O_2-induced lipid peroxidation in different rat tissues (Liver, Kidney, Brain, and Spinal) and these observed antioxidant effects of sesamin can be altered or declined by acidosis.

REFERENCES

[1] Bjelakovic, G., Nikolova, D., Simonetti, R.G. and Gluud, C. (2004). Antioxidant supplements for prevention of gastrointestinal cancers; a systemic review and meta-analysis. *Lancet.* **364(9441)**:1219-1228

[2] Bromme, D., Pentikainen, M.O., Mayranpaa, M., Aitio. And Kovanen P.T. (2004). Free radicals and cell damage. *Journal of biological chemistry.* **(277)**

[3] Federic, R. (2004). Dover publication. *The book of edible nuts.*

[4] Fenton, H.J.H. (1894). Oxidation of tartaric acid in presence of iron. *Journal chemical soc., Trans.* **65(65)**:899-911

[5] Jiang, L., Yang, K.H., Tian, J.H., Guan, Q.L., Yao, N., Cao, N., Wu, J., Ma, B. and Yang, S.H. (2010). Efficacy of antioxidant vitamins and selenium supplement in prostate cancer prevention: a meta-analysis of randomized controlled trials. *Nutrition and cancer.* **63(8)**:1196-1207

[6] Jiao, Y., Wilkinson, J., Christine, P. E. (2006). Iron chelation in the biological activity of curcumin. *Free Radicals Biological Medicine 2006*.**40**:1152-1160.

[7] Kuo, P.C., Lin, M.C., Chen, G.F., Yiu, T.J. and Tzen, J.T. (2011). Identification of methanol-soluble compounds in sesame and evaluation of antioxidant potential of its lignans. *Journal agricultural food chemistry*.**53(7)**:314-319

[8] Leclerq, G., Heuson, J.C. (1979), "Physiological and pharmacological effects of estrogens in breast cancer," .*Biochemical Biophysics Acta*, **560**:427–55

[9] Lennon, S.V., Martin, S.J., Cotter, T.G. (1991). Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. *Cell proliferation.* **24(2)**:203-214.

[10] Marnett, L.J. (1999). Lipid-peroxidation-DNA damage by malondialdehyde. *Mutation research.* **424**:83-95

[11] Muller, F.L., Lustgarten, M.S., Jang Y., Richardson, A. and Van Remmen, H. (2007). Trends in oxidative aging theories. *Free radicals biology and medicine*. **43**:477-503

[12] Ogasawara, T., Chiba, K. and Tada, M. (1998). Medicinal and aromatic plants.**10**:627-628

[13] Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical and Biochemistry* **95:** 351-358

[14] Rubbo J., Rakesh P. and Joanne, M. (1996). Biological aspects of reactive oxygen species. *Biochemical bioohysics Acta.* **1141**:385-400.

[15] Singh N., Dhalla, A.K., Seneviratne, C. and Singal, P.K. (1995). Oxidative stress and heart failure. *Molecular and cellular biochemistry.* **147(1)**:77-81

[16] Stanner, S.A., Hughes, J., Kelly, C.N. and Buttriss, J. (2004). A review of the epidemiological evidence for the antioxidant hypothesis. *Public health nutrition.*

[17] Sugano, M. and Akimoto, K. (1993). Sesamin: a multifunctional gift from nature. *Journal of Chinese nutrition society.* **18**:1-11

[18] Valko, M., Leibfritz D., Moncol, J., Cronin, MTD. Mazur, M., Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human diseases. *International journal of biochemistry and cell biology.* **39(1)**:44-84

[19] Winterbourn, C.C. (2008). Reconciling the chemistry and biology of reactive oxygen species. *Nature Chemical Biology*.**4**:278-286.