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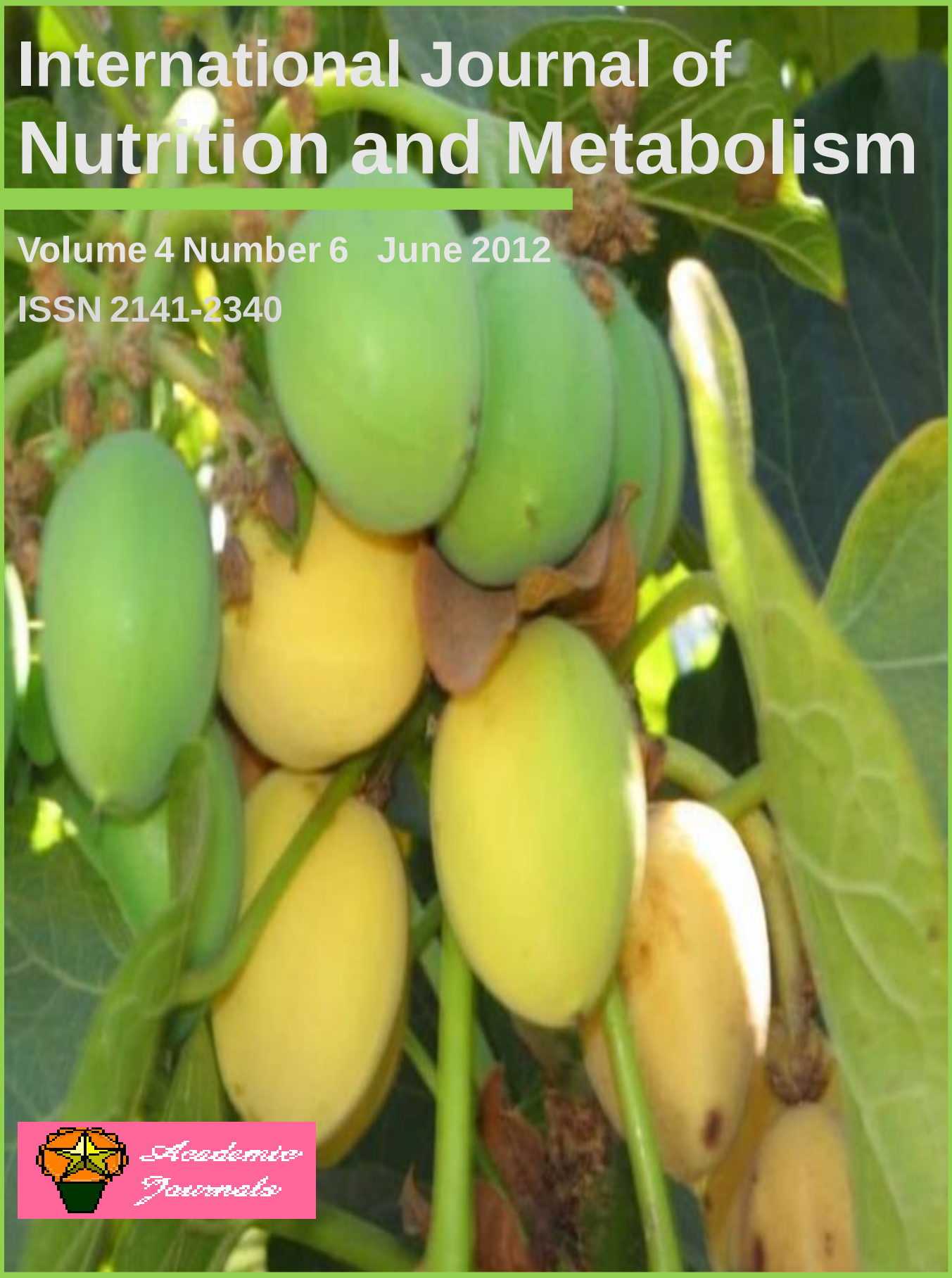

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Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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*Full Length Research Paper*

# Toxicological evaluation of oregano oil

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**The present study evaluated the mutagenic and toxicological potential of a proprietary organic oregano/olive oil mix sold under the trade name Oreganano™. The test article was investigated for its potential to induce gene mutations according to the plate incorporation and preincubation test by *Salmonella typhimurium* strains TA98, 100, 1535 and 1537 and tester strain *Escherichia coli* WP2uvrA at concentrations of 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate with and without metabolic activation. Although toxic effects were noted in all tester strains, no biologically relevant increases in revertant colony numbers of any of the five tester strains were observed. Therefore, Oreganano™ did not cause gene mutations by base pair changes or frame shifts in the genome of the strains used and were considered to be non-mutagenic in the bacterial reverse mutation assay. In a 14-day feeding study of dietary levels of 0, 1.25, 2.5 and 5.0% in Sprague-Dawley rats, there were no adverse clinical, body weight, food consumption or macroscopic changes associated with the administration of Oreganano™. Body weight gain and food consumption was statistically reduced over the 14 days in both male and female animals; however, body weight and food efficiency was unaffected. There were no macroscopic findings attributable to test article administration. Therefore, the no-observed adverse- effect level (NOAEL) was 5.0% in the diet, the highest dose tested and Oreganano™ is considered safe and suitable for consumption.**

**Key words:** Toxicology, oregano, genotoxicity.

## INTRODUCTION

The health benefits of certain herbal oil extracts has been claimed for centuries. Oil of oregano contains active ingredients that have been documented as effective against microbial agents such as bacteria, yeast, fungi and virus (Sokmen et al., 2004), including *Escherichia coli* O:157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* among others (Elgayyar et al., 2001), exerting its effects by destruction of microbial cell membranes (Nostro et al., 2007).

Further, antioxidant (Martinez-Tome et al., 2001), anti-inflammatory (Ocana-Fuentes et al., 2010), hepato-protection and anti-tumorigenic properties have been attributed to this carvocrol- and thymol-containing seasoning agent (Nostro et al., 2007). The present study evaluates the safety of oregano oil under controlled conditions according to universally accepted toxicological guidelines.

A commercially available oregano oil, Oreganano™, containing a proprietary mixture of natural O*riganum vulgare*, carvacrol (36 to 80%) and extra virgin olive oil (Factors Group, Coquitlam, BC, Canada) with a purity by certificate of analysis of 100% for the organic oregano oil (27.5 to 30 mg) and organic extra virgin olive oil (120 mg), respectively, has been newly tested for its safety

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both in a bacterial reverse mutation assay (Ames test) and a 14-day feeding study in Sprague-Dawley rats under Organisation for Economic Co-operation and Development (OECD) guidelines. These studies, conducted at Bioservice Scientific Laboratories (BSL) GmbH in Planegg, Germany (Ames) and Eurofins Product Safety Labs (14-day study), were in compliance with OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17 OECD, Paris, 1998). The studies were conducted in conformance with the OECD guidelines for Testing of Chemicals and Food Ingredients, Section 4, No. 471: Bacterial Reverse Mutation Test, and Part 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents (2008) and US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C. 4 a. Short-Term Toxicity Studies with Rodents (2003). All work undertaken by the testing laboratory was in accordance with the most recent Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and according to AAALAC standards and accreditation.

## EXPERIMENTAL METHODS

### Bacterial reverse mutation assay

The test item Oreganano™ was investigated for its potential to induce gene mutations according to the plate incorporation test (Experiment I) and the preincubation test (Experiment II) (Ames et al., 1973; Maron and Ames, 1983) using *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and tester strain *E. coli* WP2 uvrA with and without metabolic activation in triplicate in the following concentrations: Experiment I: 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate. Experiment II**:** 3.16, 10.0, 31.6, 100, 316, 1000, 2500, and 5000 µg/plate. Controls (positive, sodium azide, 4-nitro-o-phenylene-diamine, methylmethanesulfonate, 2-amino-anthracene and negative, distilled water) were tested for validity of the assay. Data were evaluated for cytotoxicity (diminution of the background lawn or a reduction in the number of revertants), and mutagenicity (mutation factor = mean revertant value of test article/ mean revertants of control).

### Fourteen days dietary toxicity study

A 14-day dietary toxicity study was conducted in CRL Sprague-Dawley CD® IGS rats to determine the potential of Oreganano™ to produce toxicity. Forty healthy rats (20 males and 20 females) were selected for the test and equally distributed into four groups (5 males and 5 females per group). Dietary levels of 1.25, 2.5 and 5.0% of Oreganano™, as well as a basal diet control (0%), were selected for the test. The test and control diets were presented to their respective groups on day 0 of the study. Additional diet was provided as needed throughout the study to insure *ad libitum* feeding. The animals were observed daily for viability, signs of gross toxicity and behavioral changes and on days 0, 7 and 14 for a battery of detailed observations. Body weights were recorded during the acclimation period including prior to test product introduction (day 0), and on days 3, 7, 11, and 14 prior to terminal sacrifice. Individual food consumption was also recorded to coincide with body weight measurements. Gross necropsies were performed on all animals. Male and female rats were evaluated separately. Mean and standard deviations were calculated for all body weight, mean daily body weight gain, mean daily food consumption and mean daily food efficiency. Data within groups was evaluated for homogeneity of variances and normality by Bartlett's test (Bartlett, 1937), analysis of variance (ANOVA), (Dunnett, 1964; 1980) in Provantis™ version 8.4.2.0, Tables and Statistics version 8.4.2.0, Instem LSS, Staffordshire UK; INSTAT Biostatistics, Graph Pad Software, San Diego, CA.

## RESULTS

### Bacterial reverse mutation test

The test item Oreganano™ was investigated for its potential to induce gene mutations according to the plate incorporation test (Experiment I) and the preincubation test (Experiment II) using *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and tester strain *E. coli* WP2 uvrA.

In two independent experiments, several concentrations of the test item were used. Each assay was conducted with and without metabolic activation. The concentrations, including the controls, were tested in triplicate. The following concentrations of the test item were prepared and used in the experiments: Experiment I: 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate. Experiment II: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate.

No precipitation of the test item was observed in any tester strain used in Experiments I and II (with and without metabolic activation). Toxic effects of the test item were noted in all tester strains used in Experiments I and II: In Experiment I (Table 1), toxic effects of the test item were observed at concentrations of 1000 µg/plate and higher (without metabolic activation) and at concentrations of 2500 µg/plate and higher (with metabolic activation), depending on the particular tester strain. In Experiment II (Table 2), toxic effects of the test item were noted at concentrations of 316 µg/plate and higher (without metabolic activation) and at concentrations of 1000 µg/plate and higher (with metabolic activation), depending on the particular tester strain.

Despite the toxic effects of the test product to the bacterial strains (which may be indicative of anti-microbial activity), no biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with Oreganano™ at any concentration level, neither in the presence nor absence of metabolic activation in Experiments I and II as per the criteria for guideline validity. The reference mutagens induced a distinct increase of revertant colonies indicating the validity of the experiments.

### Fourteen days dietary study

In a 14-day *ad libitum* feeding study, Oreganano™, as received and in the diet, was considered stable and to be both homogeneously distributed in the diets and at the

**Table 1.** Results of a plate-incorporation test (Experiment I) of Oreganano™ on *S. typhimurium/E. coli* in the presence (+) and absence (-) of S9 mixture.

| Test article | Dose level (µg/plate) | S9 mix | Revertant colony counts (mean)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | TA98 | Mutation factor | TA100 | Mutation factor | TA 1535 | Mutation factor | TA 1537 | Mutation factor | WP2 *uvrA* | Mutation factor |
| Oreganano™ | 0[b] | - | 22 | 1.6 | 105 | 1.1 | 5 | 0.8 | 9 | 1.5 | 51 | 1.2 |
| | 10.0 | | 25 | 1.8 | 94 | 1.0 | 3 | 0.5 | 9 | 1.6 | 47 | 1.1 |
| | 31.6 | | 24 | 1.7 | 102 | 1.1 | 5 | 0.8 | 6 | 1.1 | 42 | 1.0 |
| | 100 | | 23 | 1.7 | 92 | 1.0 | 4 | 0.6 | 7 | 1.1 | 39 | 0.9 |
| | 316 | | 18 | 1.3 | 94 | 1.0 | 4 | 0.7 | 6 | 1.0 | 45 | 1.0 |
| | 1000 | | 15 | 1.1 | 85 | 0.9 | 2 | 0.3 | 2 | 0.3 | 44 | 1.0 |
| | 2500 | | 14 | 1.0 | 40 | 0.4 | 0 | 0.0 | 1 | 0.2 | 33 | 0.8 |
| | 5000 | | 8 | 0.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 23 | 0.5 |
| $NaN_3$[c] | 10 | | | | 975 | 10.3 | 202 | 30.3 | 95 | 15.8 | | |
| MMS | 1µl | | | | | | | | | | 529 | 12.1 |
| 4-NOPD[c] | 10/40 | | 432 | 30.9 | | | | | | | | |
| DMSO[d] | 0 | | 14 | 1.0 | 94 | 1.0 | 7 | 1.0 | 6 | 1.0 | 44 | 1.0 |
| Oreganano™ | 0[b] | + | 26 | 1.2 | 103 | 1.1 | 5 | 0.6 | 7 | 1.3 | 59 | 1.0 |
| | 10.0 | | 29 | 1.3 | 104 | 1.1 | 7 | 0.9 | 9 | 1.6 | 49 | 0.8 |
| | 31.6 | | 28 | 1.3 | 110 | 1.1 | 8 | 1.0 | 5 | 1.0 | 43 | 0.8 |
| | 100 | | 29 | 1.3 | 107 | 1.1 | 4 | 0.5 | 6 | 1.1 | 53 | 0.9 |
| | 316 | | 25 | 1.2 | 117 | 1.2 | 6 | 0.8 | 7 | 1.4 | 54 | 0.9 |
| | 1000 | | 30 | 1.4 | 112 | 1.1 | 5 | 0.7 | 10 | 1.8 | 60 | 1.0 |
| | 2500 | | 21 | 1.0 | 80 | 0.8 | 2 | 0.3 | 2 | 0.4 | 41 | 0.7 |
| | 5000 | | 18 | 0.8 | 0 | 0.0 | 0 | 0.0 | 2 | 0.4 | 39 | 0.7 |
| 2AA[c] | 2.5/10 | | 1595 | 73.6 | 2516 | 25.8 | 75 | 9.3 | 118 | 22.2 | 175 | 3.0 |
| DMSO[d] | 0 | | 22 | 1.0 | 97 | 1.0 | 8 | 1.0 | 5 | 1.0 | 57 | 1.0 |

[a]Mean of replicate (3) plates. [b]Negative (solvent control): distilled water; [c]Positive control agents: $NaN_3$ = sodium azide; 4-NOPD = 4-nitro-o-phenylene-diamine; 2-AA = 2-aminoanthracene; MMS = methyl methane sulfonate. [d]Solvent control: DMSO[d] = dimethyl sulfoxide.

$$\text{Mutation factor} = \frac{\text{Mean revertants (Oreganano™)}}{\text{Mean revertants (solvent control)}}$$

targeted concentrations throughout the study. Diet preparations and neat test substance were not analyzed as part of this subacute dietary study and preparations were mixed as is, from the manufacturer, for both test and control diets. There were no test substance-related or other mortalities. There were no adverse clinical observations associated with Oreganano™ product. Mean body weights for male and female rats at

**Table 2.** Results of a preincubation test (Experiment II) of Oreganano™ on *S. typhimurium/E. coli* in the presence (+) and absence (-) of S9 mixture.

| Test article | Dose level (µg/plate) | S9 mix | Revertant colony counts (mean)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | TA 98 | Mutation factor | TA 100 | Mutation factor | TA 1535 | Mutation factor | TA 1537 | Mutation factor | WP2 *uvrA* | Mutation factor |
| Oreganano™ | 0[b] | - | 28 | 1.1 | 90 | 1.2 | 10 | 1.0 | 6 | 1.0 | 47 | 1.1 |
| | 3.16 | | 30 | 1.1 | 80 | 1.1 | 7 | 0.6 | 7 | 1.0 | 39 | 0.9 |
| | 10.0 | | 19 | 0.7 | 69 | 0.9 | 14 | 1.4 | 7 | 1.1 | 36 | 0.9 |
| | 31.6 | | 21 | 0.8 | 78 | 1.0 | 9 | 0.9 | 5 | 0.7 | 43 | 1.0 |
| | 100 | | 24 | 0.9 | 78 | 1.0 | 12 | 1.1 | 9 | 1.3 | 39 | 0.9 |
| | 316 | | 24 | 0.9 | 63 | 0.9 | 8 | 0.8 | 4 | 0.6 | 34 | 0.8 |
| | 1000 | | 15 | 0.6 | 26 | 0.3 | 12 | 1.2 | 2 | 0.3 | 27 | 0.6 |
| | 2500 | | 11 | 0.4 | 0 | 0.0 | 6 | 0.6 | 0 | 0.0 | 0 | 0.0 |
| | 5000 | | 4 | 0.1 | 0 | 0.0 | 0 | 0.0 | 2 | 0.4 | 0 | 0.0 |
| $NaN_3$[c] | 10 | | | | 1015 | 13.7 | 1093 | 105.7 | | | | |
| MMS | 1µL | | | | | | | | | | 548 | 12.9 |
| 4-NOPD[c] | 10/40 | | 722 | 27.4 | | | | | 121 | 18.1 | | |
| DMSO[d] | 0 | | 26 | 1.0 | 74 | 1.0 | 10 | 1.0 | 7 | 1.0 | 42 | 1.0 |
| Oreganano™ | 0[b] | + | 29 | 0.9 | 82 | 1.1 | 8 | 1.5 | 9 | 1.2 | 47 | 1.2 |
| | 3.16 | | 32 | 1.0 | 87 | 1.2 | 8 | 1.0 | 8 | 1.0 | 32 | 0.8 |
| | 10.0 | | 29 | 0.9 | 93 | 1.3 | 7 | 1.0 | 8 | 1.1 | 38 | 0.9 |
| | 31.6 | | 30 | 1.0 | 92 | 1.2 | 8 | 1.1 | 4 | 0.5 | 43 | 1.1 |
| | 100 | | 33 | 1.1 | 99 | 1.3 | 7 | 0.9 | 7 | 1.0 | 48 | 1.2 |
| | 316 | | 26 | 0.8 | 91 | 1.2 | 10 | 1.3 | 10 | 1.3 | 61 | 1.5 |
| | 1000 | | 25 | 0.8 | 83 | 1.1 | 12 | 1.6 | 4 | 0.6 | 47 | 1.2 |
| | 2500 | | 1 | 0.0 | 0 | 0.0 | 4 | 0.5 | 0 | 0.0 | 0 | 0.0 |
| | 5000 | | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| 2AA[c] | 2.5/10 | | 1387 | 44.7 | 1028 | 13.8 | 73 | 14.6 | 86 | 11.7 | 194 | 4.8 |
| DMSO[d] | 0 | | 31 | 1.0 | 75 | 1.0 | 8 | 1.0 | 7 | 1.0 | 41 | 1.0 |

[a]Mean of replicate (3) plates. [b]Negative (solvent) control: Distilled water. [c]Positive control agents: $NaN_3$ = sodium azide; 4-NOPD = 4-nitro-o-phenylene-diamine; 2-AA = 2-aminoanthracene; MMS = methyl methane sulfonate. [d]Solvent control: DMSO = dimethyl sulfoxide.

$$\text{Mutation factor} = \frac{\text{Mean revertants (Oreganano™)}}{\text{Mean revertants (solvent control)}}$$

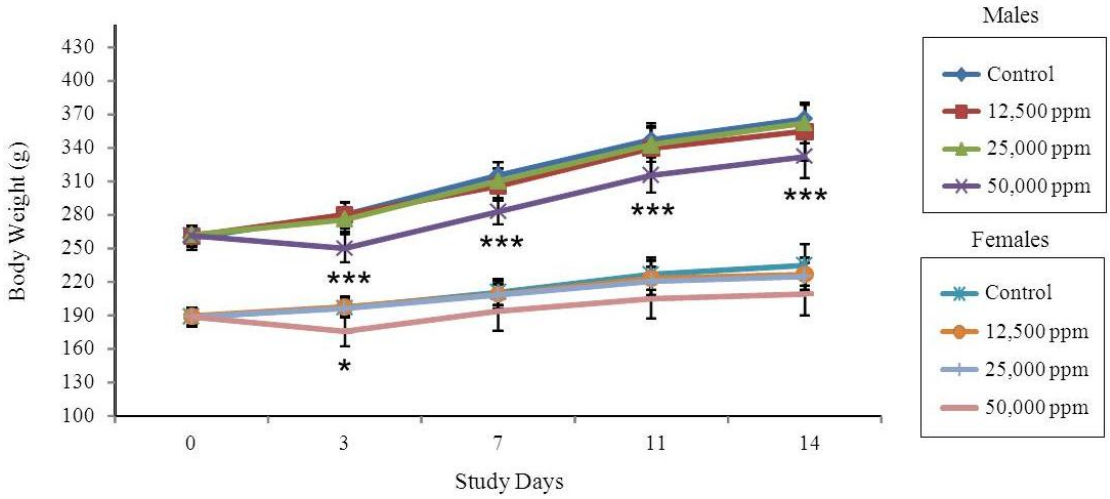


**Figure 1.** Mean body weight.

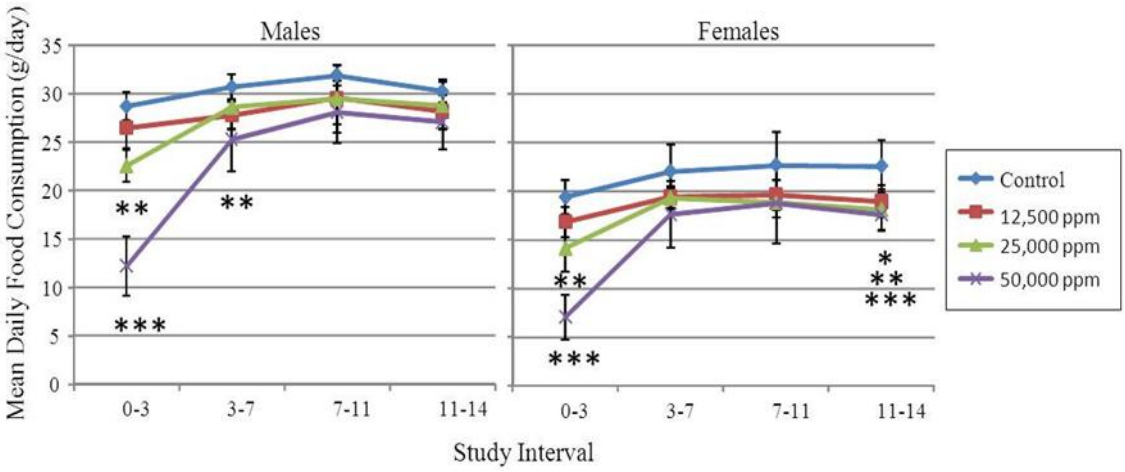


**Figure 2.** Mean food consumption.

1.25, 2.5, and 5.0% were considered comparable with control values throughout the study (Figure 1). Decreases from control were found at 5.0% in male body weight from days 3 to 11 and in female body weight on day 3. Body weight returned to comparable-to-control levels by the end of the study in both males and females. Mean daily body weight gain for male and female rats at 1.25, 2.5, and 5.0% was generally comparable with control values with the exception of decreases in male and female body weight gain at 5.0% for days 0 to 3 and overall (days 0 to 14) in males and females, and at 1.25% from days 3 to 7 in males. Mean daily food consumption for male and female rats at 1.25, 2.5 and 5.0% were generally comparable with control values throughout the study (Figure 2) with reductions at 2.5% (days 0 to 3) and 5.0% (days 0 to 7 and overall, 0 to 14) in males, and at 1.25% (days 11 to 14), 2.5% (days 0 to 3, 11 to 14 and 0 to 14) and at 5.0% (days 0 to 3, 11 to 14 and 0 to 14) in females. Overall (days 0 to 14) and mean food efficiency for male and female rats at 1.25, 2.5, and 5.0% were generally comparable with control values with the exception of reductions at days 0 to 3 in males and females, and increases in females on days 3 to 7 in females all at the 5.0% dietary level. Although significant changes from control in body weight gain and food consumption at 5% in males and females persisted overall (days 0 to 14), these decreases were considered the non-adverse residual result of losses extending from the beginning of the study, as animals of both genders recovered much of their loss as the study progressed. There were no macroscopic observations at necropsy associated with the *ad libitum* dietary intake of Oreganano™ at the levels tested. The mean overall (days 0 to 14) daily intake of Oreganano™ in male rats fed dietary concentrations of

1.25, 2.5, and 5.0% was 0, 1137.9, 2219.2, and 4092.0 mg/kg/day, respectively. For the same dietary concentrations, the mean overall daily intake of Oreganano™ in female rats was 0, 1123.7, 2134.6, and 4041.8 mg/kg/day, respectively. Therefore, the animals were considered to have received the targeted exposures with a no-adverse-effect level of 5.0% in the diet. Clinically, the recommended daily dose of Oreganano™ is approximately 0.5 mg/kg (30 mg per 60 kg human), making the highest dose tested in the present study over 8130 times suggested human intake.

## DISCUSSION

The present study examines the potential for oregano oil, long an herbal remedy, to produce toxicity when administered in the diet to young adult rats. The decreases in body weight (males), body weight gain and food consumption are attributed to initial effects at the introduction of the test article possibly owing to the initial acclimation and/or pungent odor. As such, the administration of the test substance at the highest dose appeared to notably reduce food consumption without lasting adverse effect to the animals as indicated by the maintenance of body weight and slowed reductions in body weight gain as the study progressed.

Numerous studies have reported on the *in vitro* and *in vivo* evidence for the effects of flavonoids, specifically, luteolin, (Lopez-Lazaro, 2009) to which the botanical oregano (*O. vulgare*) belongs. Biological effects consist of antioxidant, anti-inflammatory, antimicrobial, anti-cancer, antiallergy and cardiovascular protective activities for which there is evidence both preclinically and clinically (Lopez-Lazaro, 2009). Although, the true influence of oregano oil on human health requires long-term, controlled clinical studies, recent studies in mammals and humans report on the beneficial effects of lipid profiles (Ozdemir et al., 2008), angiogenic (Loboda et al, 2005), and antioxidant (El-Ashmawy et al., 2005) activities and wound healing (Al-Howiriny et al., 2009; Ragi et al., 2011) among others (Dundar et al., 2008; Force et al., 2000).

In the present study, the dietary administration of Oreganano™, a plant food concentrate, was well tolerated by rats up to a concentration of 5.0% in the diet. Dietary supplements over this level have the potential to adversely influence nutritional intake (Borzelleca, 1992). Based on the experimental conditions of this mutagenicity and 14 days test and the toxicological endpoints evaluated, these results indicate that Oreganano™ did not cause gene mutations by base pair changes or frame shifts in the genome of the tester strains used. Therefore, Oreganano™ is considered to be non-mutagenic in the bacterial reverse mutation (Ames) assay. Neither did the subacute dietary administration of Oreganano™ result in any adverse toxicological effects. Therefore, the use of appropriate levels of Oreganano™ is considered safe. A study of longer duration is appropriate to confirm and extend these results.

## ACKNOWLEDGMENTS

The Oreganano™ product used in the present study is a commercial grade product obtained from the producer (Factors Group of Nutritional Companies Inc, BC, Canada). Oreganano™ is a registered trademark of the Factors Group (BC, Canada). Simon Wood received financial support from Factors Group of Nutritional Companies, Inc. (Canada), owned by R. Gahler which retains an interest in Oreganano™.

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*Full Length Research Paper*

# Glucose-6-phosphate dehydrogenase: The balance between energy production and genetic material repair in cyanogenic toxicity response

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**The adult neurons are entirely dependent on aerobic metabolism involving the glycolytic pathway. The primary transport mechanism of glucose have been found to be dependent of exchange with glutamate, while the glutamate thus released is converted into glutamine by the surrounding astrocytes. In the metabolism of the glucose taken up by the neurons, glucose-6-phosphate dehydrogenase converts the glucose-6-phosphate into ribose sugar precursor for generation of genetic materials. In this study, we explain the basic of the rational for the conversion of glucose-6-phosphate (G-6-P) into ribose sugar as against the G-6-P proceeding into pyruvate formation for ATP generation. In toxicity studies where oxidative stress was induced by cyanide, we observed a decline in G6PDH levels. In analysis of these findings, it was observed that the G6PDH levels were secondary indicator of oxidative stress. The primary cause in the enzyme shift is for more G6P to proceed into energy production to compensate for the energy block created by cyanide while at the same time reducing the amount of G6P converted into ribose sugar for DNA repair.**

**Key words:** Glucose, glutamate, DNA repair, G6PDH.

## INTRODUCTION

Cyanide is a naturally occurring toxic substance that has been identified in a variety of food crops (Osuntokun, 1981). Cassava is the most widely consumed of these plants and it has been associated with the economic conditions in certain parts of the World; especially the tropics and sub-tropics (Okafor et al., 2002). Plants like cassava are called cyanophoric plants because they contain phytotoxins (cyanogenic glycosides). The term cyanide will usually refer to free cyanide (CN-) or hydrogen cyanide (HCN). For cyanide to exist in either state (as CN or HCN), it will depend on certain physical parameters such as pH.

It has been shown that in a medium of pH 7, most of the cyanide will be in the form of free cyanide (CN), while at pH of 11, 99% of cyanide will exist as HCN. Equilibrium is however achieved in the pH range of 9.3-9.5 (Nicholls and Soulimane, 2004).

Cyanide is highly reactive and forms salt readily with the alkali earth metals. The most reactive of these salts are those of sodium, potassium and calcium. They also dissociate easily in water to release free cyanide. The salts of copper, cadmium and molybdenum are less reactive and dissociate less easily and are often called “weak acid dissociable” (Isom et al., 1999).

### The bio-activation of cyanide

Cyanide is readily absorbed and distributed following its oral administration, such that 9% activity can be recorded in the stomach, 0.9 activity in the brain and 84% activity in the urine 24 h after administration (in the form of

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thiocyanate-SCN). The basic form in which cyanide is excreted from the body is the form of SCN (Isom et al., 1999). The enzyme rhodenese has been implicated with the function of converting cyanide into SCN, thus the major defence of the body against cyanide toxicity is the enzyme rhodenese, which will convert cyanide to SCN in the presence of thiosulphates or sulphur containing amino acids. However, since the enzyme is present in large quantities but sequestered in sites that are not readily accessible, the rate limiting factor in the conversion of cyanide to SCN is thus the relative abundance of thiosulphates and SAA (Isom and Way, 1984). Other form in which cyanide is excreted is in the form of 2-iminothiozoldine-4-Carboxylic acid; a reaction product formed as a result of the reaction with L-cysteine. The reaction is reversible by co-incubation with curcumine (Isom et al., 1999).

## CYANIDE IN ENERGY METABOLISM OF THE BRAIN

Cyanide has long been implicated with the ability to induce oxidative stress. It does so by virtue of its ability to inhibit cytochrome C oxidase, which is responsible for converting molecular oxygen into water to generate the proton gradient required to drive ATP production at complex V of the electron transport chain (Magistretti and Pellirini, 1996). When such a blockade occurs, oxygen radicals are generated at complexes I and III of the electron transport chain (ETC). In this context, we would like to describe oxidative stress in 2 typical systems (Ogundele and Olu-Bolaji, 2011);

(i) Type I: which is the type of oxidative stress observed in a system where oxygen is present but the transfer of the available is blocked
(ii) Type II: which is present in a system where oxygen is entirely absent; such is possible under low oxygen concentration in the circulatory system.

Oxygen radical generation is not characteristic of the Type I oxidative stress and not entirely characteristic of type II oxidative stress. The generated ROS in Type I then in turns induces lipid peroxidation. The most significant effect of lipid peroxidation can be felt on the membranes (lysosomal, nuclear, mitochondrial and cell membrane). The effect on nuclear membrane exposes the genetic materials to leaked endonucleases and phosphatases in the cytoplasm. The definitive response of the neurons to strike a balance between its energy requirement and repair of its genetic material is imperative (Denison et al., 2009).

### Pivotal role of glucose-6-phosphate dehydrogenase (G6PDH)

G6PDH is an enzyme that has been used as a direct indicator of oxidative stress, especially as G6PDH: LDH ratio (de Graaf et al., 2001). G6PDH catalyses the conversion of glucose-6-phosphate into ribose sugar. This represents the diversion from early stages of glycolysis to the pentose phosphate pathway (PPP), thus G6PDH does not directly represent the glycolytic pathway. We can therefore say that G6PDH shunts glucose-6-phosphate (G6P) into RNA/DNA formation as against formation of high energy pyruvate. High levels of G6PDH will indicate a diversion of G6P into RNA production and reduction in the G6P meant for pyruvate formation; which will imply the neuronal metabolic system favouring repair of the genetic materials against energy requirements of the neuron. While a reduction in G6PDh observed during oxidative stress means a reduction in the rate at which G6P is converted into ribose sugar (DNA precursor), thus, the system favours energy production over the repair of genetic materials.

In cyanide toxicity, a definitive response is imminent; whether the neuron will strike a balance between the repair of its degenerating genetic materials and its energy requirement or favour one over the other poses a major scientific question in the field of cyanide induced cell death. At this point, we would like to visualise toxicity response in terms of the metabolic requirement for cell survival as against oxygen consumption to drive ATP production. A very important indicator of oxidative stress has been the G6PDH: LDH ratio, because G6PDh is preferred against hexokinase since its a rate-limiting step in the glycolytic pathway and LDH is a key enzyme in determining the fate of the glycolytic pathway to either proceed into formation of high energy pyruvate or stop as lactate (de Graaf et al., 2001). In a previous experiment, cyanide induced oxidative stress as shown in the G6PDh: LDH ratio which follows a similar trend as the level of superoxide dismutase (SOD). However, while constructing a toxicity response model (Figure 1), the reduced level of G6PDH against LDH can imply two things;

(1) Since G6PDH catalyses the conversion of G6P into ribose sugar, a reduction in G6PDh will allow more G6P to proceed to the end of glycolysis to form high energy pyruvate (a precursor of the tri-carboxylic acid (TCA) cycle)
(2) An increase in G6PDH level will imply that more G6P has been shunted to the PPP to generate ribose sugar for DNA production).

From the accounts of cell death, a similar event has been seen to precede apoptosis and necrosis, but the intensity of such an ‘initial event’ has been implicated in deter-mining the pattern and the adopted mode of cell death. DNA cleavage has been described as the most significant event for apotosis and necrosis (Katherine et al., 2001; Denison et al., 2009). The DNA cleavage pattern has been used to distinguish between the two modes of cell death genetically (Katherine et al., 2001). If

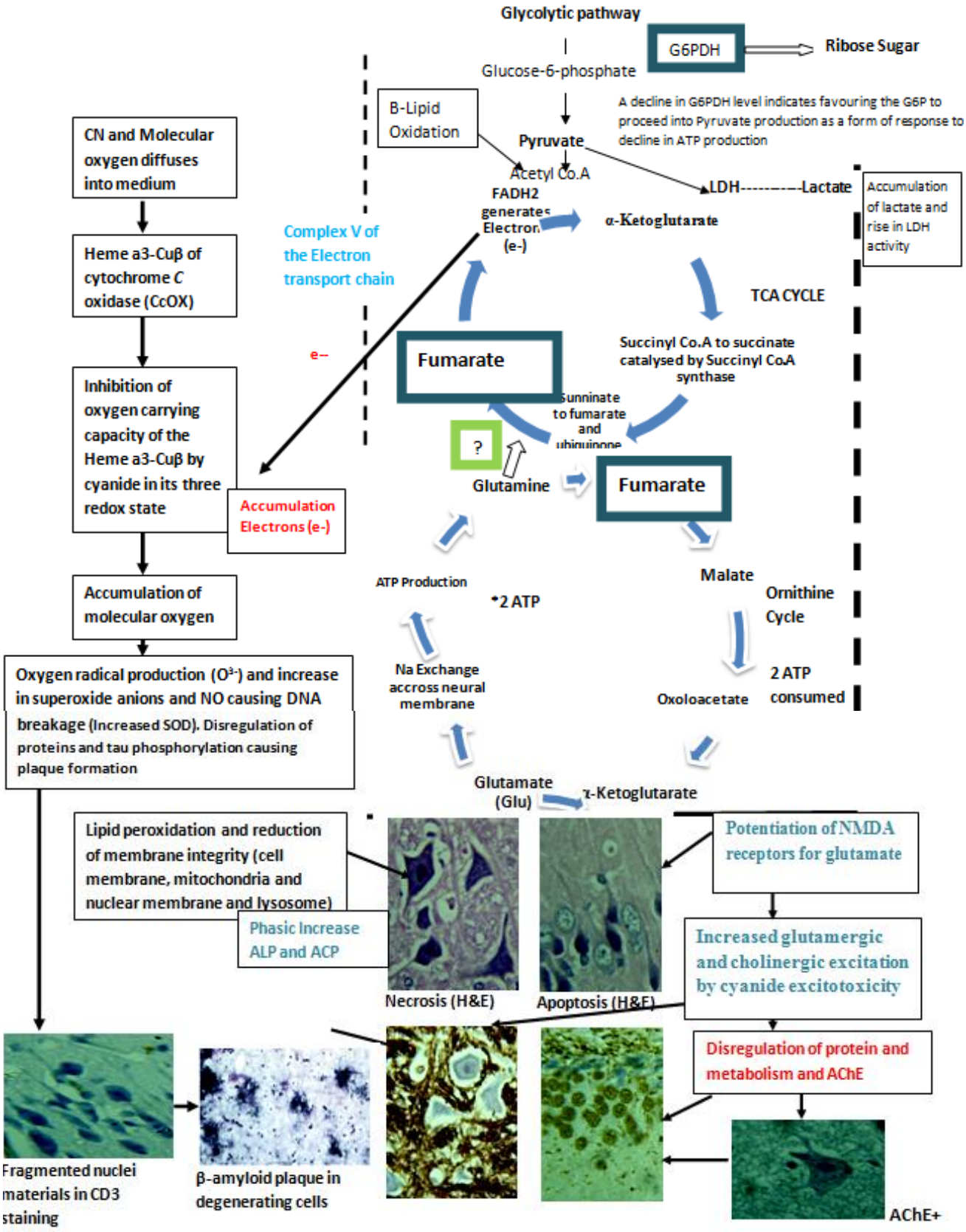


**Figure 1.** Proposed toxicity response model mechanism in cyanide neurotoxicity in the visual relay centres.

a definitive response to toxicity involves DNA breakage, is there a possibility of DNA repair machinery? A reduction in G6PDH usually termed as oxidative stress does not imply oxidative stress in its entirety but rather basic changes observed as result of effects of toxicity on the metabolic machinery of the neuron secondary to the oxidative stress. The level of G6PDh are not pre-determined but are dependent on the composition of the cytoplasm (neuronal metabolic system) such that when oxidative stress is induced the system will self-regulate to compensate for energy needs; thus, the G6PDH activity tilt the system as a regulator of energy need against DNA repair. When cyanide toxicity is induced, ATP production is impaired (indirectly by inhibition of cytochrome C oxidase), thus G6PDh levels will reduce to favour formation of pyruvate over ribose sugar for DNA repair, thereby leading to a deficiency in the DNA repair machinery. This is likely to precede the DNA cleavage characteristic of both modes of cell death (Figure 1).

The above explanation appears to be a missing link in the neglected science of necrosis. A rapid drop in G6PDh level and DNA repair machinery causes a rapid degradation of the cell, while a milder decrease in the G6PDh level can be found to induce apoptosis. This is evident in experiments performed by Katherine et al. (2001) which suggested dose dependence in the adopted mode of cell death of NP3 cells. Thus, to investigate or understand cell death pattern, the actual relationship between G6PDH and endonucleases activity should be investigated to account for the gap caused by reduced G6PDh level as a factor of DNA repair and DNA damage against the generalized term of oxidative stress. The action of cytochrome- c- oxidase (CcOX) at complex V is to transfer oxygen into water to generate the proton gradient required to drive ATP production. During the blockade of CcOX and Complex V, two major events will occur;

(i) Reduction in ATP production,
(ii) Production of oxygen radicals.

Both events will be characteristic of oxidative stress. The reduction in ATP will most likely have a direct effect on G6PDH, while the radicals will affect the membrane. We can also deduce that in a normal system, G6PDH will usually function to favour DNA repair since the neuron has more RNA than DNA. When oxidative stress is induced, reduction in ATP production will cause G6PDH to withdraw from DNA repair to favour ATP production from pyruvate to meet the energy requirement of the neuron under stress. It is also important to note that the generated radicals react with nitrogen to form NO and reactive nitrogen species (RNS). The NO thus formed is also an endogenous modulator of cell death (Isom and Way, 1984; Isom et al., 1999).

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