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Research Article

Insights Into Cod-Liver Oil with Ciperus Esculentus, Phoenix Dactylifera and Soybean on Oxidative Stress Markers Profile in Normal Male Wistar Rats

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Abstract

This study determine the effect of cod liver oil with soybean, Cyperus Esculentus, and phoenix dactylifera on oxidative stress markers, weight and fasting blood sugar level. Fifty six male healthy wistar rats with weight ranging from 162g to 301g housed in seven cages numbering eight per group were used for this study. Parameters investigated in this study includes Malondialdehyde (µg/ml), glutathione (µg/ml), fasting blood sugar (mmol/l) and weight in grammes.Group1 was assign the normal control while group2 to seven serve as the test groups Findings from this study indicate a significant decrease in the final weight in groug5 (147g) and group7 (173g) that received 1500mg/kg extract of Cyperus Esculentus, 700mg/kg phoenix dactylifera fruit daily and 472mg/kg liquid cod liver oil per body weight daily. This decrease was closely followed by group2 (147g),group6 (171g) and group3 (193g) that receive 100mg/kg extracts of Cyperus Esculentus only, combination of 1600mg/kg Cyperus esculentus,800mg/kg phoenix dactylifera, 1200mg/kg soybean and 620mg/kg phoenix dactylifera only at the end of the study compared with the control. However there was an increase in group4 final weight that received 1000mg/kg soybean extract daily and the control. The level of glutathione with antioxidant properties increased in group2, 3, 7, 4, and 6 to 2.15, 2.59, 1.96, and 1.68 µmol/ml respectively compared with the control of 1.24 µg/ml. The level of Malondialdehyde were all decreased among the test groups compared with the control. The fasting blood sugar level in group7 that received the standard drug –cod liver oil increased from the initial level of 3.28mmol/l to 4.55mmol/l at the end of the study. Insight on these nut fruits and soybean in this study have shown a beneficial effect of decreasing fasting blood sugar level and increasing glutathione level with antioxidant properties needed to promote good health and longevity.

Keywords: Oxidative stress, glutathione, cod-liver oil, Cyperus, Esculentus, Phoenix dactylifera.

Introduction

Oxidative stress mechanism is caused by reactive oxygen production in species endoplasmic reticulum and dysfunctions of the mitochondrion's serving as power plant of the cells in the body. Some amount of oxidative damages take place in normal conditions but rapidly increase during disease condition such as cardiovascular, diabetes and other leading to the reduction in life expectancy.

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The reaction of oxygen species creates a balance with antioxidants molecules in cells but when oxidative stress does occur this balance become altered due to decrease in antioxidants and accumulation of free radicals (ROS) which will then deplete the body defense system integrity to cope with detrimental consequences of physiological dysfunction. This action can thus either activate or silent the genes encoding defensive enzymes and structural proteins (Pandey *et al.,*2009 ;Blessing *et al*., 2023).

Essential minerals from phoenix dactylifera (date fruit),Cyperus Esculentus (tiger nut) are edible plant with sweet and nut-like tubers rich in anti-oxidative properties with health benefits ranging from anti-inflammatory to antithrombotic and the maintenance of the cardiovascular integrity (Buzzeti *et al;*2016 ; Adeleke *et al;*2019).

Glutathione is a strong antioxidant that defends the body against diseases; decrease proliferation of cancerous cells and promote insulin sensitivity to glucose metabolism. Glutathione has the molecular formulae ($C_{1o}H_{17}N_2O_6S$) and a molar mass of 307.32gmol^{-1} was first discovered in 1888 by a French Researcher J.de Rey-Pilhade. Glutathione reduction is the main intracellular main protein accepted as most essential intracellular hydrophilic antioxidant known for its physiologic functions such as cellular regulation, metabolism of nutrients, synthesis of protein/DNA, immune response etc. The deficiency of glutathione has been observed in the following disease condition: Alzheimer, kwashiorkor, Parkinsonism, autism, cardiovascular, liver cirrhosis, AIDS and sickle cell anemia (Wu *et al;* 2004). The major most defense mechanism endogenously against oxidative stress is the glutathione due to its ability to reversibly oxidize to glutathione disulfide during oxidative stress. Several parameters have been used to evaluate the level of oxidative damage but not all the parameters can actually fit into that role of biomarkers since most can be compromised by factors relating to tissue type, sex, dietary intake and environmental pollution (Kanti and Syed, 2010).

Malondialdehyde ($C_3H_4O_2$) is a physiologic keto aldehyde produced from peroxidation byproduct of metabolism of arachidonate during decomposition of unsaturated lipids and serve as a reliable marker for oxidative stress (Zorawar *et al;* 2014).

Materials and Methods

Samples Collection and Identification: The dark brown and yellow Cyperus Esculentus were purchased in Sabongeri Kano state of Nigeria. They were identified by a researcher in the department of plant and biotechnology of the university of science and technology, River state Nigeria. (Dutta 2011). All samples were thoroughly separated from other particles, washed and dried using a dehydrator. The Cyperus Esculentus, phoenix dactylifera and soybean were all weighed separately and measured. The date fruits were pulverized along with the inner nut seed together with an electric grinder and macerated in absolute ethanol 1500ml accompanied by shaking for three days to create room for exhaustive metabolite extraction. The concentrated filtrate was then placed in a rotary evaporator to remove the ethanol (Harbourne 1973). The concentrated extract was dried at 50 degrees in a water bath and desiccator for the removal of moist residues. The phytochemical screening was done from the aqueous extract of the fruit in the pharmacognosy faculty of pharmaceutical science of the University of Port Harcourt using specific experimental methods adopted by the association of official analytical chemist of 2016.

Experimental Animals

The animals used for this study comprises of fifty-six (56) healthy male wistar rats whose weights ranges between 162 to 301g of nine to ten weeks old. The animals were acclimatized for two weeks and housed in seven cages with eight in number per group having access to regular natural light, air, rat feeds and water ad libitum in accordance with the guidelines for the care of laboratory animals at the animal house of the university of Port Harcourt Nigeria (NRC 1985).

Experimental Design

Group1 serve as the normal control group with normal feeds and water ad libitum.

Group2 received 1000mg/kg body weight of aqueous extract of Cyperus Esculentus daily

Group3 was administered 620mg/kg body weight of phoenix dactylifera fruit extract daily

Group4 receive 1000mg/kg body weight of soybean aqueous extract daily

Group5 receive 1500mg/kg body weight extract of Cyperus Esculentus and 700mg/kg of phoenix dactylifera fruit daily

Group6 was administered 1600mg/kg Cyperus Esculentus, 800mg/kg phoenix dactylifera fruit and 1200mg/kg body weight of soybean extract daily respectively.

Group7 was administered 472mg/kg body weight of liquid cod liver oil daily.

Determination of Oxidative Stress Markers

Malondialdehyde (MDA) Ohkawa & Ohishi Method

- Principle: Under acidic condition, MDA produced from the peroxidation of fatty acid membranes react with the chromogenic reagent, 2- thio-babituric acid to yield a pink colored complex which is measured at 532nm. unit ug/ml
- Procedure: An aliquot of 0.4ml of supernatant was in mix with 1.6ml of Tris-Kcl buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a boiling water for 1hr. This was the cooled in ice and centrifuged at 4000 rpm. The clear supernatant was collected and the absorbance measured at 532nm using D/W as blank.

GSH (Reduced Glutathione Level) Sedlak & Lindsay Method

- Principle: The reduced form of glutathione comprised in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable yellow color when 5,5 dithiobis -2- nitro benzoic acid (Ellman's reagent) is added to sulfhydryl- compounds. The chromophoric product resulting from the reaction of Ellman's rgt with the reduced GSH is measured at 412nm.
- Procedure: 0.2ml of the sample was added to 1.8ml of D/W and 3ml of the precipitating solution and mixed. Allow to stand for 5 min and centrifuged at 4000rpm for 10mins. 1ml of filtrate was added to 4ml of 0.1m phosphate butter. 0.5ml of DTNB was finally added. Read and record the absorbance at 412nm using a prepared blank to 3ero the spectrophotometer. unit µg/ml

Catalase Estimation (Clairborne Method)

- Principle: catalase in the sample split hydrogen peroxide which is measured at 240nm. One unit of catalase activity equal the amount of protein that convert 1umol H2O2/min.
- Procedure: 0.2ml of sample was added to phosphate buffer containing 100mm of H2O2, in a total of 1ml.
- Incubate for 2mins at 37'c. Read and record change in absorbance at 240nm, unit u/g

Superoxide Dismutase (Mista and Fridouich Method)

- Principle: The activity of SOD to inhibit the auto-oxidation of epinephrine at PH 10.2 make this reaction a basis for a simple assay for this dismutase.
- Procedure: 0.2ml of sample was diluted in D/W to make a 1:10 dilution, 200ul of the diluted sample was added to 2.5ml of 0.05m carbonate buffer (pH10.2). Then start the reaction by adding 0.3ml of freshly prepared 0.3mm epinephrine to the mixture, which was quickly mixed by inversion. Read and record increase in absorbance at 480nm for 30secs to 2.5mins.
- Unit: u/ml

Glutathione –S- Trasferase (Habig *el al* method)

- Principle: GST demonstrate a relatively high activity with I chloro 2, 4- dinitrobenzene (CDNB). GST actively utilizes CDNB as substrate, when this substrate is conjugated with reduced glutathione, its wavelength is elongated to 340nm.
- Procedure: label the tubes as blank and test.
- Pipette 30ul of GSH (O.1M) to all tubes
- Add 150ul of CDNB (20nm) to all tubes
- Add 2.82ml of 0.1m phosphate buffer to tubes label blank & 2.79ul to the tube labeled test.
- Add 30ul of samples to the tube labeled test
- Allow for 60 seconds and read the absorbance at 340nm. unit µg/ml

Glutathione Peroxidase (Rotruck el at method)

- Principle: The assay is based on the measurement of the residual glutathione remaining after the action of glutathione peroxidase measured at 412nm.
- Unit µg/ml
- Procedure: The assay mixture containing 0.5ml of sodium phosphate buffer, 0.1ml sodium azide 0.2ml of reduced glutathione, 01ml of H2O2 and 0.5ml of 1:10

dilution of sample and 0.6ml of D/W making a total of 2. 0ml.The tubes were incubated at 37'c for 3mins and the reaction was terminated by the addition of 0.5ml of 10% TCA. To determine the residual glutathione content, 1ml of the supernatant was added to 4.0ml of disodium hydrogen phosphate and 1ml of DTNB added. The color developed was read at 412nm.

RESULTS

Table 1. *Mean Weight and Percentage Values Of Control And Test Groups*

Groups	Initial Weight (g)	Final Weight (g)	Weight gain (g)	% Increase	P-values
1	123.01±81.04	128.76±86.58	5.75	4.47	0.02
2	169.35±14.73	147.26±27.46	-22.09	-15.00	0.00
3	198.64±4.66	193.76±9.10	-4.88	-2.52	0.01
4	103.76±117.70	112.50±128.01	8.74	7.77	0.13
5	155.78±102.39	111.00±129.14	-44.78	-40.34	0.00
6	176.00±115.00	171.51±113.74	-4.49	-2.62	0.04
7	241.17±43.53	173.25±116.34	-68.47	-39.52	0.00

Key: group1=control, group2-7 –test groups

Table 2. *Characteristics Of Oxidative Stress Markers In Test Groups And Control*

Groups	GSH (µ/ml)	MDA (µmol/ml)	P-values
1	1.24±0.12	3.34±4.26	0.04
2	2.15±0.42	0.45±0.04	0.22
3	2.59±0.11	0.51±0.06	1.00
4	1.96±0.79	0.37±0.02	1.00
5	1.01±0.19	0.47±0.06	1.00
6	1.68±0.31	0.41±0.06	1.00
7	2.23±0.18	0.44±0.05	0.81

Key: GSH=Glutathione, MDA =Malondialdehyde

Table 3. *Correlation Between GSH Versus MDA*

Variables		R-values	Sig
GSH	MDA	-0.236	0.41
MDA	GSH	-0.236	0.41

No significant correlation identified.

Table 4. *FBS In Oxidative Stress Markers Test*

Groups	Initial FBS(mmol/l)	Final FBS(mmol/l)	Sugar gained(mmol/l)	% Increase
1	4.23±1.41	4.00±0.25	-0.23	-5.75
2	4.23±0.38	3.10±0.14	-1.13	-36.45
3	3.60±0.58	3.30±0.40	-0.13	-9.09
4	4.20±0.71	4.05±0.56	-0.15	-3.70
5	4.53±0.41	4.05±0.65	-0.38	-9.16
6	3.88±0.52	3.40±0.20	-0.48	-14.12
7	3.28±1.28	4.55±0.06	1.27	27.91

Discussion

Glutathione has been reported to exert a useful effect on oxidative stress due to its antioxidant properties. The level of glutathione activities was higher in group3 (2.59µg/ml), group7 (2.23µg/ml), and group2 (2.15 µg/ml) that received 100mg/kg phoenix dactylifera, 500mg/kg fresh cod-liver oil, and 1600mg/kg Cyperus Esculentus daily compared with the control of 1.24 µg/ml. However group4 with (1.96 µg/ml) received 1000mg/kg body weight of soybean daily. Furthermore in grou6 with (1.68 µg/ml) glutathione concentration level was treated at 1000mg/kg Cyperus esculentus,800mg/kg phoenix Esculentus and 800mg/kg soybean daily compared with group5 having the lowest level of glutathione (1.01 µg/ml) that received 1000mg/kg Cyperus Esculentus and 800mg/kg phoenix dactylifera daily.

The Malondialdehyde level decreased among the entire test groups compared with control. Though within the test groups, the MDA level was greater in group3 (0.51 µmol/ml) that received 1000mg/kg phoenix dactylifera fruit only and combination of 1000mg/kg Cyperus Esculentus and 800mg/kg phoenix dactylifera daily compared with other test groups. There was a significant p-value of 0.04 observed among glutathione and Malondialdehyde in the control group. Furthermore, a negative correlation and a non-significant p-value was identified among the GSH and MDA test groups. To overcome the detrimental effect of free radicals the body requires free antioxidants obtain from food such as soybean or Cyperus Esculentus synthesize by the body such as glutathione usually measured in relation to free radicals increased in the body. The content of glutathione as a major antioxidant is present in liver cells predominantly with the thiol group, assuming the role of a proton donor responsible for glutathione biological activities in the body (Abdul *et al*.,2019).The oxidation of lipids nucleic acids, proteins by free radicals result in the production of Malondialdehyde and deoxyguanosine p which disrupt the function of proteins due to the release of aldehyde into the blood to cause damage by lipid peroxidation.

FBS: At the conclusion of this experimental research the final fasting blood sugar level crashes drastically in group2 (-1.13mmol/l) that received 1600mg/kg Cyperus extract daily compared with other groups. More so group6 that received the combination of both Cyperus Esculentus, phoenix dactylifera, and soybean extracts at 1000mg/kg, 800mg/kg and 800mg/kg daily has a decrease fasting blood sugar of -0.48mmol/l. This was closely followed by group5 with -0.38mmol/l compared with the control (0.23mmol/l) group. Similar decrease in sugar level have been reported by Solomon *et al,* (2023). However, there was an increase sugar gain in group7 (1.27mmol/l) that received 500mg/kg standard drug daily compared with other test groups and the control.

Weight: There was a notable decrease in the final weight among the test groups but especially in group7 (-68.47g) that was given 500mg/kg standard drug (cod liver oil) and group5 (-44.78g) administered 1000mg/kg Cyperus Esculentus in addition with 800mg/kg phoenix dactylifera extract daily. However, there was an increase observed in group4 (8.74g) final weight that received 1000mg/kg body weight of the extract daily with a percentage increase of 7.77% compared with the control of 4.47% increase.

Conclusion

This study shows improvement in the levels of serum glutathione, an important antioxidant and a useful biomarker of oxidative stress. There was a decrease in Malondialdehyde level among the test groups compared with the control and a crash in fasting blood sugar level among all the test groups except in group7 that received the standard drug.

Conflict of interest: There is no conflict of interest among the authors

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