Online ISSN: 2249-4618
Print ISSN: 0975-5888

Global Journal of Medical Research: B

Pharma, Drug Discovery, Toxicology and Medicine

Highlights

- Genotypes of Human
- Correlation of Protein
- Oxidative Stress Induced
- Activity of Three Erythrocyte

Discovering Thoughts, Inventing Future

Volume 14 Issue 6 Version 1.0

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Volume 14 Issue 45 was originally issued as B6

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine

Volume 14 Issue 45
Originally issued as B6

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine

Volume 14 Issue 6 (Ver. 1.0)

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 6 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Comparative Analysis of Antiglycation Capacity of Aqueous and Methanolic Extracts of Vegetables

By Bilal Ahmed, Muhammad Wasim Ashraf, Abdul Ghaffar, Farah Latif & Zahid Mahmood

University of Agriculture, Pakistan

Abstract- Glycation is a reaction between amino group of blood proteins and reducing sugars in vitro conditions which are involved in a number of pathologies and disease states including Alzheimer's and diabetes. Equal concentration of different inhibitor extracts (sweet potato, turnip and methi) and glucose were used. Eight combinations of each extract were made and all these were placed at 37oC for five weeks incubation. Human normal plasma was used as a protein source. Glycation was analyzed by Thiobarbituric acid (TBA) technique which results that aqueous and methanol extracts of sweet potato and turnip showed no inhibition of non-enzymatic glycation but act as activator of reaction while aqueous extract of methi showed maximum inhibition of non-enzymatic glycation in 5th week of incubation and for methanol extract inhibition was maximum in 3rd week of incubation. In all extracts of three vegetables, extracts of methi were more effective against non-enzymatic glycation. These findings suggest that in future methi can be used for lowering glucose level in the body as it is efficient in lowering the glycation level in different conditions when level of glucose is high.

GJMR-B Classification : NLMC Code: QV 38.5

COMPARATIVEANALYSISOFANTIGLYCATIONCAPACITYOFAQUEOUSAND METHANOLICEXTRACTSOFVEGETABLES

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Comparative Analysis of Antiglycation Capacity of Aqueous and Methanolic Extracts of Vegetables

Bilal Ahmed [α], Muhammad Wasim Ashraf [σ], Abdul Ghaffar [ρ], Farah Latif [ω] & Zahid Mahmood [¥]

Abstract- Glycation is a reaction between amino group of blood proteins and reducing sugars in vitro conditions which are involved in a number of pathologies and disease states including Alzheimer's and diabetes. Equal concentration of different inhibitor extracts (sweet potato, turnip and methi) and glucose were used. Eight combinations of each extract were made and all these were placed at 37ºC for five weeks incubation. Human normal plasma was used as a protein source. Glycation was analyzed by Thiobarbituric acid (TBA) technique which results that aqueous and methanol extracts of sweet potato and turnip showed no inhibition of non-enzymatic glycation but act as activator of reaction while aqueous extract of methi showed maximum inhibition of non-enzymatic glycation in 5th week of incubation and for methanol extract inhibition was maximum in 3rd week of incubation. In all extracts of three vegetables, extracts of methi were more effective against non-enzymatic glycation. These findings suggest that in future methi can be used for lowering glucose level in the body as it is efficient in lowering the glycation level in different conditions when level of glucose is high.

I. Introduction

Non-enzymatic glycation (glycosylation) is a multistage condensation reaction starting between reducing sugar and amino group (mainly in Lys and Arg) of different proteins (Stoynev *et al.*, 2004) there are twofold meaning of non-enzymatic glycation: on one hand, early glycation product measurement which give estimation of glucose exposure and previous metabolic control of the subject; while on the other hand, intermediate and the late glycation reaction products measurement (Lapolla *et al.*, 2005) ending up with complex heterocyclic compound formation called advanced glycation end products (AGEs) (Stoynev *et al.*, 2004) lead in progression of atherosclerosis, Alzheimer's (Stoppa *et al.*, 2006) and particularly in diabetes mellitus which is a endocrine disorder (Forbes *et al.*, 2004) characterized by hyperglycemia and many chronic complications affecting the blood vessels, eyes, skin, nerves, and kidneys (Ahmad and Ahmed, 2006). Non-enzymatic glycosylation (Glycation) process, also known as Maillard reaction, (Hatfield, 2007) may contribute to formation of discoloration, off-flavors and decreased nutritional value (Nursten, 2005).

The intermediate appearance leads to the Amadori compound formation (an aldosylamine; aldose initial reaction with amino groups results in the formation of Schiff's base, which slowly rearrange itself for the production of 1-amino-1-deoxyketose, an aldosylamine) occurs in glycation early stages, however in late stage of glycation, irreversible formation of advanced glycation end products (AGEs) occur after a repeated reactions complex cascade as condensation, cyclization, dehydration, fragmentation and oxidation (Kikuchi *et al.*, 2003). A state hyperglycemia found in diabetes, where non-enzymatic glycation, lipid oxidation and oxidation of protein occur. As a result, accumulation of advanced glycation end product (AGEs) in diabetic subject's tissues and the plasma. Accumulation of this AGE has been linked to pathogenic complication the development in diabetes (Lalla *et al.*, 2001).

II. Materials and Methods

Research work was planned to find out the inhibition of glycation with natural inhibitor i.e. Sweet potato, turnip and methi.

a) Selection of Conditions and concentrations

To study the inhibitory effects on glycation or glycation inhibition *invitro*, eight combinations of each inhibitor were made with plasma and glucose, and were placed at 37ºC for five weeks (Zhang and Swaan, 1999). Plasma was used as a protein source. Samples were drawn after 1st, 2nd, 3rd, 4th and 5th week of incubation to perform the experiments for glycation and glycation inhibition. Along with temperature (37ºC) different concentrations of glucose and inhibitor were used.

b) Estimation of Browning

Browning was estimated by taking absorbance at 370nm using spectrophotometer. After every week one sample was drawn and took 0.1 ml from it. Rest of the sample was kept in refrigerator at -20ºC. In 0.1 ml of sample 4ml of distilled water was added and 4.1 ml volume was obtained. Then absorbance was taken at 370nm by spectrophotometer. Blank samples will be run with each condition of glucose and inhibitor concentration.

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c) Total proteins estimation (g/dL)

Total proteins in all samples before and after dialysis were determined by Biuret method using Biuret reagent (Gornall *et al.*, 1949). 1ml of Biuret reagent was added in blank, standard and all samples tubes. Placed the tubes at 37°C for 15 minutes and reading was taken at 540nm. The standard curve was made with the half of absorbance of standard solution.

III. Dialysis

Glycated plasma samples were dialyzed against dist. H2O for twenty-four hours with constant stirring at room temperature to remove the free glucose by using dialyzing membrane.

a) Measurement of Glycation level

The glycation level was measured by TBA method (Furth, 1988).

b) Thiobarbituric acid (TBA) colorimetric technique

TBA technique (Furth, 1988) was used for the determination of both enzymatic and non-enzymatic glycation. The standard curve was made by using fructose standard solution.

IV. Results and Discussion

a) Estimation of Browning

Combination of plasma with buffer and glucose showed maximum browning (0.233) at 1st week of incubation while value of browning decreases to (0.196) at 2nd week. In 3rd week of incubation was at its minimum value (0.184). In the 4th week it increases to (0.229) and in the 5th week browning was (0.221). In the next combination of plasma with inhibitor sweet potato, glucose and buffer gives maximum level of browning. Plasma with buffer and glucose combination showed browning (0.168) at 1st week of incubation while value of browning moves to maximum which was (0.177) at 2nd week. In 3rd week, incubation was at its minimum value (0.148). In the 4th week it increases to (0.158) and in the 5th week browning was (0.152). Combination of plasma with Turnip as inhibitor, glucose and buffer in the next showed maximum browning in the 1st week of incubation which was (0.582) then it move to its lowest value of combination which was (0.307) in the 2nd week. In the 3rd week it gets (0.368) then in the 4th week it was (0.353) and it shows 2nd highest value of browning in the 5th week which was (0.385). Combination of plasma with buffer and glucose showed maximum browning (0.286) at 1st week of incubation while value of browning moves to minimum of its combination which was (0.253) at 2nd week. In 3rd week of incubation it starts increasing gradually which was (0.259).

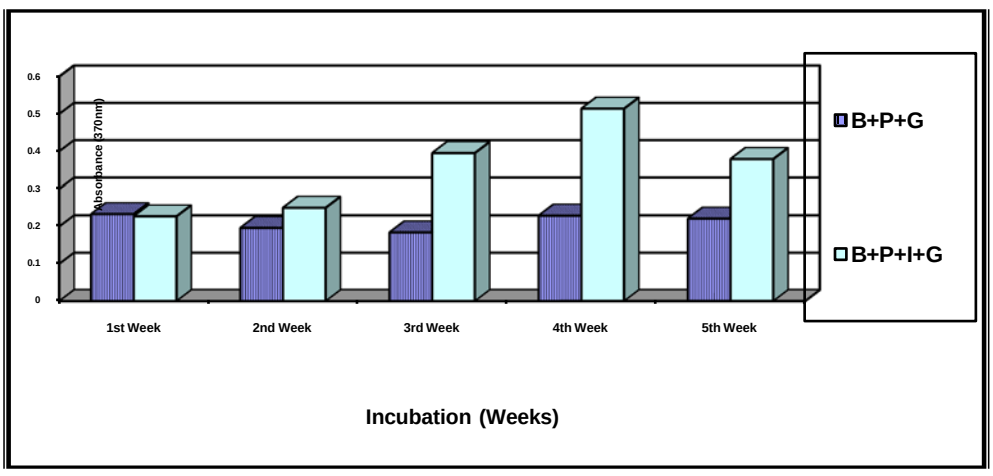

Figure 1: Determination of Browning by the Aqueous Extract of Sweet Potato (S P) at 37°C

In the 4th week it gets (0.265) and in the 5th week of incubation browning was (0.276). In the next combination of plasma with Methi as inhibitor, glucose and buffer showed browning in the 1st week of incubation which was (0.196) then it move to its maximum value of combination which was (0.225) in the 2nd week. Combination of plasma with buffer and glucose showed browning (0.155) at 1st week of incubation while value of browning increases to (0.161) at 2nd week. In 3rd week of incubation browning moves to maximum of combination which was (0.191). In the next combination of plasma with Turnip as inhibitor, glucose and buffer showed browning in the 1st week of incubation (0.565) then the value of browning increases to (0.635) in the 2nd week. In the 3rd week it was lowest of combination (0.478) then in the 4th week it showed highest browning of its combination (0.673) and value of browning in the 5th week was (0.512).

b) Thiobarbituric Acid Test

Incubation of plasma with glucose and buffer showed maximum glycation level at 1st week of

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combination which was (.365 mole/mole) while decreased glycation level (.280 mole/mole) recorded in 2nd week. Combination of plasma, sweet potato as inhibitor, glucose and buffer showed highest value of glycation (.646 mole/mole) at 3rd week of incubation which gradually decreases in coming two weeks. In case of glycation inhibition, inhibitor act as activator of glycation reaction as it showed minimum value (.394 mole/mole) in 1st week of incubation. Incubation of plasma with glucose and buffer showed maximum glycation level at 4th week of combination which was (.274 mole/mole) while decreased in glycation level (.169 mole/mole) recorded in 1st week. Combination of plasma, turnip as inhibitor, glucose and buffer showed highest value of glycation (.908 mole/mole) at 3rd week of incubation which decreases in coming week. In case of glycation inhibition, inhibitor act as activator of glycation reaction as it showed minimum value (.572 mole/mole) in 4th week of incubation.

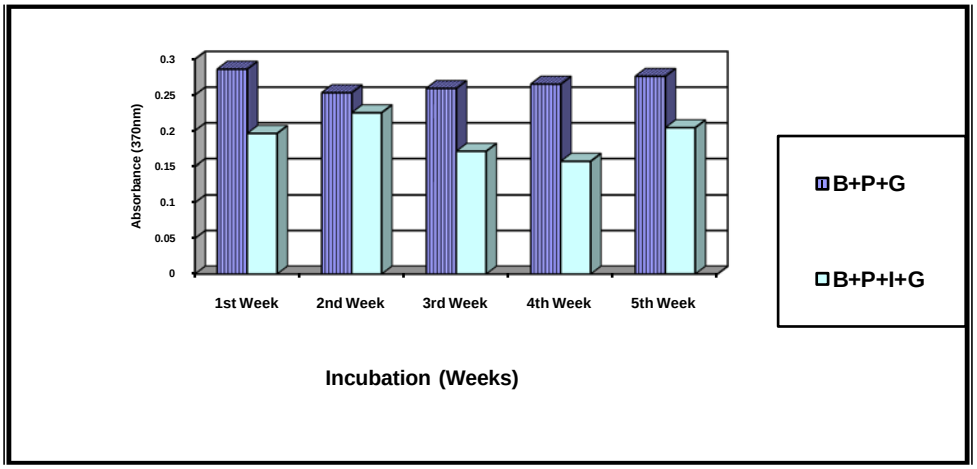

Figure 2 : Determination of Browning by the Aqueous Extract of Methi (M) at 37°C

Incubation of plasma with glucose and buffer showed maximum glycation level at 5th week of combination which was (.342 mole/mole) while decreased glycation level (.274 mole/mole) recorded in 4th week of incubation. Combination of plasma, methi as inhibitor, glucose and buffer showed highest value of glycation (.266 mole/mole) at 4th week of incubation with a gradual increase from 1st week.

V. Conclusion

In case of non-enzymatic glycation, methanol extract of methi showed maximum inhibition of glycation in 3rd week of incubation as compare to aqueous extract which showed minimum value of inhibition in 5th week of incubation. On thorough study it is concluded that methanol extract of methi is more effective in glycation inhibition.

VI. Discussion

Bierhaus *et al.* (1998) explored that products mostly derived from carbohydrate starts accumulating in tissue proteins at high rate with increasing age and in diabetes which are products of oxidation and glycation reaction. Marles and Farnsworth, (1995) demonstrated that the hypoglycaemic activity of *Trigonella foenum-graecum* is because of its active components chemical nature of. Chemical compounds isolated from *Trigonella foenum-graecum* include alkaloids, saponins and steroids etc. Zia *et al.* (2001) said that *Trigonella foenum-graecum* (Fenugreek) (Leguminosae) is also being used as an herbal medicine. Seeds of *Trigonella foenum-graecum* are known for their antidiabetic, tonic carminative effects. The oral route of administration for methanolic extract produced hypoglycaemic effect at the dose of 1 g: kg body weight. In aqueous and methanolic extract, presence of hypoglycaemic activity is because of active compounds which are polar in nature.

Reference Références Referencias

1. Ahmad and Ahmed (2006) demonstrated that diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eyes, nerves, blood vessels, skin, and kidneys.
2. Ahmed, N. and A. J. Furth. 1992. Failure of common glycation assays to detect glycation by fructose. Clin. Chem. 38:1301-1303.
3. Bierhaus, A., M. A. Hofmann, R. Ziegler and P. P. Nawroth. 1998. Diabetes and glycation. Cardiovascular Research. 37: 586-600.
4. Forbes., (2004) demonstrated that advanced glycation end product (AGE) formation may contribute to the progression of atherosclerosis, particularly in diabetes.
5. Furth, A. J. 1988. Methods for assaying nonenzymatic glycosylation. Anal Biochem. 175(2): 347-360.

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6. Gillery, P. 2001. Advanced glycation end products (AGEs), free radicals and diabetes. Journal of Social Biology. 4: 387-390.
7. Gornall, A. G., C. S. Bardwill and M. M. David. 1949. Determuination of serum proteins buy means of biuret reactions J. Biol. Chem. 177: 571-766.
8. Grover, J. K., S. Yadav and V. Vats. 2002. Evaluation of antihyperglycemic and hypoglycemic effect of Trigonella foenum-graecum Linn, Ocimum sanctum Linn and Pterocarpus marsupium Linn in normal and alloxanized diabetic rats. Journal of Ethnopharmacol. 79: 95-100.
9. Hatfield (2007) stated that Glycation (nonenzymatic glycosylation) processes, also known as the Maillard reactions, are a series of reactions between carbohydrates and free amino groups of proteins.
10. Kikuchi, S., K. Shinpo, M. Takeuchi, S. Yamagishi, Z. Makita, N. Sasaki and K. Tashiro. 2003. Glycation—a sweet tempter for neuronal death. Brain Research Reviews. 3: 306–323.
11. Lalla, E., I. R. Lasmaster and A. M. Schmidt. 2001. Receptors of advanced glycation end products inflammation and accelerated periodontal disease in diabetes. Ann. Periodontal. 6(1): 113-118.
12. Lapolla and joney j. (2005) non-enzymatic glycation has a twofold meaning: on one hand, measurement of early glycation products can estimate the extent of exposure to glucose and the subject's previous metabolic control; on the other hand, measurement of intermediate and late products of the glycation reaction is a precious instrument in verifying the relationship between glycation products and tissue modifications.
13. Makita, Z., S. Radoff, E. J. Rayfield, Z. Yang, E. Skolnik, V. Delaney, E. A. Friedman, A. Cerami and H. Vlassara. 1999. Advance glycosylation end products in patients with diabetic nephropathy. Journal of Diabetes Complications. 325: 836-842.
14. Marles, R. J. and N. R. Farnsworth. 1995. Antidiabetic plants and their active constituents. Phytomedicine. 2: 137-189.
15. Morten A.k and Bernargi E. (2006) explained that glycation is a non-enzymatic process in which proteins react with reducing sugar molecules and thereby impair the function and change the characteristics of the proteins. Glycation is involved in diabetes and aging where the accumulation of glycation products causes side effects.
16. Nursten. (2005) said that Maillard reactions are a complex set of reactions, typically occurring between carbonyl compounds and amino groups originating from proteins, peptides or amino acids. Maillard reactions may lead to formation of off-flavours, decreased nutritional value and discolouration.
17. Stoppa. (2006) found that non-enzymatic glycation is implicated in the development of various diseases such as Alzheimer's and diabetes mellitus. An increase in the generation of reactive oxygen species can occur by non-enzymatic glycation and glucose autoxidation.
18. Stoynev S. and Ahmed D. (2004) said that non-enzymatic glycosylation (glycation) of proteins is a multistage chemical process starting as a condensation reaction between reducing sugars and primary amino groups (mainly from the side chains of Lis and Arg) and ending up with formation of complex heterocyclic compounds called advanced glycation end products (AGEs).
19. Thomas E. and Morey C. (2005) studied prolonged hyperglycemia, dyslipidemia and oxidative stress in diabetes result in the production and accumulation of AGEs.
20. Zhang, E. Y. and P. W. Swaan. 1999. Determination of Membrane protein Glycation in Diabetic Tissue. AAPS Pharmaceutical Sciences. 1: 20-35.
21. Zia, T., S. N. Hasnain and S. K. Hasan. 2001. Evaluation of the oral hypoglycaemic effect of *Trigonella foenum-graecum* L. (methi) in normal mice. Journal of Ethnopharmacology. 75: 191-195.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 6 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Glutathione S-Transferase Activity of Three Erythrocyte Genotypes of Human Participants Treated with Pyrimethamine/Sulphadoxine and Quinine

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Keywords: glutathione S-transferase activity, erythrocy-tes, pyrimethamine/sulphadoxine, quinine, 1-chloro-2, 4- dinitrobenzene (CDNB).

GJMR-B Classification : NLMC Code: QV 4

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Glutathione *S*-Transferase Activity of Three Erythrocyte Genotypes of Human Participants Treated with Pyrimethamine/Sulphadoxine and Quinine

Paul Chidoka Chikezie

Abstract- Studies to ascertain levels of erythrocyte glutathione *S*-transferase (Ery-GST) activity of non-malarious and malarious male participants of HbAA, HbAS and HbSS erythrocyte genotypes treated with pyrimethamine/sulphadoxine mixture and quinine were carried out. Incubation of erythrocytes with 1-chloro-2, 4-dinitrobenzene (CDNB) caused quantitative conjugation of reduced glutathione (GSH) to produce S-(2, 4-dinitrophenyl) glutathione, which formed the bases for the measurement of Ery-GST activity using a spectrophotometer. Blood samples were drawn from treated non-malarious and malarious participants at time intervals of 0, 3, 6 and 18 h and measured for Ery-GST activity. The control values of Ery-GST activity of non-malarious and malarious participants were within the ranges of 3.27 ± 0.13 – 12.50 ± 1.58 IU/gHb and 2.75 ± 0.16 – 12.21 ± 1.20 IU/gHb respectively. Ery-GST activity of malarious participants was significantly ($p < 0.05$) lower than that of the malarious participants, except that of parasitized HbSS erythrocytes. Generally, Ery-GST activities in the presence of the two antimalarials exhibited a biphasic profile. The first phase showed decreasing levels of Ery-GST activity at $t < 6$ h following the administration of pyrimethamine/sulphadoxine mixture and quinine to the non-malarious and malarious participants. In the second phase, Ery-GST activity increased when experimental $t > 6$ h. The overall pattern of Ery-GST activity within the experimental time ($0 < t < 18$ h) showed evidence of antimalarial induced disturbance of erythrocyte homeostasis, which could be of relevance from toxicological standpoints and for monitoring therapeutic events in malarial disease.

Keywords: glutathione S-transferase activity, erythrocytes, pyrimethamine/sulphadoxine, quinine, 1-chloro-2, 4-dinitrobenzene (CDNB).

I. Introduction

Pyrimethamine (250 mg)/sulphadoxine (50 mg) mixture is commonly used for prophylaxis and treatment of certain strains of *Plasmodium falciparum* that are resistant to chloroquine (Bray *et al.,* 1998) and usually sold under the trade mark name of Fansidar™. The drug combination effectively blocks two enzymes involved in the biosynthesis of folinic acid within the parasite (Milhous *et al.,* 1985). Quinoline blood schizontocides behave as weak bases when concentrated in food vacuoles of susceptible *Plasmodia*, where it causes increase in vacuolar pH, inhibits peroxidase activity of haem and thereby, disrupts non-enzymatic polymerization of ferroprotoporhyrin IX (FPIX) - haemin to haemozoin. The failure to inactivate FPIX kills the parasite via oxidative damage to membranes, digestive proteases and possibly other critical biomolecules of the parasite (Ducharme and Farinotti, 1996).

Enzyme studies have revealed a collection of protein molecules with common characteristic high affinity for reduced glutathione (GSH). Several of these protein molecules have been isolated from rat and human liver (Ketley *et al.,* 1975; Awasthi*et al.,* 1981; Hayes and Pulford, 1995), pigeon, locust gut, housefly and other sources (Ketley*et al.,* 1975) and characterized. These protein molecules are classified based on their enzymatic activities as glutathione *S*-transferase (GST: EC: 2.5.1.18) (Jacoby, 1976). The functions of GSTs are classified into two general categories (Harvey and Beutler, 1982).As intracellular binding proteins (Mannervik and Danielson, 1988; Hiller *et al.,* 2006), GSTs on a broad scale function as solubilizing and transport proteins, analogous to the extracellular functions of albumin (Boyer and Oslen, 1991; Oakley *et al.,* 1999). Also, GSTs catalyze the conjugation of electrophilic groups of hydrophobic drugs and xenobiotics to form glutathione-thioethers (Board *et al.,* 1990). Thio-ethers are eventually converted to mercapturic acid by the sequential actions of gamma-glutamyl transpeptidase, depeptidase and N-acetylase (Habig*et al.,* 1974; Mannervik and Danielson, 1988).

GST activity has been implicated in the acquisition of drug resistance (Black and Wolf, 1991). However, the role of GST activity in malaria resistance has not been studied, except by Dubois *et al.,* (1995) who reported that drug-resistant *Plasmodium berghei* resulted from altered GST activity (Srivastava *et*

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al., 1999). The present study ascertained the comparative levels of erythrocyte glutathione *S*-transferase (Ery-GST) activity of non-malarious and malarious male participants of HbAA, HbAS and HbSS erythrocyte genotypes treated with pyrimethamine/ sulphadoxine mixture and quinine.

II. Materials and Methods

a) Anti-malarials

Fansidar™ (Swiss (Swipha) Pharmaceuticals Nigeria Ltd) and quinine (BDH, UK), were purchased from Cimpok Pharmaceuticals, Amakhohia, Owerri, Nigeria.

b) Selection of participants/experimental design

Fifteen (n = 15) malarious males (59 - 79 kg) infected with antimalarial susceptible strain of *Plasmodium falciparum* and twenty (n = 20) non-malarious male participants (61 - 73 kg), both of confirmed HbAA, HbAS and HbSS genotypes enrolled for this study. The malarious participants were individuals attending clinics at the Federal Medical Center (FMC), St. John Clinic/Medical Diagnostic Laboratories, Avigram Medical Diagnostic and Research Laboratories, and Qualitech Medical Diagnostic Laboratories. All laboratory investigations were carried out at Avigram Medical Diagnostic and Research Laboratories, Owerri, Imo State, Nigeria. The malarious participants were within the age brackets of 21 - 34 years old, whereas the non-malarious participants were within the age brackets of 20 - 28 years old. All participants were administered with single dose of pyrimethamine/sulphadoxine mixture and quinine, each according to the following specifications, [pyrimethamine] = 14.9 mg/kg: [sulphadoxine] = 2.9 mg/kg and [quinine] = 5.9 mg/kg. Specifically, nine (n = 9) and six (n = 6) of the malarious participants received pyrimethamine/sulphadoxine mixture and quinine respectively. For comparative study, the 20 non-malarious participants were administered with the same doses of the two antimalarials, of which eleven (n = 11) and nine (n = 9) of the participants received pyrimethamine/sulphadoxine mixture and quinine respectively.

The participants were randomly selected between June and August 2012. Exclusion criteria include; gastrointestinal tract infection, protein energy malnutrition, renal diseases, cirrhosis, hepatitis, obstructive jaundice, cancer, diabetes mellitus, hypertension, obesity, smoking, alcoholism, persons living with HIV, patients taking anti-malaria drugs and vitamin supplements, patients who have been treated for malaria in the past 2 months (Onyesom and Onyemakonor, 2011; Idonije*et al.,* 2011).

c) Ethics

The Ethical Committee of University of Port Harcourt, Port Harcourt, Nigeria, approved the study in compliance with the Declaration on the Right of the Patient (WMA, 2000). Before enrolment for the study, the patients/participants involved signed an informed Consent Form.

d) Collection of blood specimen and preparation of erythrocyte haemolysate

Blood samples were drawn, using 5.0 mL capacity disposable syringes, from treated non-malarious and malarious participants at time intervals of 0, 3, 6 and 18 h. Erythrocytes were separated from the blood samples and washed by centrifugation methods of Tsakiris *et al.,* (2005) with modifications according to Chikezie, (2011). Within 15 min of collection of blood samples, portions of 3.0 mL of the samples were introduced into centrifuge test tubes containing 3.0 mL of buffer solution pH = 7.4: 250 mM tris (hydroxyl methyl) amino ethane-HCl (Tris-HCl)/140 mM NaCl/1.0 mM $MgCl_2$/10 mM glucose). The erythrocytes were separated from plasma by centrifugation at 1200 x *g* for 10 min and washed 3 times by the same centrifugation method with the buffer solution. The pelleted erythrocytes were re-suspended in 3.0 mL of phosphate buffer saline (PBS) solution and passed twice through newly packed columns (3.5 cm in a 30 mL syringe) of cellulose-microcrystalline cellulose (ratio 1:1; *w/w*) to obtain erythrocyte suspension sufficiently devoid of leucocytes and platelets. The pelleted erythrocytes were finally re-suspended in 6.0 mL of PBS to obtain approximately 10% haematocrit according to Chikezie *et al.,* (2012). A 2.0 mL portion of the separate pelleted erythrocyte genotypes were lysed by freezing/thawing as described by Galbraith and Watts, (1980) and Kamber *et al.,* (1984). The erythrocyte haemolysate was used for the measurement Ery-GST activity.

e) Malaria parasite density test

Portion of 2.0 mL of the blood samples were collected into EDTA bottles for malaria parasite tests. Measurement of parasite density of peripheral blood smear was by Giemsa stained techniques. The films were examined microscopically using ×100 objective under oil immersion (Cheesbrough, 1998). Participants with parasitaemia levels within the range of 1000 to 9999/µL were used for the present study.

f) Erythrocytes haemolysate haemoglobin concentration

A modified method (Baure, 1980), based on cyanomethaemoglobin reaction was used for the determination of haemolysate haemoglobin concentration. The expressed values were in grams per deciliter (g/dL). A 0.05 mL portion of erythrocyte haemolysate

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NaCN and 300 mg $K_4Fe(CN)_6$ per liter). The mixture was left to stand for 10 min at room temperature ('25' '±'and '5°C') and absorbance read at λmax = 540 nm against a blank. The absorbance was used to evaluate haemolysate haemoglobin concentration by comparing the values with the standards.

g) Erythrocyte glutathione S-transferase

Ery-GST activity was measured by the method of Habig *et al.,* (1974) as described by Pasupathi *et al.,* (2009) with minor modifications according to Chikezie *et al.,* (2009). The reaction mixture contained 1.0 mL of 0.3 mM phosphate buffer (pH = 6.5), 0.1 mL of 30 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 1.7 mL of distilled water. After pre-incubating the reaction mixture at 37°C for 5 min, the reaction was started by the addition of 0.1 mL of erythrocyte haemolysate and 0.1 mL of GSH substrate. The absorbance was measured at time intervals of 30 s for 5 min at λmax = 340 nm. Ery-GST activity was expressed in international unit per gram haemoglobin (IU/gHb) using an extinction coefficient (∑) of 9.6 mM^{-1} cm^{-1} in a reaction mixture in which 1 mole of GSH was oxidized (Equation 1).

Calculation of Ery-GST activity

$$E_A = \frac{100}{(Hb)} \times \frac{OD/min}{\Sigma} \times \frac{V_C}{V_H} \quad \textit{Equation 1}$$

Where,

E_A = Enzyme activity in IU/gHb

[Hb] = Haemolysate haemoglobin concentration (g/dL)

OD/min = Change per min in absorbance at 340 nm.

V_C = Cuvette volume (total assay volume) = 3.0 mL.

V_H = Volume of haemolysate in the reaction system (0.05 mL).

h) Statistical analyses

The experiments were designed in a completely randomized method and data collected were analyzed by the analysis of variance procedure while treatment means were separated by the least significance-difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 version (2006). The correlation coefficients between the results were determined with Microsoft Office Excel, 2010 version.

III. Results

The reference values of Ery-GST activity of male participant of HbAA, HbAS and HbSS genotypes is presented in Table 1. Ery-GST activity of the human erythrocyte genotypes was in the order: HbSS > HbAS > HbSS. The control values of Ery-GST activities of non-malarious and malarious male participants were within the ranges of 3.27 ± 0.13 – 12.50 ± 1.58 IU/gHb and 2.75 ± 0.16 – 12.21 ± 1.20 IU/gHb respectively.

Table 1 : Erythrocyte glutathione *S*-transferase activity of male participants administered with pyrimethamine/sulphadoxine mixture and quinine at *t* = 0 h

	Ery-GST Activity (IU/gHb)			
	NMAL		MAL	
Genotype	PS (*n* = 11)	Q (*n* = 9)	PS (*n* = 9)	Q (*n* = 6)
HbAA	3.40 ± 0.05[b,c]	3.27 ± 0.13[b,c]	2.81 ± 0.76[b,c]	2.52 ± 0.23[b,c]
HbAS	4.25 ± 0.10[b]	4.30 ± 0.07[b]	2.75 ± 0.16[b]	2.79 ± 0.11[b]
HbSS	12.50 ± 1.58[a]	11.65 ± 1.20[a]	12.19 ± 1.76[a]	12.21 ± 1.13[a]

Means with the different letters are significantly different at p > 0.05. NMAL: non-malarious participants; MAL: malarious participants; PS: pyrimethamine/sulphadoxine mixture; Q: quinine; n: number of male participants.

A comparative overview of Ery-GST activities of three erythrocyte genotypes of participants treated with pyrimethamine/sulphadoxine mixture and quinine, within the experimental time intervals of 0 h < *t* < 18 h, are summarized in Figures 1, 2 and 3. The Ery-GST activity was presented as relative enzyme activity (%) at the given experimental time intervals to that of the enzyme activity at *t* = 0 h.

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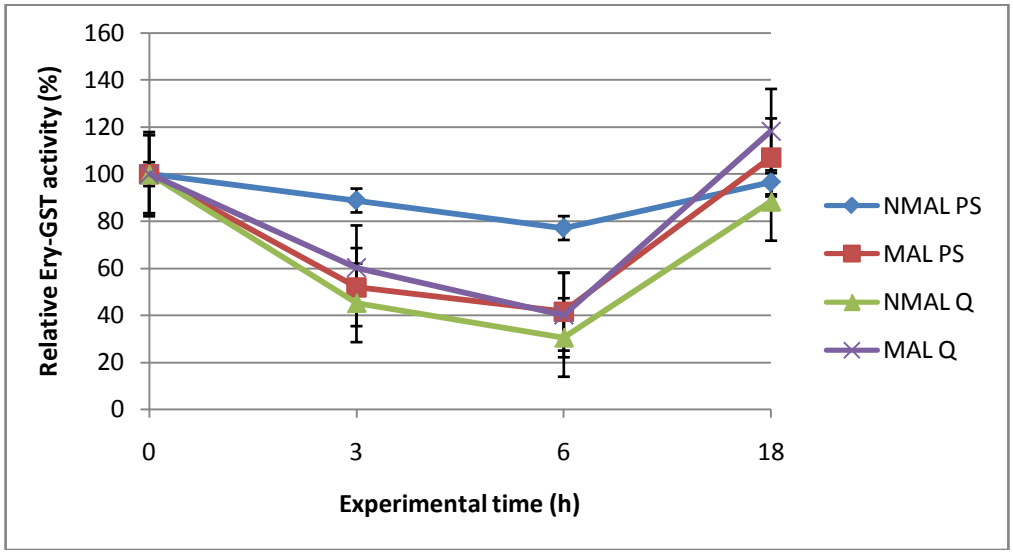

Figure 1 : Relative erythrocyte glutathione *S*-transferase activity of HbAA genotype of male participants administered with pyrimethamine/sulphadoxine mixture and quinine. NMAL PS: non-malarious male participants administered with pyrimethamine/sulphadoxine mixture; MAL PS: malarious male participants administered with pyrimethamine/sulphadoxine mixture; NMAL Q: non-malarious male participants administered with quinine; MAL Q: malarious male participants administered with quinine

Prior to administration of the two antimalarials to the participants, Ery-GST activities of the three genotypes were in the increasing order: HbSS > HbAS > HbSS. The profiles of Ery-GST activities of the three erythrocyte genotypes were irrespective of malarial status of the participants. However, there was no significant difference ($p > 0.05$) in Ery-GST activity between HbAA and HbAS erythrocytes (Figures 1 and 2). Furthermore, Ery-GST activities of parasitized erythrocytes were significantly ($p < 0.05$) lower than that of non-malarious participants, except Ery-GST activity of HbSS erythrocyte genotype.

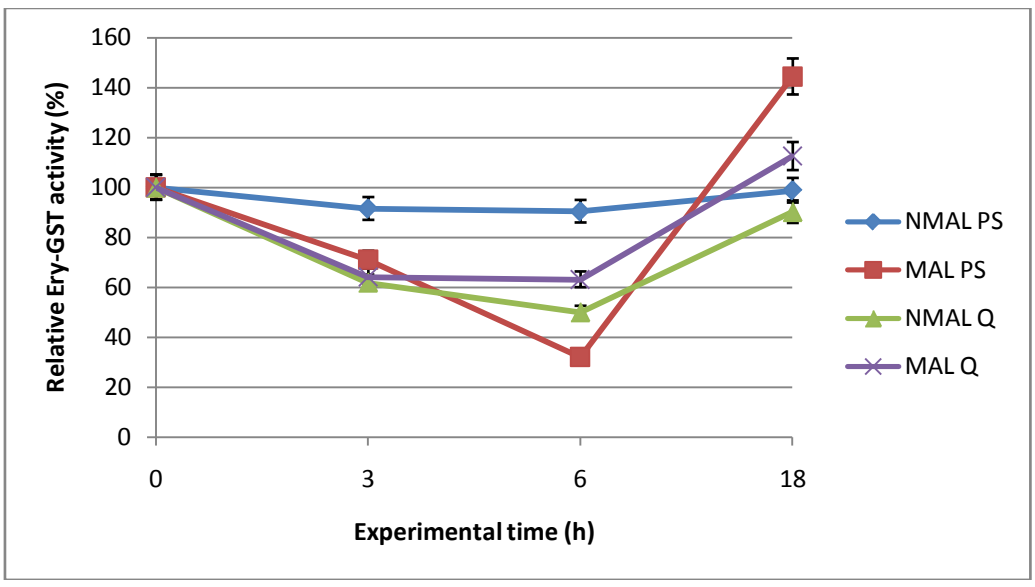

Figure 2 : Relative erythrocyte glutathione S-transferase activity of HbAS genotype of male participants administered with pyrimethamine/sulphadoxine mixture and quinine. NMAL PS: non-malarious male participants administered with pyrimethamine/sulphadoxine mixture; MAL PS: malarious male participants administered with pyrimethamine/sulphadoxine mixture; NMAL Q: non-malarious male participants administered with quinine; MAL Q: malarious male participants administered with quinine

Ery-GST activity of malarious participants of HbAS genotype gave the lowest level enzyme activity at $t = 6$ h following the administration of quinine (Figure 2), whereas malarious participants of HbSS genotype gave peak Ery-GST activity = 12.58 ±1.50 IU/gHb at $t = 18$ h (Figure 3).

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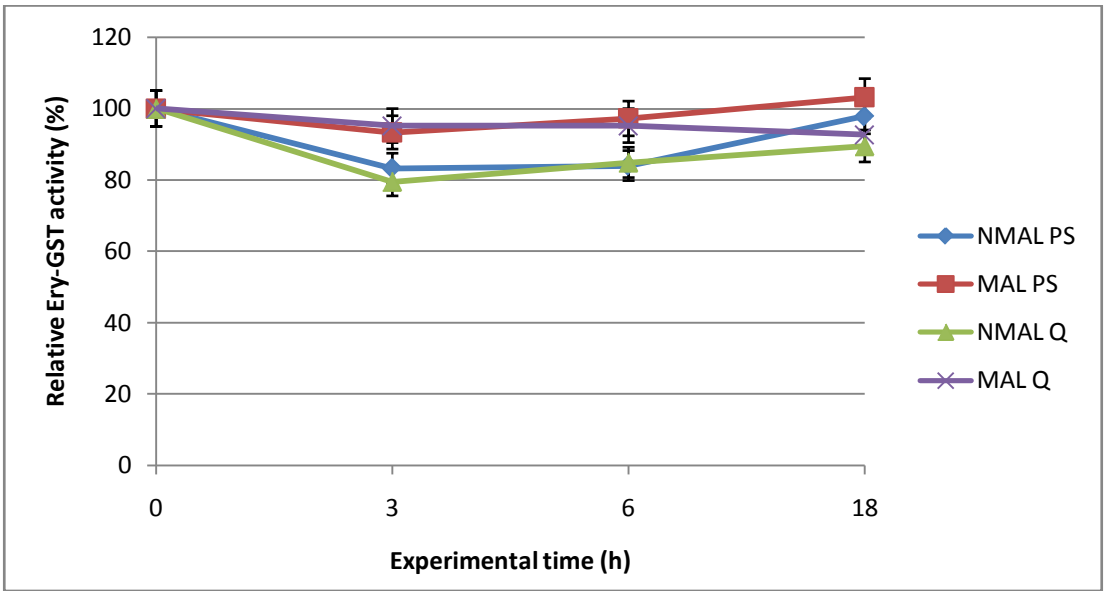

Figure 3 : Relative erythrocyte glutathione S-transferase activity of HbSS genotype of male participants administered with pyrimethamine/sulphadoxine mixture and quinine. NMAL PS: non-malarious male participants administered with pyrimethamine/sulphadoxine mixture; MAL PS: malarious male participants administered with pyrimethamine/sulphadoxine mixture; NMAL Q: non-malarious male participants administered with quinine; MAL Q: malarious male participants administered with quinine

Generally, the patterns of Ery-GST activities in the presence of the two antimalarials exhibited a biphasic profile. The first phase showed decreasing levels of Ery-GST activity within $t < 6$ h following the administration of pyrimethamine/sulphadoxine mixture and quinine to the non-malarious and malarious male participants. In the second phase, Ery-GST activity increased when the experimental $t > 6$ h.

IV. Discussion

Human GST activity, though not routinely assayed in clinical laboratories, could serve as a useful marker enzyme in diagnostic pathology. For instance, over-expression of GST in erythrocytes of patients with chronic renal failure (Galli *et al.,* 1999) and uremia (Galli *et al.,* 1999; Carmagnol *et al.,* 1981) have received immense attentions and documentations. Patients with hepatocellular damage present elevated plasma GST activity (Mulder *et al.,* 1999; Beckett and Hayes, 1993). In addition, low GST activity consequent upon impaired placental detoxification pathways may represent a risk factor for recurrent early pregnancy loss (Zusterzeel *et al.,* 2000) and as an indicator of oxidative stress at birth (Neefjes *et al.,* 1999). The level of expression of GST could provide useful diagnostic parameter in carcinoma of the breast (Forrester *et al.,* 1990) and bladder (Engel *et al.*, 2002).

The present report showed that Ery-GST activity of the human erythrocyte genotypes was in the order: HbSS > HbAS > HbSS (Table 1), which was in concordance with previous findings (Anosike *et al.,* 1991). According to Shalev *et al.,* (1995), comparative raised levels of Ery-GST activity of HbSS genotype was the outcome of corresponding raised levels of oxidants in this erythrocyte genotype. The intermediate level of Ery-GST activity of HbAS erythrocytes was a reflection of the hybrid nature of heterogeneous erythrocyte (Anosike *et al.,* 1991).

Previous investigations by Sarin *et al.,* (1993) revealed that parasitaemia caused decreased levels of enzyme activities associated with the glutathione system such as glutathione peroxidase (GPx), glutathione reductase (GRx) and GST activities of erythrocyte lysates. Accordingly, the present study showed that Ery-GST activities of parasitized erythrocytes of HbAA and HbAS genotypes exhibited significant ($p < 0.05$) decreased levels of enzyme activity compared to corresponding Ery-GST activity of non-malarious participants (Figures 1 and 2), which corroborated the findings of Sohail *et al.,* (2007). Therefore, low level of Ery-GST activity probably served as host defense strategy against the malarial parasites through up-regulation of oxidative protection mechanisms. In addition, Ery-GST activity served as a biomarker for diagnostic and therapeutic events in malaria. For similar purposes and reasons, reports have equally shown that patients infected with the malarial parasites (Becker *et al.,* 2004; Kavishe *et al.,* 2006) and causative organism of visceral Leishmaniasis (Neupane *et al.,* 2008) exhibited lower plasma levels of reactive oxygen and nitrogen species (RONS) antagonist such as glutathione (GSH), catalase and α-tocopherol than in the control groups. Therefore, inoculation of malarial parasites into biologic systems, most probably,elicitsthe production of reactive

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oxygen species (ROS) as a part of host defense strategy against the invading parasites (Becker *et al.,* 2004). The non-significant difference ($p > 0.05$) in Ery-GST activity between the non-malarious and malarious participants of HbSS genotype (Figure 3), implied that the host HbSS erythrocytes did not turn on the oxidative up-regulatory pathways that are involved in the control measures and elimination of the parasite. Expectedly, the perpetual high oxidative state of HbSS erythrocytes (Anosike *et al.,* 1991) provided and sustained the requisite anti-fecundity capabilities of this erythrocyte genotype against the malarial parasites.

Furthermore, low levels of Ery-GST activity of malarious participants was in connection with malarial pathophysiology described elsewhere (Dubios *et al.,* 1995, Liebau *et al.,* 2002). The ingestion and degradation of large quantities of haemoglobin by malarial parasite elicits the generation of potentially parasitotoxic FPIX. Accordingly, FPIX efficiently binds to *P. falciparum* GST (pfGST) (Harwaldt *et al.,* 2002) as well as to Ery-GST, preferably to the GST-GSH complex (Hiller *et al.,* 2006), and thereby, engenders uncompetitive inhibition of the GSTs.

In the first phase enzyme activity profile, Ery-GST showed decreasing level of activity with progression of experimental time, which was in concordance with previous reports (Mannervik and Danielson, 1988; Ayalogu *et al.,* 2001; Hiller *et al.,* 2006). The second phase showed evidence of recovery and activation of Ery-GST activity, exemplified by increasing level of the enzyme activity with increasing experimental time. The positive activation of Ery-GST activity in the second phase of Ery-GST activity profile was the outcome of generation and accumulation of ROS associated with the molecular events of the first phase enzyme activity profile. Therefore, ROS induced positive activation of Ery-GST activity served as a measure to detoxify and neutralize the cytotoxic ROS, in efforts to restore erythrocyte homeostasis. In agreement with the present findings, Hayes and Pulford, (1995) had proposed that cellular GST activity was under the regulatory mechanism of ROS and activation of GST activity can be considered as an adaptive response for the detoxification of cytotoxic carbonyl-, peroxide and epoxide-containing metabolites released in the cell by oxidative stress.

The overall pattern of Ery-GST activity within the experimental time ($0 < t < 18$ h) showed evidence of antimalarial induced disturbance of erythrocyte homeostasis, which could be of relevance from toxicological standpoints and for monitoring therapeutic events in malarial disease.

Reference Références Referencias

1. Anosike EO, Uwakwe AA, Monanu MO, Ekeke GI. (1991). Studies on human erythrocyte glutathione-S transferase from HbAA, HbAS and HbSS subjects *Biochima Biomedica Acta* 50:1051-1055.
2. Awasthi YC, Garg HJ, Dao DD, Partridge CA, Srivastiva SK. (1981). Enzymatic conjugation with 1-chloro-2,-4-dinitrobenzene. The fate of glutathione conjugates in erythrocytes and the effect of glutathione depletion on hemoglobin. *Blood* 58(4):733-773.
3. Ayalogu EO, Igboh NH, Dede EB. (2001). Biochemical changes in and liver of albino rats exposed to petroleum samples (gasoline, kerosene, and crude petroleum). *Journal of Applied Science and Environmental Management* 5(1):97-100.
4. Baure JD. (1980). Laboratory investigation of hemoglobin. *In: Gradwohl's Clinical Laboratory Methods and Diagnosis.* Sonnenwirth AC, Jarett L, (Eds). St. Louis Mosby. MO. pp. 809-902.
5. Becker K, Tilley L, Vennerstron JL, Roberts D, Rogerson S, Ginsburg H. (2004). Oxidative stress in malarial – infected erythrocytes host – parasite interactions. *International Journal of Parasitology* 34:163-189.
6. Beckett GJ, Hayes JD. (1993). Glutathione S-transferases: biomedical applications. *Advanced Clinical Chemistry* 30:281–380.
7. Black SM, Wolf CR (1991).The role of glutathione dependent enzyme in drug resistance. *Pharmacological Therapeutics* 51:139-154.
8. Board P, Coggan M, Johnston P, Ross V, Suzuki T, Webb G. (1990). Genetic heterogeneity of the human glutathione transferases; a complex of gene families. *Pharmacological Therapeutics* 48(3):357-369.
9. Boyer TD, Oslen E. (1991). Role of glutathione-S transferase in heme transport. *Biochemical Pharmacology* 42:188-190.
10. Bray PG, Mungthin M, Ridley RG, Ward SA. (1998). Access to hematin: the basis of chloroquine resistance. *Molecular Pharmacology* 54:170–179.
11. Carmagnol F, Sinet PM, Rapin J, Jerome H. (1981). Glutathione *S*- transferase of human red blood cells; assay, values in normal subjects and in two pathological circumstances: hyperbilirubinemia and impaired renal function. *Clinica Chima Acta* 117:209 217.
12. Cheesbrough M. (1998). District laboratory practice in tropical countries. Cambridge University Press, Cambridge. pp. 246-250.
13. Chikezie PC, Akuwudike AR, Chikezie CM. (2012). Membrane stability and methaemoglobin content of human erythrocytes incubated in aqueous leaf extract of *Nicotiana tabacum* product. *Free Radical and Antioxidants 2* (4):56-61.
14. Chikezie PC, Chikezie CM, Uwakwe AA, Monago CC. (2009). Comparative study of glutathione *S*-transferase activity of three human erythrocyte

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genotypes infected with *Plasmodium falciparum. Journal of Applied Science and Environmental Management* 13(3):13-18.

15. Chikezie PC. 2011. Glutathione *S*-transferase activity of human erythrocytes incubated in aqueous solution of five antimalarial drugs. *Free Radical and Antioxidants* 1(2):25-9.
16. Dubois VL, Platel DFN, Pauly G, Tribouley DJ. (1995). *Plasmodium berghei;* Implication of intracellular glutathione and related enzymes inchloroquine resistance *in vivo*. *Experimental Parasitology* 81:117-124.
17. Ducharme J, Farinotti R. (1996). Clinical pharmacokinetics and metabolism of chloroquine: focus on recent developments. *Clinical Pharmacokinetics* 31:257-274.
18. Engel LS, Taioli E, Pfeiffer R, Garcia-Closas M, Marcus PM. (2002). Pooled analysis and meta-analysis of glutathione S-transferase M1 and bladder cancer: A HUGE review. *American Journal of Epidemiology* 156:95-109.
19. Forrester LM, Hayes JD, Millis R, Barnes D, Harris AL, Schlager JJ, Powis G, Wolf CR. (1990). Expression of glutathione *S*-transferases and cytochrome P450 in normal and tumor breast tissue. *Carcinogenesis* 11:2163–2170.
20. Galbraith DA, Watts DC. (1980). Changes in some cytoplasmic enzymes from red cells fractionated into age groups by centrifugation in Ficoll™/Triosil™ gradients. Comparison of normal human and patients with Duchenne muscular dystrophy. *Biochemical Journal* 191:63-70.
21. Galli F, Rovidati S, Benedettis S, Buoncristiani U, Covarelli C, Floridi A, Canestrari F. (1999). Over expression of erythrocyte glutathione *S*-transferase in uremia and dialysis. *Clinical Chemistry* 45:1781-1788.
22. Habig WH, Pabst MJ, Jacoby WB. (1974). Glutathione *S*-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 249:7130-7139.
23. Harvey JW, Beutler E. (1982). Binding of heme by glutathione *S*-transferase: The first step in mercapturic acid formation. *Journal of Biological Chemistry* 245:7130-7139.
24. Harwaldt P, Rahlfs S, Becker K. (2002). Glutathione *S*-transferase of the malaria parasite *Plasmodium falciparum* characterization of a potential drug target. *Biological Chemistry* 383:821-830.
25. Hayes JD, Pulford DJ. (1995). The glutathione *S*-transferase superfamily. Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Critical Review of Biochemistry and Molecular Biology* 30:445-600.
26. Hiller N, Fritz Wolf K, Deponte M, Wende W, Zimmerman H, Becker K. (2006). *Plasmodum falciparum* glutathione *S*-transferase structural and mechanistic studies on ligand binding and enzyme inhibition. *Protein Science* 15:281-289.
27. Idonije OB, Festus O, Okhiai O, Akpamu U. (2011). Comparative study of the status of a biomarker of lipid peroxidation (malondialdehyde) in patients with *Plasmodium falciparum* and *Plasmodium vivax*malaria infection. *Asian Journal of Biological Science* 4:506-513.
28. Jacoby WB. (1976). Glutathione-S transferase: A group of multifunctional detoxification proteins. *In: Advances in Enzymology* (Meister A. (Ed) John-Wiley Interscience. New York. 40:383-414.
29. Kamber K, Poyiagi A, Delikonstantinos G. (1984). Modifications in the activities of membrane-bound enzymes during *in vivo* ageing of human and rabbit erythrocytes. *Comparative Biochemistry and Physiology* B. 77B:95-99.
30. Kavishe RA, Koenderink JB, McCall MB, Peters WH, Mulder B, Hermsen CC, Sauerwein RW, Russel FG, Van Der Ven JA. (2006). Severe *Plasmodium falciparum* malaria in Cameroon: Associated with the glutathione S-transferase M1 null genotype. *American Journal of Tropical Medicine and Hygiene* 75(5):827-829.
31. Ketley JN, Habig, WH Jacoby WB. (1975). Binding of non- substrate ligand to glutathione-S-transferase from human erythrocyte. *Archive of Biochemistry and Biophysics* 188:287-293.
32. Liebau E, Bergmann B, Campbell AM, Teesdale–Spittle P, Brophy PM, Luersen K, Walter R (2002). The glutathione S-transferase from *Plasmodium falciparum*. *Molecular Biochemistry and Parasitology* 124:85-90.
33. Mannervik B, Danielson UH. (1988). Glutathione transferases-structure and catalytic activity. *CRC Critical Review of Biochemistry* 23:283-337.
34. Milhous WK, Norman FW, Jean HB, Robert ED. (1985). *In vitro* activities and mechanism of resistance to antifol antimalarial drugs. *Antimicrobial Agents Chemotherapy* 1985:525-530.
35. Mulder TPJ, Court DA, Peters WHM. (1999). Variability of glutathione *S*-transferase α in human liver and plasma. *Clinical Chemistry* 45:355-359.
36. Neefjes VME, Evelo CTA, Bears LGM, Blanco CE. (1999). Erythrocyte glutathione S-transferase as a marker of oxidative stress at birth. *Archive of Diseases Child Fetal Neonatal Education* 81:F130 - F133.
37. Neupane DP, Majhi S, Chandra L, Rijal S, Baral N. (2008). Erythrocyte glutathione status in human visceral Leishmaniasis. *International Journal of Clinical Biochemistry* 23(1):95-97.

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38. Oakley AJ, Lo Bellow M, Nucceteli M, Mazzetti AP, Parker MW. (1999). Theligandin (non-substrate) binding site of human pi class glutathione transferase is located in the electrophile binding sited (H-Site). *Journal of Molecular Biology* 291:913-926.
39. Onyesom I, Onyemakonor N. (2011). Levels of parasitaemia and changes in some liver enzymes among malarial infected patients in Edo-Delta Region of Nigeria. *Current Research Journal of Biological Science* 3(2):78-81.
40. Pasupathi P, Chandrasekar V, Kumar US. (2009). Evaluation of oxidative stress, antioxidant and thyroid hormone status in patients with diabetes mellitus. *Journal of Medicine* 10:60-66.
41. Sarin K, Kumar A, Prakash A, Sharma A. (1993). Oxidative stress and antioxidant defense mechanism in *Plasmodium vivax* malaria before and after chloroquine treatment. *International Journal of Malaria* 30(3):127-133.
42. Shalev O, Repka T, Goldfarb A, Grinberg L, Abrahamov A, Olievieri NF. (1995). Deferiprone (L1) Chelates pathologic iron deposits from membranes of intact thalasaemic and sickle red blood cells both *in vitro* and *in vivo*. *Blood* 86:2008-2013.
43. Sohail M, Kaul A, Raziuddin M, Adak T. (2007). Decreased glutathione S-transferase activity: Diagnostic and protective role in vivax malaria. *Clinical Biochemistry* 40(5-6):377-382.
44. Srivastava P, Puri SK, Kamboj KK, Pandey VC. (1999). Glutathione-Stransferase activity in malarial parasites. *Tropical Medicine and International Health* 4(4):251-254.
45. Tsakiris S, Giannoulia-Karantana A, Simintzi I, Schulpis KH. (2005). The effect of aspartame metabolites on human erythrocyte membrane acetylcholinesterase activity. *Pharmacological Research* 53:1-5.
46. WMA. (2000). World medical association declaration of Helsinki ethical principles for medical research involving human subjects. 52nd WMA General Assembly, Edinburgh, Scotland.
47. Zusterzeel PLM, Nelen WLD, Roelofs MJ, Peters WHM, Blom HJ, Steegers EAP. (2000). Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss. *Molecular Human Reproduction* 6(5):474-478.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 6 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Correlation of Protein Carbonyl and Malondialdehyde in Oxidative Stress Induced Senescence of RBC Membrane in Type 2 Diabetes Mellitus

By Dr. Asfia Afreen & Dr. Dinesh Javarappa
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Abstract- Diabetes mellitus is a group of metabolic disease characterised by a state of chronic hyperglycemia. The biochemical process of Advanced Glycation appears to be enhanced in the Diabetes melieu as a result of not only hyperglycemia but also other stimuli such as oxidative stress and lipid peroxidation.

A case control comparative study was done with Type 2 Diabetes mellitus and normal controls at BMCH & RC, chitradurga. According to the criteria, blood sample were collected under aseptic precautions and evaluation of fasting blood sugar, HbA1C, Protein carbonyl along with RBC membrane ghost preparation and estimation of malondialdehyde(MDA) were done.

Keywords: diabetes mellitus, oxidative stress, reactive oxygen species, protein carbonyl and malondialdehyde (MDA).

GJMR-B Classification : NLMC Code: WD 200

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Correlation of Protein Carbonyl and Malondialdehyde in Oxidative Stress Induced Senescence of RBC Membrane in Type 2 Diabetes Mellitus

Dr. Asfia Afreen [α] & Dr. Dinesh Javarappa [σ]

Abstract- Diabetes mellitus is a group of metabolic disease characterised by a state of chronic hyperglycemia. The biochemical process of Advanced Glycation appears to be enhanced in the Diabetes melieu as a result of not only hyperglycemia but also other stimuli such as oxidative stress and lipid peroxidation.

The aim of the study is to establish a link between the oxidative stress induced by changes with protein carbonyl content and MDA damaging the RBC membrane composition in Type 2 DM in comparison to normal controls.

The correlation of Malondialdehyde (MDA) and Protein carbonyl levels in relation to control of Type 2 Diabetes mellitus based on HbA_1C level indicate that there is an autooxidation of glucose which results in persistent production of malondialdehyde(MDA) and ROS which can release advance glycation end products (AGE) and advanced lipoxidation end products(ALE) along with increased carbonylation of proteins leading to protein damage, oxidative modification of aminoacid residues ,aminoacid fragmentation and increased proteolytic susceptibility. Protein carbonyl can be generated by via non specific oxidation of aminoacid by via nonspecific oxidation of aminoacid or via catalysed oxidation of specific aminoacid key to protein function by oxygen and glycation.

A case control comparative study was done with Type 2 Diabetes mellitus and normal controls at BMCH & RC, chitradurga. According to the criteria, blood sample were collected under aseptic precautions and evaluation of fasting blood sugar, HbA_1C, Protein carbonyl along with RBC membrane ghost preparation and estimation of malondialdehyde(MDA) were done. It was found that there was significant increase of protein carbonyl in serum of Type2 DM cases (1.20±0.08) in comparison to control groups (0.90±0.06) with a statistical significance of ($p<0.001$) along with Malondialdehyde (MDA) of RBC membrane which was also significantly increased (4.23±0.21) in Type 2 Diabetes Mellitus in comparison to normal control (3.28±0.19) with a statistical significance of $P<0.001$. In our study, the positive correlation of membrane Malondialdehyde(MDA) and protein carbonyl was established with 74% of cases of Type 2 Diabetes Mellitus falling into the HbA1C control group of 7-8% indicating that protein carbonyl, Malondialdehyde (MDA) levels are early indication of progressive diabetic changes.

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Keywords: diabetes mellitus, oxidative stress, reactive oxygen species, protein carbonyl and malondialdehyde (MDA).

I. Introduction

Diabetes mellitus is the major health problem affecting people all over the world. It is one of the most extensively investigated human diseases. Diabetes Mellitus is a metabolic disease characterized by a state of chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both. The vast majority of diabetes falls into two broad categories. During diabetes mellitus, persistent hyperglycemia produces free radicals especially reactive oxygen species (ROS), glucose autooxidation and protein glycosylation. Increase in the levels of ROS in diabetes mellitus is due to their increased production and/or decreased destruction by non enzymatic or enzymatic reactions like catalase, reduced glutathione (GSH), superoxide dismutase (SOD) antioxidants.[1] The impairment caused by increased ROS is thought to result in random damage to proteins , lipids and DNA. Oxidative stress and oxidative damage to tissues are common end points of chronic diseases such as atherosclerosis, rheumatoid arthritis and diabetes. Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications.[2]

Over the last few decades several age related alterations of erythrocytes have been investigated,[3] of these oxidative damage to the erythrocyte membrane components is presently thought to play a key event during senescence of pathological red cells in thallesemia, sickle cell anaemia etc. The oxidative damage is probably initiated by reactive oxygen species (ROS) and other oxidants endogenously. [4] The study was undertaken to evaluate the effect of oxidative stress on erythrocyte membrane in Type 2 Diabetes mellitus and compare them with normal subjects.

II. Materials and Methods

The study was approved by the Ethics committee; a written informed consent was obtained from all participants in this study. A total of 100 patients

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with type 2 diabetes mellitus were recruited from the institute's medicine department. The diagnosis of type 2 diabetes mellitus was confirmed by glycosylated hemoglobin (>7). Hundred age and sex matched apparently healthy individuals with normal plasma glucose and with no symptoms suggestive of DM were taken as controls. Both cases and controls were subjected to estimation of biochemical parameters. Fasting plasma glucose was estimated by using commercially available kit in automated analyzer.[5] The estimation of glycosylated hemoglobin was done by cation exchange resin method[6], RBC membrane were prepared by Dodge et al [7], protein carbonyl estimation was done by Levine et al [8] method and MDA was estimated by Ohkawa et al method.[9]

III. Statistical Analysis

Statistical analysis of data was performed using SPSS (Version 15.0). Chi-square and Fisher Exact test has been used to find the significance of protein carbonyl and MDA levels between cases and controls. R environment Ver 2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.,

IV. Results

A Comparative study consisting of 50 Diabetic Mellitus patients and 50 controls was undertaken to investigate the oxidative stress parameters in type 2 DM cases when compared to controls. The mean age of the diabetics was 41.52 ±5.47years whereas it was 55.58±12.84 years respectively. Both among the cases and controls the sex distribution was same i.e. 80% and 20% males and females respectively. The maximum number of the age group of 41-45 i.e. 32%. The mean FBS levels among cases and controls were 197.50±8462 and 93.48±7.54 mg/dl and respectively. There is significant difference between levels of protein carbonyl and MDA levels among diabetics and controls. The mean protein carbonyl in cases and controls were 1.20±0.08 and 0.90±0.06 nmols/mg of protein respectively ($p<0.001$). The mean MDA in cases and controls were 4.23±0.21 and 3.28±0.19 nmols/mg of protein respectively ($p<0.001$).

V. Discussion

Diabetes Mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The biochemical process of advanced glycation appears to be enhanced in the diabetic milieu as a result of not only hyperglycemia but also other stimuli such as oxidative stress and lipid peroxidation. Protein carbonyl content in the cells is one of the indications of oxidative damage to protein and can be generated via nonspecific oxidation of aminoacids exposure of protein to oxygen radicals results in protein damge, this includes oxidative modification of many amino acid residue fragmentation, aggregation and increased proteolytic susceptibility. Like most biological membranes the plasma membrane of erythrocytes is rich in protein owing to this unique feature membrane proteins of erythrocytes are primary target for ROS & RNS.[10] The protein carbonyl content was increased in cases in comparison to controls. Cellular proteins are believed to be the targets of free radical induced oxidation injury. Protein carbonyl content in the cells is one indication of oxidative damage to proteins and can be generated by via non specific oxidation of aminoacids or via catalysed oxidation of specific aminoacid key to protein function by oxygen and glycation. Persistent hyperglycaemia in diabetes mellitus leads to increased formation of free radicals through various mechanisms. These free radicals attack and damage lipids, proteins and nucleic acids resulting in various late diabetic complications.[11] In the present study MDA content of cases was significantly raised in comparison to controls which exhibits the free radical injury due to peroxidative breakdown of phospholipids, fatty acids and accumulation of MDA resulting in senescence of RBC membrane.

VI. Conclusion

The present study suggested that excess free radicals are generated due to persistant hyperglycemia, which induces changes in membrane lipid peroxidation and oxidation of proteins and fragmentation which are potential risk factors for the development and progression of oxidative damage resulting in senescence of RBC membranes.

Reference Références Referencias

1. Kangralkar VA, Shivraj Patil D, Bandivadekar R M. Oxidative stress and Diabetes : A Review. Int. J. Pharma Applications 2010;1: 38-45.
2. Mousa SA. Oxidative stress in diabetes mellitus. Roman J. Biophys 2008;18(3):222-2362) Fujino T, Ando K, Beppu M, Kikugawa K. Enzymatic removal of protein aggregates from erythrocyte membranes. J. Biochem 2000 Jun; 127(6):1081-86.
3. Fujino T, Ando K, Beppu M, Kikugawa K. Enzymatic removal of protein aggregates from erythrocyte membranes. J. Biochem 2000 Jun;127(6):1081-86.
4. Celedone G, Rodriguez I, Espana J, Lissi E. Contribution of hemoglobin and membrane constituents modification to human erythrocyte damage produced by peroxyradicals of different charge and hydrophobicity. Free Radic. Res 2001; 34:17-31.
5. Burtis CA .Carbohydrates .In: Sacks DD ednt. Clinical Chemistry. Chapter 22.6th edn. Saunders 2008:390-91.

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6. Gowenlock AH, Mc Maurray JR, Mc Lauchun DM. Tests in disorders of glucose metabolism. In: WeinerK edn. Practical Clinical Biochemistry. Chapter 25.6 edn. CBS publishers.1996:333-49.
7. Dodge JT, Mitchell C, Hanahan D J. The preparation and chemical characteristics of hemoglobin free ghosts of human erythrocyte. Arch Biochem Biophys 1963 Jan;100:119-13..
8. Levine RL, Williams JA, Stadtman ER, Shacter: E. Carbonyl assays for determination of oxdatively modified proteins. Methods Enzymol 1994;233:346-357.
9. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal.Biochem., 1979;95:351-358.
10. Stryer, Lubert. Portrait of an allosteric protein. In: Biochemistry. Chapter 7. 4th edn. WH Freeman_Co.1995:p 154.
11. Ayaz K Mallick, Ravindra Maradi, Vivek R.Joshi, Gaurav Shorey, Marya Ahsan. Study on Malondialdehyde as a marker of lipid peroxidation in male and female patients with type 2 Diabetes Mellitus. International Journal of Phamaceutical Sciences Review and Research May-june 2011:vol 8, issue 2.article 033.198-201.

Table : 1

Variables	Cases	Control	Diference	P value
PROTEIN CARBONYL (nmoles/mg of protein)	1.20±0.08	0.90±0.06	0.30	<0.001**
MDA (nmoles/mg of protein)	4.23±0.21	3.28±0.19	0.95	<0.001**

***(P<0.001)=significant*

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 6 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

The Cardioprotective Effects of Irbesartan and Candesartan in Isoproterenol Induced Cardiomyopathy in Rats

By Jan J. Alshmani & Ansam N. Alhassani
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Abstract- The presence of a wide selection of angiotensin receptor blockers and the conflicting evidence regarding their cardioprotective effect, led to the attempt to evaluate the impact of irbesartan and candesartan on cardiac hypertrophy and remodeling. Female Albino rats were divided into 3 groups. The first group served as the control group and was given 1 ml distilled water via oral gavage and 0.5 ml distilled water subcutaneously. The second group was the isoproterenol (ISO) group and was given a daily S.C. injection of ISO at a dose of 5 mg/kg. The third group served as the treatment group and it was subdivided into 2 groups, both received ISO as stated previously along with a treatment drug which was administered via oral gavage and they included: ISO-Irb(irbesartan 50 mg/kg/day), and ISO-Cand(candesartan 2.6 mg/kg/day). All groups were treated for a period of 14 days. The assayed parameters included; mean serum Matrix metalloproteinase 9 (MMP-9), Cardiac troponin I (cTn-I), and Heart weight to Body weight (Hw/Bw) ratio.

Keywords: angiotensin, isoproterenol, cardiomyopathy, ARBs, MMP-9, cTn-i, candesartan, irbesartan.

GJMR-B Classification : NLMC Code: QV 37.5

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The Cardioprotective Effects of Irbesartan and Candesartan in Isoproterenol Induced Cardiomyopathy in Rats

Jan J. Alshmani [α] & Ansam N. Alhassani [σ]

Abstarct- The presence of a wide selection of angiotensin receptor blockers and the conflicting evidence regarding their cardioprotective effect, led to the attempt to evaluate the impact of irbesartan and candesartan on cardiac hypertrophy and remodeling. Female Albino rats were divided into 3 groups. The first group served as the control group and was given 1 ml distilled water via oral gavage and 0.5 ml distilled water subcutaneously. The second group was the isoproterenol (ISO) group and was given a daily S.C. injection of ISO at a dose of 5 mg/kg. The third group served as the treatment group and it was subdivided into 2 groups, both received ISO as stated previously along with a treatment drug which was administered via oral gavage and they included: ISO-Irb(irbesartan 50 mg/kg/day), and ISO-Cand(candesartan 2.6 mg/kg/day). All groups were treated for a period of 14 days. The assayed parameters included; mean serum Matrix metalloproteinase 9 (MMP-9), Cardiac troponin I (cTn-I), and Heart weight to Body weight (Hw/Bw) ratio.Irbesartan co-administered with ISO significantly reduced mean serum MMP-9 concentration, while candesartan significantly reduced MMP-9, and cTn-I concentrationscompared to the ISO group respectively. The Hw/Bw ratio was significantly reduced by both drugs. In conclusion both treatment drugs possessed some degree of cardioprotection; candesartan being the most beneficial in ameliorating isoproterenol induced cardiac injury.

keywords: angiotensin, isoproterenol, cardiomyopathy, ARBs, MMP-9, cTn-i, candesartan, irbesartan.

I. Introduction

The human heart is an exceptional organ, that's designed to function continuously for an average 70 year life span of a normal individual, thus a human heart beating at a rate of 70 beats per minute will exceed 2.5 billion beats throughout the life span of a human being (McCartan et al., 2012), this exceptional muscular pump displays extraordinary capacity to adapt to a broad range of genetic and extrinsic factors to sustain its contractile functions, failure to do so results in cardiac dysfunction and cardiomyopathy (Harvey and Leinwand, 2011). Cardiomyopathies are defined as "a heterogeneous group of diseases involving the myocardium which are associated with mechanical and/or electrical dysfunction that usually exhibits inappropriate ventricular hypertrophy or dilation and are due to a variety of causes that frequently are genetic" (Maron et al., 2006). They can be classified either into primary, or secondary; or according to the type of cardiomyopathy into dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC) (Wexler et al., 2009)

DCM is a progressive, irreversible condition with an estimated prevalence of 1:2500, and is considered one of the leading causes of heart failure (Burke, 2011). HCM is regarded as a leading cause of death among athletes, and has an incidence of 1:500 (Maron, et al., 2006), while RCM and ARVC are considered rare types of cardiomyopathy (Wexler et al., 2009). Many biomarkers have been associated with cardiac remodeling and cardiomyopathy (Gopal and Sam, 2013), among these is the cTn-I and MMP-9, their elevation is involved in cardiac injury and cardiomyopathy (Herman et al., 1999; Fairweather et al., 2011), in addition the renin angiotensin system (RAS) can induce left ventricular hypertrophy and fibrosis (Ocaranza et al., 2002), due to the direct effect of Ang II on myocardial cell hypertrophy through its action on the AT_1 receptor (Mehta and Griendling, 2007).

II. Materials and Methods

Thirty six female albino rats, 8-12 weeks old, weighing 140-200 grams, were used. The animals were housed in groups of four per cage, on sawdust in the animal house facility, under conditions of controlled ambient temperature of 22-25 oC with a 12 hour light/ dark cycles. The animals were supplied with rodent chow and free access to tap water.

a) The Rats were allocated into 3 groups as follow

Group 1: (Control group) This group included 8 rats and served as the control group; they received 1ml distilled water orally via oral gavage and 0.5 ml distilled water subcutaneously for a period of 14 days. Group 2: (ISO group) included 8 rats and served as a model of isoproterenol induced cardiomyopathy. The animals were injected with isoproterenol hydrochloride in a dose of 5mg/kg/day (Tipnis et al., 2000; Heather et al., 2009; Chowdhury et al., 2013), S.C. for a period of 14 days to induce distinguishable cardiac hypertrophy and cardiomyopathy. Group 3: (Treatment group) included 20 rats, and served as the treatment group; they were

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further subdivided into 2 subgroups all of which received isoproterenol as stated previously for group 2, along with the treatment drug administered via oral gavage, and they include: Group 3.1 (ISO-Irb. group): This group included 10 rats that were given irbesartan 50mg/kg/day. Group 3.2(ISO-Cand. group): This group included 10 rats that were given candesartan 2.6 mg/kg/day. All groups were treated for a period of 14 consecutive days.

Isoproterenol hydrochloride solution was prepared by reconstitution of isoproterenol hydrochloride powder with distilled water daily under sterile conditions immediately before injection (Grimm et al., 1998). The rats were first weighed and then isoproterenol was injected S.C into each rat except control group which was injected with distilled water S.C. The subcutaneous route was used because of the higher levels of cTn-I associated with this route, and a greater degree of cardiac injury (Brady et al., 2010). Immediately after the injection, the rats received the corresponding treatment drug according to the stated dose for each group, (except for the control group and the ISO group). After 14 days, 24hr of the last dose, the rats were anesthetized by injecting thiopental sodium 100mg/kg/I.P (Grimm et al., 1998), then dissected to expose the beating heart, after which blood was withdrawn directly from the right ventricle. The withdrawn blood was placed in a graduated glass conical bottom centrifuge tubes and allowed to settle for 20 min after which it was centrifuged at 3000 RPM for 10 minutes. The obtained serum was placed in eppendorf tubes and stored at -20 oC for further analysis; the heart was extracted, dried with filter paper and weighed.

b) Serum Measurements

Rat Matrix Metalloproteinase 9 and Cardiac troponin I serum concentrations were measured bydouble-antibody sandwich enzyme-linked immune-osorbent assay (ELISA), purchased from Uscn life science/ Germany and QAYEE-BIO/ Germany respectively.The Hw/Bw ratio was calculated by dividing the heart weight (mg) over the body weight (gm.). (Suckowet al, 2005).

$$\boldsymbol{Hw\backslash Bw\ ratio} = \frac{\textbf{Heart weight in } \boldsymbol{mg}}{\boldsymbol{Body\ weight\ in\ gm}}$$

c) Statistical Analysis

All data are expressed as Mean ± standard deviation. Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 16. Data analysis was made using one-way analysis of variance (ANOVA). Comparison between groups was done by using Post Hoc LSD test. P<0.05 was considered statistically significant.

III. Results

By the end of the study the following mortality was recorded: 2 of 10 rats in the ISO-Cand group. These animals were excluded from the study.

The table below shows the effect of co-administration of the treatment drugs with isoproterenol on the studied parameters. Irbesartan in its respective group, significantly reduced mean serum MMP-9 concentration to 8.10±2.32 ng/ml, while candesartan significantly reduced both serum concentrations of MMP-9 (8.25±1.96 ng/ml) andcTn-I (67.47±10.06 ng/ml,). The Hw/Bw ratio was significantly reduced by both treatment drugs.

Table 1: The effect of Irbesartan, and Candesartanco-administered with Isoproterenol on serum matrix metalloproteinase 9, cardiac troponin I, and heart weight to body weight ratio

		Biomarkers		
		MMP-9 ng/ml	cTn-I ng/ml	Hw/Bw ratio
Groups	Control	7.66±1.50	70.35±13.27	3.15±0.35
	ISO	11.38±3.41*	85.58±10.95*	4.53±0.31*
	ISO-Irb	8.10±2.32[a]	80.42±14.07	3.76±0.29[a]
	ISO-Cand	8.25±1.96[a]	67.47±10.06[ab]	3.65±0.20[a]
	P-Value	**0.015**	**0.019**	**<0.001**

- *Values are expressed as mean ± standard deviation*
- *Difference between individual groups were detected using post hoc LSD test*
- *p<0.05 is considered significant*
- **indicates a significant difference from the control at P<0.01*
- *[a]indicates a significant difference from the ISO group at p<0.01*
- *[b]indicates a significant difference between ISO-Cand and the ISO-Irb group*
- *P value refers to the significance of the difference detected by ANOVA.*
- *MMP-9: Matrix metalloproteinase 9. cTn-I: Cardiac troponin I. Hw/Bw: Heart weight to body weight.*

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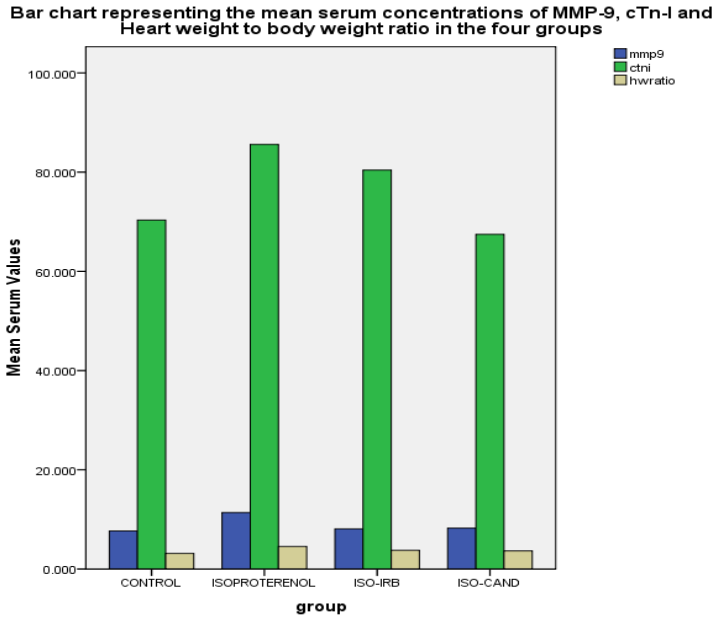

Figure 1 : Bar chart comparing the mean serum levels of MMP-9, cTn-I and cholesterol in the control group, ISO group, ISO-Irb group, and ISO-Cand group

IV. Discussion

Isoproterenol through its non-selective β-adrenoceptor activation causes severe cardiac injury and myocardial hypertrophy through inflammation, cytosolic Ca2+ overload and generation of reactive oxygen species (ROS) (Serra et al., 2008).

The mean serum MMP-9 concentration was significantly increased in the ISO group when compared to the control group, which is consistent with Li et al., (2008) and Cheng et al., (2009) as wasthe mean serum cTn-I concentration which is consistent with York et al., (2007). The elevated levels of cTn-I and MMP-9 are associated with cardiomyopathy and cardiac remodeling (Babuin and Jaffe, 2005; Roldán et al., 2008), and may reflect the myocardial injury produced by the administration of isoproterenol in the present study. Irbesartan in its respective groups, produced a significant reduction in MMP-9 serum concentrations which is in agreement with Montalescotet al., (2009), while candesartan in its respective group significantly reduced both mean serum MMP-9 and cTn-I concentrations, which is consistent with Palaniyappan et al., (2009), who found that candesartan is capable of normalizing MMP-9 (activity, protein, and mRNA) in rats after reperfused myocardial infarction.

The effects of ARBs on MMP-9 and cTn-I may be mediated through the inhibition of Ang II, Deschamps and Spinale, 2006 stated that Ang II stimulation of neonatal rat ventricular myocytes can trigger the mobilization of cytoplasmic Nuclear Factor-κB to the nucleus which in turn increases MMP-9 transcription.

Isoproterenol increased the mean Hw/Bw ratio significantly above control and this is consistent with Boluyte et al., (1995). This increase was significantly reduced in both treatment subgroups, and is consistent with the findings of Richer et al., (1999), Shirai et al., (2005).The effectiveness of ARBs in reducing heart weight to body weight ratio can be explained on the bases of their ability to block the action of Ang II, since accumulating evidence suggest that Ang II is involved in pathologic cardiac hypertrophy processes including myocyte hypertrophy, myocyte gene reprogramming, fibroblast proliferation, and extracellular matrix protein accumulation (Gray et al., 1998; Kim and Iwao 2000; Ichihara et al., 2001).

The observed differences among individual ARBs seen in this study may be attributed to the different binding affinity to the AT1 receptor (Kakuta et al., 2005).

The observed differences among individual ARBs seen in this study may be due to the different binding affinity to the AT1 receptor (Kakuta et al., 2005). Burnier (2001) stated that candesartan has the best Ang II antagonistic activity profile. Verdecchia et al., (2009) concluded that despite the shared mechanism of action, each ARB is characterized by specific pharmacological properties that could influence its clinical efficacy. . In conclusion both treatment drugs expressed cardioprotective abilities, candesartan being the most beneficial since it was capable of normalizing serum cTn-I levels as well as the MMP-9 and Hw/Bw ratio.

V. Acknowledgment

The professional assistance of Dr. Marwan Alnamir, and Dr. Nithal Abdulkader are gratefully acknowledged. This work was supported by Hawler Medical University/ College of Pharmacy.

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References References References

1. Babuin L. and Jaffe A.S. (2005), Troponin: the Biomarker of choice for the detection of Cardiac Injury, CMAJ, 173(10):1191–1202.
2. Boluyt M.O., Long X., Eschenhagen T., Mende U., Schmitz W., Crow M.T., and Lakatta E.G. (1995), Isoproterenol infusion induces alterations in expression of hypertrophy-associated genes in rat heart, Am J. Physiol., 269(2 Pt 2):H638-647.
3. Brady S., York M., Scudamore C., Williams T., Griffiths W. and Turton J. (2010), Cardiac troponin I in isoproterenol-induced cardiac injury in the Hanover Wistar rat: studies on low dose levels and routes of administration, ToxicolPathol., 38(2):287-291.
4. Burke A.P. (2011), Dilated Cardiomyopathy Pathology, Burke A.P., (Editor), Medscape: Drugs, Diseases and Procedures.
5. Burnier M. (2001), Angiotensin II Type 1 Receptor Blockers, Circulation, 103(6): 904-912.
6. Cheng Y.S., Dai D.Z., and Dai Y. (2009), Isoproterenol disperses distribution of NADPH oxidase, MMP-9, and pPKCε in the heart, which are mitigated by endothelin receptor antagonist CPU0213, ActaPharmcol. Sin., 30(8):1099-1106.
7. Chowdhury D., Tangutur A.D., Khatua T.N., Saxena P., Banerjee S.K. and Bhadra M.P. (2013), A proteomic view of isoproterenol induced cardiac hypertrophy: Prohibitin identified as a potential biomarker in rats, J. Transl Med., 11(Issue1):1-13.
8. Deschamp A.M. and Spinale F.G. (2006), Pathways of matrix metalloproteinase induction in heart failure: bioactive molecules and transcriptional regulation, Cardiovasc Res., 69(3):666-76.
9. Fairweather D., Abston E.D. and Coronado M.J. (2011), Biomarkers of Heart Failure in Myocarditis and Dilated Cardiomyopathy, Chapter 16, D. Cihakova (Editor), InTech Europe Pub., Croatia, P:323-348.
10. GopalD.M., and Sam F. (2013), New and emerging biomarkers in left ventricular systolic dysfunction--insight into dilated cardiomyopathy, J. of Cardiovasc. Trans. Res., 6:516-527.
11. Gray M.O., Long C.S., Kalinyak J.E., Li H.T., and Karliner J.S., (1998), Angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of TGF-beta 1 and endotheline-1 from fibroblasts, Cardiovasc Res., 40(2):352-63.
12. Grimm D, Elsner D, Schunkert H, Pfeifer M, Griese D, Bruckschlegel G, Muders F, Riegger G.A.J. and Kromer E.P. (1998), Development of heart failure following isoproterenol administration in the rat: role of the renin-angiotensin system, Cardiovasc Res., 37(1):91-100.
13. Harvey P.A. and Leinwand L.A. (2011), Cellular mechanisms of cardiomyopathy, JCB, 194(3):355-365.
14. Heather L.C., Catchpole A.F., Stuckey D.J., Cole M.A., Carr C.A. and Clarke K. (2009), Isoproterenol induces in vivo functional and metabolic abnormalities; similar to those found in the infarcted rat heart, J PhysiolPharma., 60(3):31-39.
15. Herman E.H., Zhang J., Lipshultz S.E., Rifai N., Chadwick D., Takeda K., Yu Z.X., and Ferrans V.J. (1999), Correlation between serum levels of cardiac Troponin-T and the severity of the chronic cardiomyopathy induced by doxorubicin, J. ClinOncol., 17(7):2237-43.
16. Ichihara S., Senbonmatsu T., Price E. Jr., Ichiki T., Gaffney F.A., and Inagami T. (2001), Angiotensin II type 2 receptor is essential for left ventricular hypertrophy and cardiac fibrosis in chronic angiotensin II-induced hypertension, Circulation, 104(3):346-51.
17. Kim S. and Iwao H. (2000), molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases, Pharmacol Rev., 52(1):11-34.
18. Kakuta H., Sudoh K., sasamata M., and Yamagishi S. (2005), Telmisartan has the strongest binding affinity to angiotensin II type 1 receptor: comparison with other angiotensin II type 1 receptor blockers, Int J Clin Pharmacol Res., 25(1):41-46.
19. Li L., Zhang Y., Li Y., Yu B., Xu Y., Zhao S., and Guan Z. (2008), Mesenchymal stem cell transplantation attenuates cardiac fibrosis associated with isoproterenol-induced global heart failure, Transpl. Int., 21(12):1181-1189.
20. Maron B.J., Towbin J.A., Thiene G., Antzelevitch C., Corrado D., Arnett D., Moss A.J., Seidman C.E., and Young J.B. (2006), Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention, Circulation, 113(14):1807-1816.
21. McCartan C., Mason R., Jayasinghe S.R., and Griffiths L.R. (2012), Cardiomyopathy Classification: Ongoing Debate in the Genomics Era, Biochem Res Int., Vol. 2012, Article ID-796926, 10P.
22. Mehta P.K. and Griendling K.K. (2007), Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system, Am J Physiol Cell Physiol, 292(1):C82-C97.
23. Montalescot G., Drexler H., Gallo R., Pearson T., Thoenes M., and Bhatt D.L. (2009), Effect of Irbesartan and Enalapril in non-ST elevation acute coronary Syndrome: Results of the Randomized, double-blind ARCHIPELAGO study, Euro. Heart J., 30(2):2733-2741.

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24. Ocaranza M.P., Diaz-Araya G., Chiong M., Muňoz D., Riveros J.P., Ebensperger R., Sabat S., Irarrȧzaval P., Jalil J.E. and Lavandero S. (2002), Isoproterenol and Angiotensin I-Converting Enzyme in Lung, Left Ventricle, and Plasma During Myocardial Hypertrophy and Fibrosis, J. Cardio. Pharma., 40(Issue 2):246-254.
25. Palaniyappan A., Uwiera R.R.E., Idikio H., and Jugdutt B.I. (2009), Comparison of Vasopeptidase inhibitor Omapatrilat and angiotensin receptor blocker candesartan on extracellular matrix, myeloperoxidase, cytokines, and ventricular remodeling during healing after reperfused myocardial infarction, Mol Cell Biochem., 321(1-2):9-22.
26. Richer C., Fornes P., Cazaubon C., Domergue V., Nisato D., and Giudicelli J.F. (1999), Effects of long-term angiotensin II AT1 receptor blockade on survival, hemodynamics and cardiac remodeling in chronic heart failure in rats, Cardiovasc Res., 41(1):100-108.
27. Roldán V., Marín F., Gimeno J.R., Ruiz-Espejo F., González J., Feliu E., García-Honrubia A., Saura D., Morena G., Valdés M. and Vicente V. (2008), Matrix metalloproteinases and tissue remodeling in hypertrophic cardiomyopathy, Am Heart J., 156(1):85-91.
28. Serra A.J., Higuchi M.L., Ihara S.S.M., Antônio E. L., Santos M.H.H., Bombig M.T.N.M., and Tucci P.J.F. (2008), Exercise training prevents β-adrenergic Hyperactivity-induced Myocardial Hypertrophy and Lesions, Euro. Heart J., 10:534-539.
29. Shirai K., Watanabe K., Ma M., Wahed M.I., Inoue M., Saito Y., Suresh P.S., Kashimura T., Tachikawa H., Kodama M. and Aizawa Y., (2005), Effects of angiotensin-II receptor blocker candesartan cilexetil in rats with dilated cardiomyopathy, Mol Cell Biochem., 269(1-2):137-42.
30. Tipnis U.R., He G.Y., Li S., Campbell G., and Boor P.J. (2000), Attenuation of isoproterenol-mediated myocardial injury in rat by an inhibitor of polyamine synthesis, CardiovascPathol., 9(5):273-280.
31. Verdecchia P., Angeli F., Repaci S., Mazzotta G., Gentile G., and Reboldi G. (2009), Comparitive assessment of angiotensin receptor blockers in different clinical settings, Vasc Health Risk Manag., 2009(5):939-948.
32. Wexler R., Elton T., Pleister A., and Feldman D. (2009), Cardiomyopathy: An Overview, Am Fam Physician, 79(9):778-784.
33. York M., Scudamore C., Brady S., Chen C., Wilson S., Curtis M., Evans G., Griffiths W., Whayman M., Williams T., and Turton J. (2007), Characterization of troponin responses in isoproterenol-induced cardiac injury in hanoverwister rats, Toxi. Path., 35(4):606-617.

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Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

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	A-B	**C-D**	**E-F**
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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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