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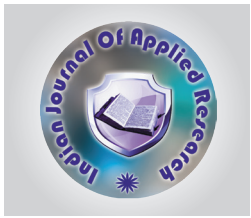

Biochemichal Studies on Effect of Age on Erythrocytes of Rats

KEYWORDS: Colonclassification, PHP.

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ABSTRACT *The determination of some biochemical alteration in rat blood under the effect of Age have an important role in biotransformation and cell defense mechanism , this attributed to the most enzyme activities which declined with advanced age .*

The present study was carried out on a total number of Sixty male rats were selected and kept under good hygienic measurements and divided into three groups each one contains twenty rats for 1, 3 and 6 months. The result showed the most enzyme activities were declined with advanced age as (Glucose-6-phosphate dehydrogenates, Reduced glutathione, Glutathione peroxidase, Cholinesterase and superoxide dismutase while Gamma glutamyl transferase was increased with aging and normocytic normochromic anemia with increasing age.

INTRODUCTION

The erythrocytes represent an important source of antioxidant capacity of the blood. the red blood cell, under physiological condition, is continuously exposed to oxidants such as the super-oxide radical (O2-) and hydroxyl radical. these radicals are also produced under experimental and\ or clinical use of some oxidizing drugs such as phenyl hydrazine and primaquin.However, the red blood cell has a protective mechanism against this oxidative damage by some ways of certain enzyme systems (**Suzuki et al., 1984)** mentioned that main enzymes are glutathione peroxides, catalase and super oxide dismutase, are known to protect the red blood cell against such damage. In addition, glutathione reductase is important in maintaining an adequate level of reduced glutathione which is required to protected the red blood cell from oxidation. erythrocytes are permanently in contact with potentially damaging levels of oxygen, but their metabolic activity is capable of reversing this injury under normal conditions. However, it is well known that variety of physiological and pathological factors may increase reactive oxygen species and induce the oxidative stress **(Puppo and Halliwell ,1988).** erythrocytes are equipped by many defense systems representing their antioxidant capacity (**Kurata et al. ,1993**). this protective system includes superoxide dismutase (SOD), catalase (CAT), reduced glutathione, glutathione peroxidase (GPx), glutathione-S-transferase, and glutathione reductase. two enzymes are shared in H2O2 detoxification: CAT and GPx, but their relative significance in H2O2 scavenging is still not clear (**Kurata et al., 1993;Gaetani et al., 1996; Guilivi et al., 1994; Mueller et al., 1997; Johnson et al., 2000 and Nakababu et al., 2003).** The erythrocyte survival may be related to age of animals, rapid destruction of fetal erythrocyte was reported to occur in newborn puppies between birth and 2 weeks of age **(Lee et al., 1971).**

Aging is the accumulation of changes in a person over time **(Bowen, et al ., 2004)**. there is strong evidence that the red blood cell is an appropriate model to study the mechanisms underlying the cellular and tissutal aging .during aging of RBcs occure many changes in its component whatever in vivo or in viro **(Bosman et al .,2005)**.

RBCs storage under blood bank conditions and aging this is a new marker to cancer progression if the transfusion blood has been stored for nine days or longer **(Tomimaru et al ., 2010 ; Asahara et al ., 1999 ; Sugita et al ., 2008 and Shiba et al ., 2009)** .

(Worlford et al., 1986) found that the blood parameters of different experimental animals were affected by aging as in rats as the blood parameters mostly decreased with increased age and **(Carl et al ., 2014**) found that blood from young mice reverses aging in old mice, rejuvenating their muscles and brains that it could lead to treatments for disorders like Alzheimer's disease and heart disease.

MATERIAL AND METHODS

Expermintal Animals:

A total number of 60 male Spraque Dawely rats aged rats weighed 150-200g while young rats weighted 7-57 g . The animals were obtained from Animal Health Research Center in Cairo .

Diet & Management of rats:

Rats were kept for 4 weeks for acclimatization at the Animal House of Faculty of Veterinary Medicine (Suez Canal University). Before the beginning of the experiment they were housed in separate metal cages under controlled environmental and nutritional conditions (20°C-25°C and 55-60% relative humidity). Throughout the period of experiment they were maintained on a standard ration as the following table show according to **Tae -Yoal et al., 1995 and Brichard et al., 1996,** The animals had free access to water and food.

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Experimental design:
Rats were divided into three groups according to age each group consist of 20 rats placed in individual cages and classified as follows :

Group (a): n=20, Group (b): n=20 and Group (c): n=20

Sampling:
The first sample collection to the group (a) which contain 20 rat with one month of age ,second collection after 2 month to the group (b) which contain 20 rat with three month of age , at the end of experiment period collect the last sample collection from the group (c) which contain 20 rat with six month of age from the medial canhus of rat eyes using micro-hematocrite tubes according to **(Wolford et al., 1986).** During collection of each sample was divided to three parts:

1) The first part was obtained in clean dry rubber stoppered vial contained 1 mg disodium ethylene diamine tetra acetate (EDTA) as anticoagulant for each 1 ml collected blood . the sample were gently mixed and used for measuring the erythrocytic count (RBCs) hematocrit value (PCV%) and hemoglobin concentration (Hb). reduced glutathione , superoxide dismutase and measuring glucose-6-phosphate dehydrogenase.

2) The second part was collected without anticoagulant in a centrifuge rubber stoppered tube and allowed to clot at room temperature. The samples were centrifuged at 3000 r.p.m. for 15 minute and the obtained non- hemolyzed sera were used for measuring Gamma glutamyl transferase activity & Choline estrase.

3) The third part were collected in a dry tube containing heparin in concentration of 1 unit heparin for each 1 ml collected blood and samples gently mixed . these samples served for measuring the glutathion peroxidase.

METHODS
Determination of reduced glutathione:

The concentration of reduced glutathione was determined by enzyme-linked immunosorbent assay (ELISA) kite according to the method described by **(Beutler ,1957).**

Determination of Superoxide Dismutase Activity:

The Superoxide Dismutase Activity was determined by enzyme-linked immunosorbent assay (ELISA) kite according to the method described by **(Misra and Fridovich ,1972)** .

Determination of glucose-6-phosphate dehydrogenase:
The concentration of glucose-6-phosphate dehydrogenase was determined by enzyme-linked immunosorbent assay (ELISA) kite according to the method described by **(Kornberg et al .,1955).**

Determination of glutathion peroxidase:
The concentration of glutathion peroxidase was determined by kinetic method based on the procedure of **Paglia and Valentine (1967).**

Determination of cholinesterase:
The concentration of cholinesterase was determined by fluorescence detection method based on the procedure of (Gilberstadt ,1984).

Determination of Gamma Glutamyl Transferase :
The concentration of Gamma Glutamyl Transferase was determined by colorimeric technique according to the method described by (Nemesanzky and Lott ,1985).

Determination of Determination of complete blood picture:

The samples analysied by Sysmex XS-800*i*(automated analyzer)**.**

RESULTS
G6PD activity**,** GSH level**,** SOD activity**,** GPX activity and CHE level the mean values were significantly decreased with increasing age while GGT level the mean values were significantly increases with increasing age and there were normocytic normochromic anemia ,the obtained results revealed that a highly significant decrease in the mean values of packed cell volume and hemoglobin concentration with increasing age .while a non- significant decrease in the mean values of mean corpuscular volume ,mean corpuscular hemoglobin , mean corpuscular hemoglobin concentration , white blood cells and platelet with increasing age .

Figure (1) Effect of age on G6PD activity in erythrocytes of rats:

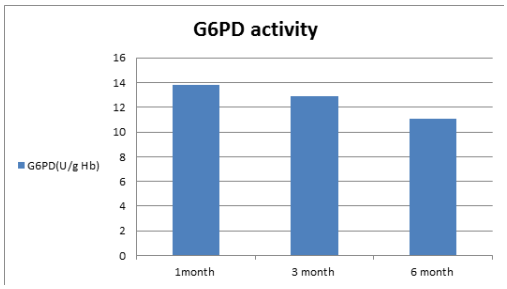


Figure (2) : Effect of age on GSH level in erythrocytes of rats :

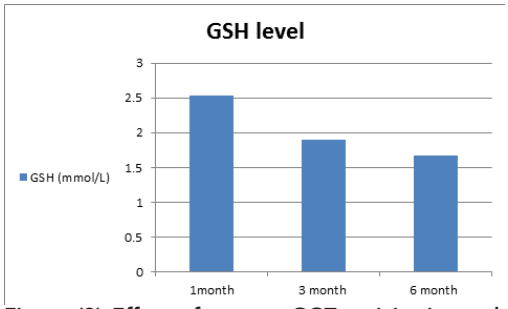


Figure (3) Effect of age on GGT activity in erythrocytes of rats :

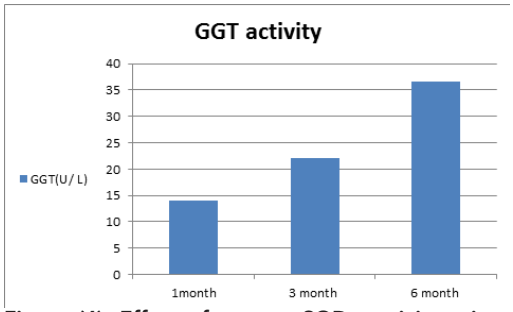


Figure (4): Effect of age on SOD activity in erythrocytes of rats :

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Figure (5): Effect of age on GPX activity in erythrocytes of rats:

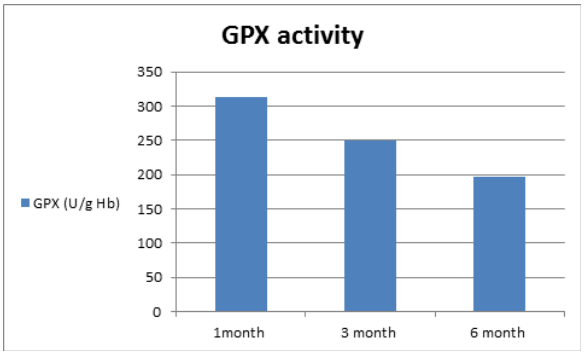


Figure (6): Effect of age on CHE level in erythrocytes of rats :

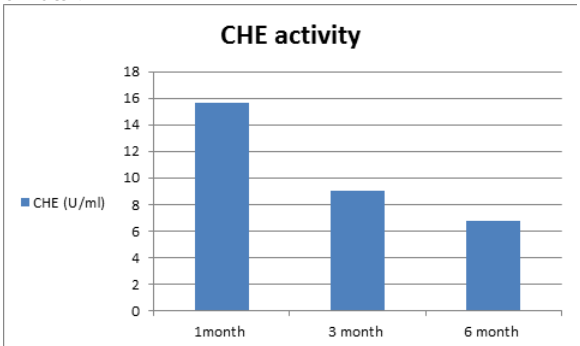
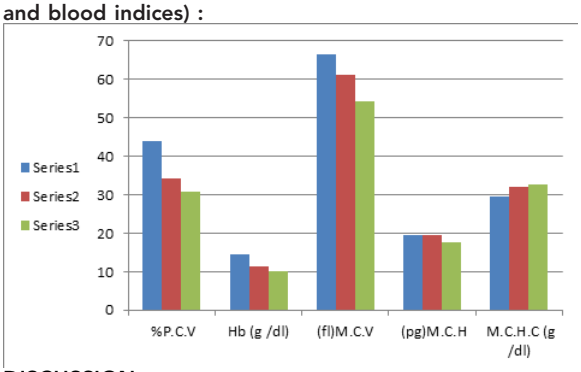


Figure (7): Effect of age on blood picture of rats (Hb and blood indices) :



DISCUSSION

The study has been conducted to evaluate the effect of aging on Biochemical alternations which occur in the RBCS as following:

Glucose-6-phosphate dehydrogenase :

The results show that the Glucose-6-phosphate dehydrogenase was significantly decreased with increasing age as there is high significant between group (c) and another two group while no significant between group (a) and group (b) .

Dealing with the significant decrease that occurred with aging several authors gave possible explanation . as the work of **(Marks et al .,1958)**showed that the level of certain enzymes in mature erythrocytes decrease with the aging of these cells , in vivo ,like the activity of Glucose-6-phosphate dehydrogenase was progressively lowered with aging of red blood cells. the significant decrease may be attributed to the neonatal erythrocytes can synthesis the enzyme than older cells .this was explained by **(Smith et al .,1970)**who stated that there may be an increased synthesis of the adult enzyme in the neonatal erythrocytes and he added that the increased neonatal enzymes activity could be explained on the basis of a younger cell population on presence of reticulocytes, since the enzyme activity of erythrocytes which have just been released from the bone marrow is much higher than that of older cells .

Apparently similar result to this finding was given by **(Joseph et al ., 1972)** who found that the activity of Glucose-6-phosphate dehydrogenase was higher in neonatal than in those of infants and adults erythrocytic level of this enzyme .**(Valentine et al., 1967) and (Kirkman and Kennedy,1968)** who showed that all these enzymes were higher in young erythrocytes than old erythrocytes .

Reduced glutathione:

The effect of age on Reduced glutathione level in blood of rats was significantly decreased with increasing age as there is a significant decrease between group (a) and the other two groups while no significant between group (b) and group (c).

this significant decrease in reduced glutathione level with increasing age may be attributed to that reduced glutathione was exhausted by the high concentration of superoxide radicals which oxidized thiol of reduced glutathione as suggested by **(Asada and Kanematsu ,1976)** who stated that superoxide radicals oxidize thiols.

$$GSH + O_2^- + H \rightarrow GS + H_2O_2$$

and lower glutathione concentration with increasing life of the animal would be expected **(Harisch et al ., 1980)** . This decrease may be due to oxidation of sulf hydryl group and diminished level of GSH **(Sarker et al., 1998)**

the significant decrease in reduced glutathione level may be attributed to that reduced glutathione regeneration rates were significantly lower in normal adult humans .in accordance with **(Agar et al ., 1974)** who reported that although new born human have higher activities of Glucose-6-phosphate dehydrogenase and glutathione reductase , that reduced glutathione regeneration rates were significantly lower in normal adult humans . a similar explanation by **(Smith et al ., 1970)** who stated that this decrease may be attributed to the general decline of enzyme activity with increasing age which is consistent with the hypothesis that protein synthesis declines during later development and aging , he also added that Glucose-6-phosphate dehydrogenase and glutathione reductase were elevated in neonatal erythrocytes than in infant and adult. this significant decrease was attributed to the decrease of Glucose-6-phosphate dehydrogenase activity with increasing age **(Marks et al .,1958)** and Glucose-6-phosphate dehydrogenase provide NADPH which convert oxidized from a glutathione to reduced form of glutathione by help of glutathione reductase **(Kosower et al ., 1967).**

Superoxide dismutase :

The effect of age on Superoxide dismutase activity showed significantly decreased with increasing age as there is a significant decrease between group (a) and the other two groups while no significant between group (b) and group (c).

the decrease of superoxide dismutase activity with aging by (**Reiss and Gershon,1976)** concluded that liver superoxide dismutase activity was decreased in old rats .in this respect **(Harisch et al ., 1980)** explained the high concentration of Superoxide dismutase radicals was attributed

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

to decrease of Superoxide dismutase activity with aging . **Webster and Toothill ,(1986)** found that Superoxide dismutases activity decreased significantly with the duration of storage , this may be attributed to that superoxide dismutases activity decreased with aging .

While the increase in Superoxide dismutase was found by **(Hoshino et al., 1985)**. That the activity of superoxide dismutase in the soluble fraction of rat liver cells was increased by adding thiol (SH)compounds such as glutathione ,cystein and 2- mecaptopropionyleglycine . **Jansson et al., (1985)** mentioned that red blood cell superoxide dismutase is increased in iron deficiency anemia. This finding suggested an increased formation of Superoxide dismutases compensatory to an increased oxidant stress.

Gamma Glutamyl transferase:
The effect of age on γGT showed significantly increases with increasing age table as there is a significant increases between group (c) and the other two groups while no significant between group (a) and group (b).

this increase may be attributed to the activities of fetal liver and brain are higher than those of adult rat **(Meister and Tate , 1975)** who added that in contrast the Gamma Glutamyl transferase of fetal rat kidney activity exhibits little or no activity but after birth the activity increased with aging **(Nash and Tate , 1982).** Who suggested that Gamma Glutamyl transferase is initially synthesized in rat kidney.

(Rhone et al., 1976) showed that serum GGT within its laboratory normal range might be an early and sensitive marker for oxidative stress.

another view the primary role of GGT ectoactivity is to metabolize extracellular reduced glutathione (GSH), allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis; in

This way, a continuous "GSH cycling" across the plasmamembrane occurs in a number of cell types **(Forman et al., 1997)**. Thus, ectoplasmic GGT favors the cellular supply of GSH, the most important non-protein antioxidant of the cell.

While **(Hanes et al ., 1952)** showed that GGT decreased under the effect of age due to GGT may play a significant role in amino acid transport in neonatal rat liver than in the adult tissue .thus the GGT activities of neonatal were higher than those of adult rats **(Tate et al ., 1976)** .

Glutathione peroxidase :
The effect of age on Gpx showed high significantly decreased with increasing age as there is a high significant decrease between three groups .

Few studies have examined the activity of GPx, which depends on **Se** for its activity, in adults older than 65 years, and fewer studies have examined GPx activity in adults older than 65 years with comorbid illness and disability. This population best reflects the accumulation of free radical damage that results in age-associated conditions, in keeping with the free radical theory of aging **(Harman, 1988).** the same opnion found from **(Sara et al ., 2008)** as they hypothesized that free radical damage contributes to aging. Age-related decline in activity of the antioxidant enzyme glutathione peroxidase (GPx) may contribute to increased free radicals. We hypothesized that GPx activity decreases with age in a population of older women with disability .As they explain that This is the first study to examine the association between age and GPx activity in an older adult cohort with disability and chronic disease. These findings suggest that, after age 65, GPx activity declines with age in older women with disability. This decline does not appear to be related to diseases that have been previously reported to alter GPx activity. Longitudinal examination of GPx activity and other antioxidant enzymes in diverse populations of older adults will provide additional insight into age- and disease-related changes in these systems.

Cholinesterase :
The effect of age on CHE showed significantly decreased with increasing age as there is a significant increases between group (a and the other two groups while no significant between group (b) and group (c).

Acetylcholinesterase (AChE) can be used as a marker of cell aging in human red blood cells (RBCs),suggested by **(Yeato et al ., 1998)**. The results indicated that old human red blood cells showed significantly lower AChE activity compared to young human red blood cells of both sexes.

(Moudgil et al., 1973) found in his study the activity of acetylcholinesterase and its induction by estradiol in the cerebral hemisphere and cerebellum of immature (9-), adult (29-) and old (65-week) female rats were studied to understand the mechanism of aging The activity of the enzyme of the cerebral hemisphere is highest in the immature rat and decreases thereafter. There is no such change in its activity in the cerebellum as a function of age.

(August, 1998) found that Total acetylcholinesterase (AChE) and the molecular forms of the enzyme from six brain regions were compared in young adult (6 mo) and aged (24 mo) Fischer 344 rats. Total AChE activity was significantly reduced with aging these results indicate that aged rats exhibit reduced brain AChE**.**

Effect of age on blood picture of rats :
A highly significant decrease in the mean values of packed cell volume and hemoglobin concentration with increasing age as there is a significant decrease between group (a) and group (c) while high significant between group (b) and group (a) and group (c) in RBCS while about hemoglobin concentration and there is a significant decrease between group (a) and group (b) while a non- significant decrease between group (b) and group packed cell volume (c) .while a non- significant decrease in the mean values of mean corpuscular volume ,mean corpuscular hemoglobin , mean corpuscular hemoglobin concentration , white blood cells and platelet with increasing age. The values of hematological parameters are affected by a number of factors These factors include age, sex nutritional and environmental factors. the senile rats had hematological disorders manifested by drop in hemoglobin content, RBCs count and hematocrit value This is consistent with data from **(Videan et al ., 2008)** who found a significant age related increase in anemia risk, based on significant decreases in hemoglobin and hematocrit. Also, **(Wolford et al ., 1987)** reported that hematological parameters changes with aging. These hematological changes are reflected by a decline in marrow cellularity, increased risk of myeloproliferative diseases and a decline in adaptive immunity **(Prabhakar, 2009).** may attributed to protein synthesis which decrease with age . this was explaned by **(YON –Hahn , 1966).** Who stated that protein synthesis

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

declines during later development and aging . in this respect **(London et al .,1950)**. added that the mature red blood cells can't synthesize the hem . because they lack mitochondria.

The hemoglobin is considered as a conjugated protein, this given by **(Schalm et al., 1975)** who stated that hemoglobin is a conjugated protein and consisting of hem and globin . thus the hemoglobin concentration decreased with aging .

The highly significant decrease in packed cell volume with increasing age may be attributed to the decrease in erthrocytic formation and decrease in hemoglobin synthesis (**Halvorsen et al ., 1975**).

Apparently similar result were obtained by **(Wolford et al ., 1986)** who found thst a decreased in hematocrite value ,hemoglobin concentration and red blood cells counts with increasing age in the rats, and **(Noonan et al ., 1978)** who found that decreased erythrocytic counts , hemoglobin concentration and packed cell volume with aging in hereford cattle

CONCLUSION

The obtained data revealed significant changes in the levels of most enzymes studied besides the changes in the blood pictures of rats .As enzymes play an important role in bio-transformations and cell defence mechanism as the role of superoxide radicals which is considered a fatal radical for the intact cell , in animals which kept under stress conditions .

And this data make an evidence that the levels and activity of RBCS enzymes can be used as a marker of cell aging in animals and human red blood cells (RBCs).

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