

Fertility indicators and glutathione status in Sprague Dawley rats

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Key words

Fertility, glutathione, rats

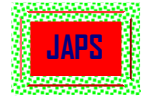
1 SUMMARY

The objective of this study was to assess the relationship between glutathione status and male fertility. Forty male Sprague Dawley rats were divided into two groups; twenty rats administered paracetamol (Glutathione depletory) and the other group of rats (the control), got 0.9% normalized saline for ninety day. At day 15, 30, 60, and 90, four rats from each group were assessed for semen quality, sperm motility, morphology, glutathione concentration and histology of testis, liver and epididymis. Results showed that semen homogeneity disappear and liquefaction occurs after 30 seconds, sperm motility dropped to below 20%, glutathione levels dropped to 50nmoles/l sperm, abnormalities rose to 95 % by day 90, of these 75% had bent tail and 80% had abnormal mid-piece The study group had 80% quantal pregnancy, >60% libido, >80% mount and intromissions and a total of 12 pups from the four rats, gestation period was increased by an average of 10 days as compared to the control group with 100% quantal pregnancy, <80% libido, mounts, intromissions and a total of 35 pups. However al pups were 100% free from abnormality. These results show that glutathione depletion affects male fertility especially on the quality of sperms. Normal sperm dropped to less than 5% by day 15. Sperm droplets were not marked and were not affected with time. The study group showed marked accelerated increase in sperm abnormality from 50% at day 15 to 90% at day 90. For the control group it rose from 10 to 35% on day 15 and 90, respectively. Significant reduction layer of spermatogonia, spermatocytes and spermatids, were observed on day 30 to day 90. However in the control group slight lymphocytic infiltration and liver necrosis and a slight reduction in spermatogonia, spermtocytes and spermatids were seen on day 60 to 90. It could be concluded that glutathione depletion affects the physiology of male reproductive system with consequent detrimental effects on male fertility.

2 INTRODUCTION

Excessive concentrations of ROS in the semen depresses total antioxidant capacity (TAC) and lowers the levels of individual antioxidants (Lewis *et al.*, 1997). ROS are generated by the cellular components of semen, and include a superoxide anion radical (O_2^-), hydrogen

peroxide (H_2O_2), and lipid hydroperoxides formed via lipid peroxidation of the membrane lipids of the spermatozoa (Alvarez & Storey, 2005). The effects of lipid peroxidation include irreversible loss in motility, damage to the sperm DNA and reduced fertility (Maxwell &



Watson, 1996). Oxidative energy production is inevitably associated with the generation of reactive oxygen species (ROS), excessive concentrations of which can lead to cellular pathology. Glutathione as a substrate of glutathione peroxidase present in the sperm head slows down the peroxidation process and hence maintains sperm motility (Christopherson, 1968), thus playing an active role in sperm fructolysis (Slaweta & Laskowska, 1987). The cell membrane of mammalian spermatozoa has a high content of polyunsaturated fatty acids (PUFA) (Zalata *et al.*, 1998), and the lipids incorporated in the sperm plasma membrane amount to 60 – 65% of its content. In spermatozoa, a high ratio of glutathione/glutathione disulfide (GSH/GSSG) is known to be a protective factor against oxidative stress (Sikka, 1995).

3 MATERIALS AND METHODS

3.1 Animals: Forty adult male Sprague-Dawley rats of 12-14 weeks of age and weighing 380-430 g were randomly selected. They were kept at the animal house at the University of Zimbabwe in a 12-h light/dark cycle with controlled temperature and humidity for the whole research period of 15 weeks. The rats were maintained in wire-floor cages over laboratory-grade pine shavings as bedding and were allowed to adapt for at least 2 weeks prior to the commencement of the study.

3.2 Experimental protocol: The rats were randomly divided into two groups of 20 each. Group one, which was the control got 0.9% normal saline. Group two received paracetamol syrup and timely killed for histological studies, measurement of glutathione levels, sperm analysis as well as fertility study. The rats received daily doses of normal saline or paracetamol syrup based on body weight for up to ninety days applied using the rule of thumb that a rat consumes 5 grams of food and 10 millilitres of water daily per 100 grams of body weight. Four rats from each group were taken at day 15, 30, 60 and 90 for histological analysis, glutathione measurements and sperm analysis. Semen harvesting and sampling from the epididymis was done according to Brown *et al.* (1994).

3.3 Semen analysis: The semen sample was first evaluated by simple visual inspection. A normal sample has a grey-opalescent appearance, is

homogenous and liquefies within 60 min at room temperature 37°C under the influence of enzymes of prostatic origin (Rat Sperm Morphological Assessment Guideline document, 2000). Sperm motility was rated on a visual scale. Sperm were classified as normal, abnormal head, abnormal mid piece, abnormal tail, detached head, proximal cytoplasmic droplet, distal cytoplasmic droplet, and bent tail using criteria described by Oettle and Soley (1988).

Various reports have confirmed the role of glutathione in improving some fertility indicators in human and animals. Spermatozoa are highly susceptible to oxidative damage due to the high content of polyunsaturated fatty acids within their plasma membrane and such damage may underlie certain aspects of male infertility. Increasing knowledge of the mechanisms whereby ROS and endogenous antioxidant systems influence reproductive processes can assist to optimise the application of exogenous antioxidants to fertility treatment (Taylor, 2001). Infertility problems in human are increasing to alarming figures and the investigation of the possible roles of antioxidant is of growing interest among researchers and scientists. This study assessed the role of glutathione depletion in fertility in male rats.

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3.4 Samples collection and measurement of glutathione: Blood samples were harvested by cardiac puncture and venipuncture of the posterior lateral tail veins. Ten labeled samples [1-10 vortex] were set up. Nine milliliters of the blank solution (phosphate buffer) was added to 1 ml of standard GSH in sample 1. Total glutathione in blood was determined by spectrophotometry.

3.5 Determination of fertility: A total of eight rats (four from each group) were used, each male rat was placed in a labeled cage with at least a Para 1 female rat and 21-27 days after the beginning of the mating experience, the female rats were checked daily for parturition. The male rats were considered fertile if the mating resulted in at least one pregnancy. Sexual behavior was assessed through observation of libido (assessed by % mounting and % intromission).

3.6 Data analysis: All data were analyzed by two-way ANOVA using SPSS. Post hoc analyses compared treatment groups for the effects of time

and for the effects of pretreatment/treatment at the same time point, all values were expressed as the mean \pm SD.

4 RESULTS

Liquefaction time in the study group decreased from 60 to 30 seconds at day 60 and remained unchanged despite continued treatment until day 90. In the control group liquefaction time decreased gradually down to 40 seconds on day 90 (Fig. 1).

Sperm motility in the study group dropped by 30% on day 15 and there was no marked change up to day 30. On day 60 motility further dropped to 20% but there wasn't any further decrease. In the control group motility dropped gradually to 50% on day 30 then rose slightly to 55% on day 60 and remained at 50% afterwards. In group A (part of the control group) motility increased from 50 to 75% on

day 30 then decreased sharply to below 50% on day 90. In group 2B (part of the study group) motility never reached 50% from day 15 then dropped to below 20% on day 90 of paracetamol treatment (Fig. 2).

This figure shows that in the study group progressiveness dropped sharply from 5 at day zero to 2 at day 30, and remained constant until day 60. Progressiveness finally dropped to 1 at day 90. In the study group progressiveness remained at 5 until day 15 and gradually dropped and leveled at 3 between day 60 and 90 (Fig. 3).

Average liquefaction time in the two groups over 90 days

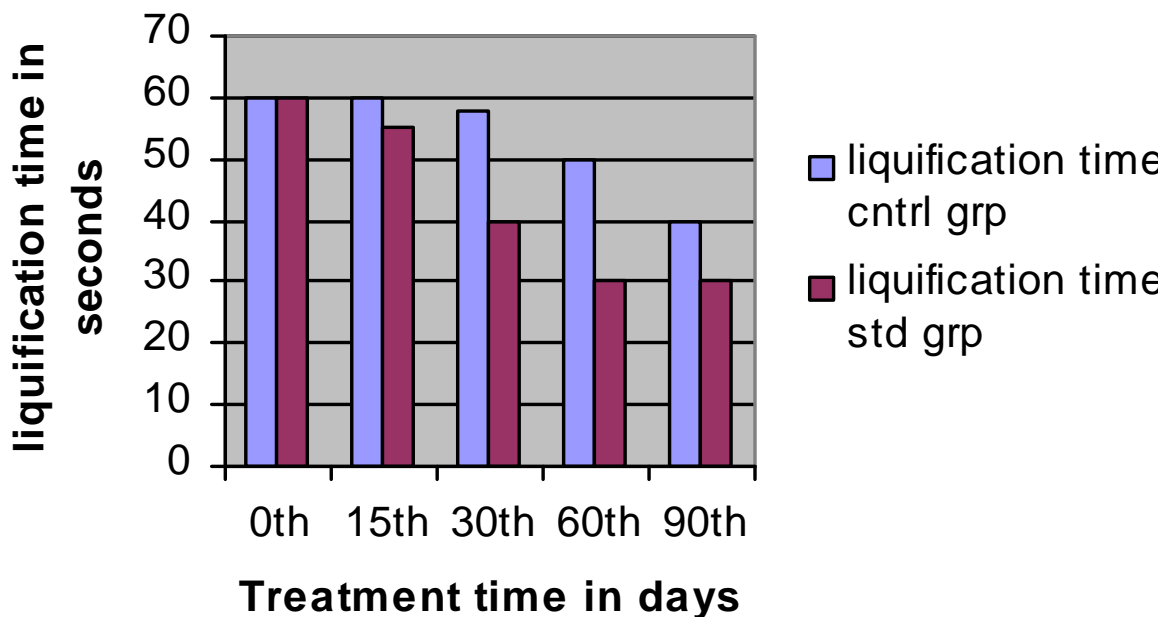
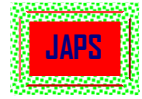


Figure 1: Average liquefaction time in rats over 90 days. Blue columns is for control group; red columns for the standard treatment.

5 DISCUSSION

The effects of glutathione depletion on male fertility resulting from the commonest OTC (over the counter drug) acetaminophen has not been explored by previous studies. Most studies have dealt on the immediate effects of glutathione on fertility in general using unique chemicals such as diethyl maleate and butathionine. Mammalian spermatozoa are highly susceptible to lipid peroxidation, which

leads to structural damage to the sperm cell accompanied by lowered motility and metabolism. In this study GSH dropped to 50nm/l in the study group as compared to 300nm/l in the control group and this showed that paracetamol is a GSH depleter. However a fall in GSH in the control group over time might be attributed to the aging process as



Average % sperm motility

illustrated by Rossi *et al.* (2002) that GSH levels can drop to 250nm/l with age.

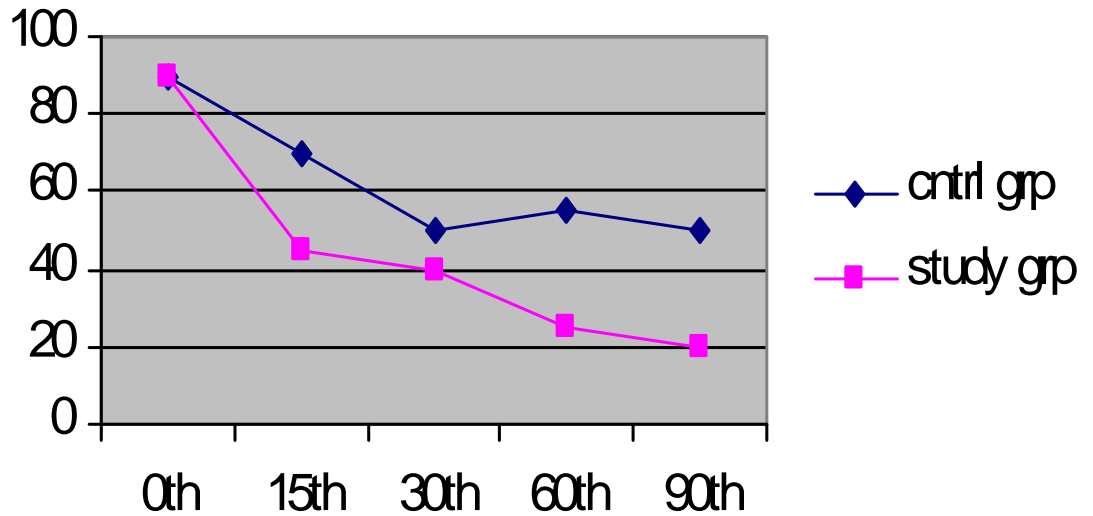


Figure 2: Average motility of rat sperm over 90 treatment days. Blue line is for the control group; red line for the treated group.

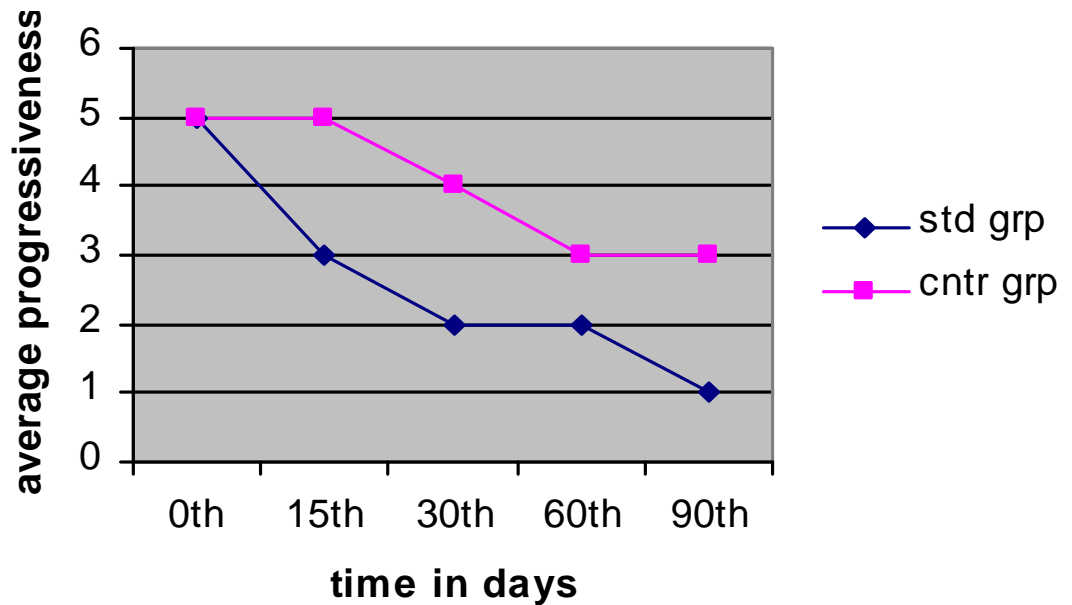
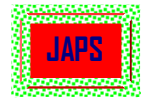


Figure 3: Progressiveness of rat semen over 90 treatment days. Red line for control group; blue line is for the standard treatment group.

GSH depletion affects sperm motility more than any other variable, which is important as previous studies show that motility is the major factor determining the fate of sperms (Seed *et al.*, 1996). In this study sperm motility might have been complemented by massive morphological abnormalities especially on the sperm tail and the

mid piece with 60% and 75% abnormality, respectively. The mid piece being the powerhouse of the sperm is the main part affected by GSH depletion due to high ROS production and the higher amount of PUFA (Garrido *et al.*, 2004). This might be attributed to the effects of hormones other than GSH but since GSH has pleomorphic roles,



more studies are needed to establish the link between the role of hormones and GSH.

In the general appearance of semen marked difference was seen on liquefaction time and homogeneity. This showed that glutathione was reduced in the group treated with glutathione since GSH plays an important role as a co-factor in prostatic enzymes responsible for semen homogeneity and liquefaction lagging time. Lack of homogeneity and reduced liquefaction time affects sperm motility and half-life.

In histological results paracetamol did not cause severe liver necrosis despite marked GSH reduction as reported in previous investigations. This suggests a need for future studies on the pharmacodynamics of paracetamol in other parts of the body other than the liver. The age of the rat at the start of dosing may also influence the outcome of the study. For example, it is known that for some chemicals younger animals are more susceptible than more mature animals (Linder *et al.*, 1990; Brown *et al.*, 1994). In Swiss Albino mice, paracetamol caused reduction in liver GSH-pX activity (Kanbur *et al.*, 2009)

ROS production is high in the reproductive tissues (Kumagai *et al.*, 2002; Agarwal *et al.*, 2003) and the increases in ROS concentration may affect DNA integrity (Ford, 2004; Rivlin *et al.*, 2004). Antioxidants counteract the effects of oxidative stress and age-related decreases in antioxidant enzymes in Leydig cells (Chen *et al.*, 2001) and sertoli cells are characterized by high levels of intracellular GSH (Peltola *et al.*, 1996; Mruk *et al.*, 2002). High levels of glutathione peroxidase have been found in testes (Fujii *et al.*, 2003), and in Sertoli cells (Luo *et al.*, 2006). The protective activity of

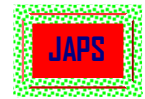
glutathione peroxidase (GPX) in developing spermatids and spermatozoa has been noted in several species (Miura *et al.*, 2002; Krishnamoorthy *et al.*, 2005); in the sperm maturation process (Pfeifer *et al.*, 2001), and having a role against oxidative stress (Kumagai *et al.*, 2002). Reduced glutathione levels may be important in the initiation of chromatin compaction and DNA stabilization during spermiogenesis. It must be taken into consideration that there is not yet a standardized methodology for semen analysis and experimental models in rat's reproductivity, and these differ from one laboratory to the other. Thus, basal values of seminology and GSH measured previously may (at least in part) differ and may also derive from artifacts.

The results of this study demonstrate that paracetamol depletes glutathione and this depletion affected spermatogenesis. This in turn results in poor sperm quality and ultimately reduced fertility index as well as concentrations of GSH and, particularly, GSSG in blood. These findings suggest that almost all differences found in other investigations may be attributable to these artifacts more than to patho-physiologic in vivo factors.

In conclusion, the study demonstrated that the effect of GSH depletion affected greatly sperm motility, morphology, and homogeneity, liquefaction and fertility index. However, some similar changes were also noted in the control group and this might be attributed to the aging process and biological factors. More studies need to be done on the male human subject since of the 40% cases of infertility, 10% are of unknown etiology. Male infertility can be reduced if ways of supplementing glutathione are discovered in addition to identifying other potential depletors of glutathione.

6 REFERENCES

- Agarwal, A., Saleh, R.A., Bedaiwy, M.A., 2003: Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil. Steril.* 79: 829–843.
- Alvarez, J.G. and Storey, B.T., 2005: Differential incorporation of fatty acids into and peroxidative loss of fatty acids from phospholipids of human spermatozoa. *Mol. Reprod. Dev.* 42: 334–346.
- Brown, C.D., Forman, C.L., McEuan, S.F., Miller, M.G. 1994. Metabolism and testicular toxicity of 1,3-dinitrobenzene in rats of different ages. *Fundam Appl Toxicol.* 23: 439-446.
- Chen, H., Cangello, D., Benson, S., Folmer, J., Zhu, H., Trush, M.A., Zirkin, B.R., 2001: Age-related increase in mitochondrial superoxide generation in the testosterone-producing cells of Brown Norway rat testes: relationship to reduced steroidogenic function? *Exp. Gerontol.* 36: 1361–1373.
- Christopherson, B.O., 1968: The inhibitory effect of reduced glutathione on the lipid peroxidation of microsomal fraction and mitochondria. *Biochem. J.*, 106: 515-522.
- Fujii, J., Iuchi, Y., Matsuki, S., Ishii, T., 2003: Cooperative function of antioxidant and redox systems against oxidative stress in



- male reproductive tissues. *Asian J. Androl.* 5, 231–242.
- Garrido, N., Meseguer, M., Alvarez, J., Simon, C., Pellicer, A., Remohi, J. 2004: Relationships among standard semen parameters, glutathione peroxidase/glutathione reductase activity, and mRNA expression and reduced glutathione content in ejaculated spermatozoa from fertile and infertile men. *Fertil Steril.* 82 Suppl 3:1059–66.
- Kanbur M, Eraslan G, Beyaz L, Silici S, Liman BC, Altinordulu S, Atasever A. (2009). The effects of royal jelly on liver damage induced by paracetamol in mice. *Exp Toxicol Pathol.* 61(2):123-32.
- Krishnamoorthy, G., Murugesan, P., Muthuvel, R., Gunadharini, D.N., Vijayababu, M.R., Arunkumar, A., Venkataraman, P., Aruldas, M.M., Arunakaran, J., 2005: Effect of Aroclor 1254 on Sertoli cellular antioxidant system, androgen binding protein and lactate in adult rat in vitro. *Toxicology* 212: 195–205.
- Linder, R.E., Strader, L.F., Barbee, R.R., Rehnberg, G.L., Perreault, S.D., 1990: Reproductive toxicity of a single dose of 1,3-dinitrobenzene in two ages of young adult male rats. *Fundam Appl Toxicol.* 14: 284–298.
- Luo, L., Chen, H., Trush, M.A., Show, M.D., Anway, M.D., Zirkin, B.R., 2006: Aging and the brown Norway rat Leydig cell antioxidant defense system. *J. Androl.* 27: 240–247.
- Maxwell, W.M.C. and Watson, P.F., 1996. Recent progress in the preservation of ram semen. *Anim. Reprod. Sci.* 42, 55–65.
- Miura, M., Sasagawa, I., Suzuki, Y., Nakada, T., Fujii, J., 2002: Apoptosis and expression of apoptosis-related genes in mouse testis following heat exposure. *Fertil. Steril.* 77, 787–793.
- Mruk, D.D., Silvestrini, B., Mo, M.Y., Cheng, C.Y., 2002: Antioxidant superoxide dismutase—a review: its function, regulation in the testis, and role in male fertility. *Contraception* 65 (4): 305–311.
- Oettle, E.E., and Soley, J.T., 1988: Peroxidative loss of fatty acids from phospholipids of human spermatozoa. *Mol Reprod Dev.* 42: 334 – 346.
- Peltola, V., Huhtaniemi, I., Metsa-Ketela, Ahotupa, M., 1996. Induction of lipid peroxidation during steroidogenesis in the rat testis. *Endocrinology* 137: 105–112.
- Rossi, R., Milzani, A., Dalle-Donne, I., Giustarini, D., Lusini, L., Colombo, R., Di Simplicito, P., Rao, M.V. and Sharma, P.S. 2002: Polyunsaturated fatty acids of germ cell membranes, glutathione and glutathione-dependent enzyme-PHGPx: from basic to clinic. *Contraception.* 65(4): 301-4.
- Sikka, S.C., 1995: Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Frontiers Biosci.* 1:78–86.
- Slaweta, R., and Laskowska, T., 1987: The effect of glutathione on the motility and fertility of frozen bull semen. *Anim. Reprod. Sci.*, 13: 249-253.
- Seed, J., Chapin, R. E., Clegg, E. D., Dostal, L. A., Foote, R. H., Hurtt, M. E., Klinefelter, G. R., Makris, S. L., Perreault, S. D., Schrader, S., Seyler, D., Sprando, R., Treinen, K. A., Veeramachaneni, D. N., Wise, L. D., 1996: Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. *Reproductive Toxicology* 10(3): 237-244
- Taylor, C.T., 2001. Antioxidants and reactive oxygen species in human fertility. *Environmental Toxicology and Pharmacology* 10: 189–198.
- Zalata, A., Christophe, A., Depuydt, C., 1998: The fatty acid composition of phospholipids of spermatozoa from infertile patients. *Mol Hum Reprod.* 4: 11–8.