Degenerative effect of paraquat-induced oxidative stress and the ameliorative effect of *Aloe vera* gel on the egg and wing morphometry in *Drosophila melanogaster*

Usha Rani[1], Krishan Kumar Selwal[1], Manvender Singh[2] and Veer Bhan[2*]

[1]Department of Biotechnology, Deenbandhu Chhotu Ram University of Science & Technology, Murthal (Sonepat)-131039, India

[2]Department of Biotechnology, University Institute of Engineering & Technology, Maharshi Dayanand University, Rohtak-124001, India

[*]**Corresponding Author:** veerbhan.uiet@mdurohtak.ac.in

Associate Professor, Department of Biotechnology, University Institute of Engineering & Technology, Maharshi Dayanand University, Rohtak-124001, India

Article History: **Received:** 23.04.2022 **Revised:** 18.06.2022 **Accepted:** 27.07.2022

Abstract:

Paraquat (PQ) is known to induce oxidative stress (OS), which causes damage to the cell membrane, DNA, lipids, and proteins. In the present study, we analyzed the degenerative effect of PQ-induced OS and the ameliorative effect of *Aloe vera* gel (AVG) on egg morphometry in *Drosophila melanogaster* (Dm). Flies were sorted into control (CTR), PQ-treated, and AVG+PQ co-treated. The control group was fed on Standard *Drosophila* Medium (SDM). The AVG-supplemented group was fed on SDM and Aloe vera gel (5mL/L). PQ-treated and AVG+PQ-treated groups were exposed to an 18mM/L concentration of PQ for 8 hours and were allowed to recover from PQ stress by feeding on their respective food for 48 hours. The eggs laid by mated females were analyzed morphometrically under a stereo-zoom microscope. A significant degenerative effect was observed for the eggs of *Drosophila* flies exposed to PQ without *Aloe vera* gel supplementation. In comparison, a substantial reduction in egg degeneration was observed where flies were supplemented with *Aloe vera* gel and PQ exposure. The result of the present study showed the degenerative effect of PQ and the ameliorative effect of AVG in Dm.

Degenerative effect of paraquat-induced oxidative stress and the ameliorative effect of Aloe vera gel on the egg and wing morphometry in Drosophila melanogaster

Section: Research Paper

Keywords: Paraquat, Aloe vera gel, antioxidant, *Drosophila melanogaster*, oxidative stress

Introduction:

Aerobic metabolism develops reactive oxygen species (ROS), which are by-products that accumulate during regular oxygen metabolism. ROS production is necessary for gene expression (Finkel & Holbrook, 2000), cell signaling (Thannickal & Fanburg, 2000; Ravindra et al., 2004; Sittipunt, 2005), and redox homeostasis. Antioxidant systems that scavenge free radicals are essential for maintaining a healthy ROS level. The balance between generating and eliminating ROS is crucial for almost every mammalian metabolic function. Maintenance of this balance is an essential constitutive process and mainly influences cell proliferation, differentiation, apoptosis, and death (Suntres, 2002). Oxidative stress (OS) arises when ROS generation outpaces the antioxidants' capacity to scavenge ROS. Unfortunately, an increase in ROS concentration or a decrease in scavenging capacity can readily disrupt this equilibrium. ROS levels that are too high are hazardous to human health. They can accumulate oxidative damage in distinct subcellular compartments that exert very toxic effects on DNA, proteins, and lipids. ROS-mediated damage can ultimately influence physiological functions, such as cell and redox-sensitive signaling pathways, and lead to pathological conditions (Smith et al., 1978).

Regarding the female reproductive system, ROS and antioxidants have been recognized as critical factors involved in ovarian physiological metabolism. Nutritional status influences reproduction at the level of gametogenesis, particularly ovarian function, in a wide range of organisms, from invertebrates, like worms and insects, to large domestic animals, like sheep and pigs (Drummond-Barbosa & Spradling, 2001; Sohrabi *et al.,* 2015; Hohos & Skaznik-Wikiel, 2017; Wang *et al.,* 2017). For example, a high-fat diet leads to poor ovarian function, as evidenced by more ovarian follicle death and fewer maturing follicles in rabbits (Cordier *et al.,* 2013) or an increase in immature, at the expense of developing, follicles in mice (Solon-Biet *et al.,* 2015). Conversely, ovaries from mice fed a calorically restricted diet contained a larger pool of developing follicles and fewer atretic follicles compared to female mice fed *ad libitum*, ultimately leading to improved fertility and fecundity (Selesniemi *et al.*, 2008). Over the last decade, *Drosophila melanogaster*, the fruit fly, has emerged as an essential player in metabolism and physiology studies (Baker & Thummel, 2007; Rajan & Perrimon, 2013; Trinh & Boulianne, 2013), with many focused on the effects of maternal nutrition on reproduction and offspring health (Brookheart & Duncan, 2016). In the present study, *Drosophila melanogaster* was used as an *in-vivo* model organism to understand the dietary influences of Aloe vera gel on ovarian function due to morphological similarities between fruit fly and human ovaries as well as stages of *Drosophila* oocyte development, which will provide helpful information on the overall influence of diet on ovarian output (i.e., oocyte production) in fruit flies.

Materials and Methods:

Oregon-K strain of Dm was grown on SDM. Flies were sorted into Control (CTR), PQ-treated, and AVG+PQ co-treated. The control group was fed on Standard *Drosophila* Medium (SDM). The AVG-supplemented group was fed on SDM and Aloe vera gel (5mL/L). PQ-treated and AVG+PQ-treated groups were exposed to an 18mM/L concentration of PQ for 8 hours and were allowed to recover from PQ stress by feeding on their respective food for 48 hours. The eggs and wings were analyzed under the stereo-zoom microscope. Poel's salt solution was applied during the eggs' analysis to maintain the egg membrane's osmolarity.

Result and discussion:

PQ's effect on the developmental morphology of eggs:

For morphometrical analysis, eggs were collected from CTR, AVG-supplemented, PQ-exposed, and AVG+PQ co-treated flies, **Figures 1 and 2** depict the eggs laid by a *Drosophila* female fly and the structure of a single egg, respectively. Eggs from the CTR group showed average growth and appearance, whereas eggs of PQ-exposed flies showed an abnormal appearance (~46%) due to shorter and malformed chorionic appendages (**Figure 3**). The size of the eggs from the PQ-exposed flies was also reduced compared to eggs oviposited by control females. Most exposed eggs showed significant developmental abnormalities in the chorionic appendages and outer chorion structure (~91%; n ~ 45). The cuticle layer of the 3rd instar larvae was degraded in the initial three days when metamorphically emerging into 3rd instar larvae. 3rd instar larvae of control flies developed a mature cuticle layer with polar cells. Their polar cells were identified with the acid staining. In contrast, eggs from the PQ-treated flies lacked the cuticle layer as it was degraded in the initial three days when metamorphically emerging into 3rd instar larvae (**Figures 2 and 3**). **Figure 4** describes the negative image of an egg from the control *Drosophila* fly, showing yolk spheres, vitelline membrane, and PF.

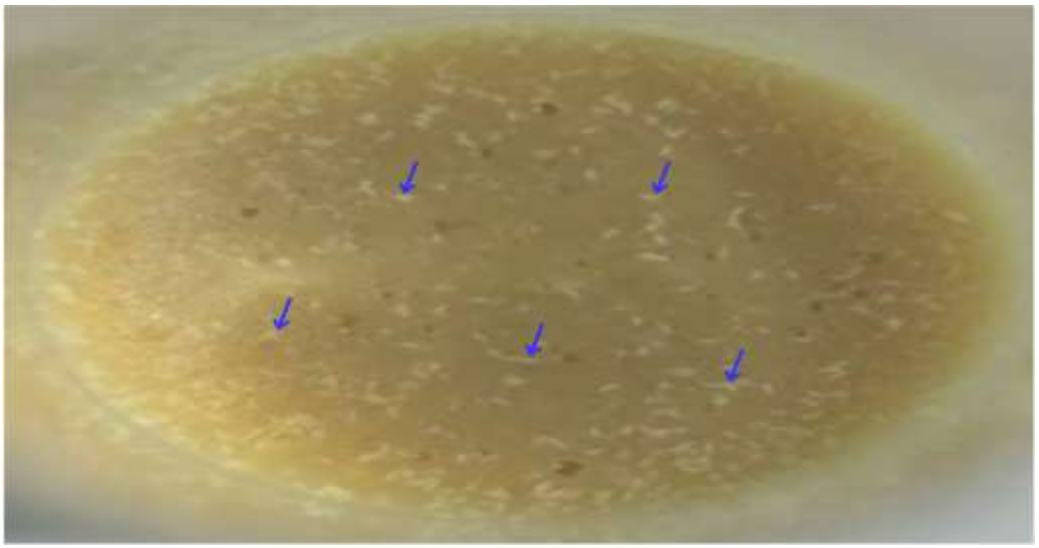

Figure 1: Eggs laid by *Drosophila* are indicated by blue arrows.

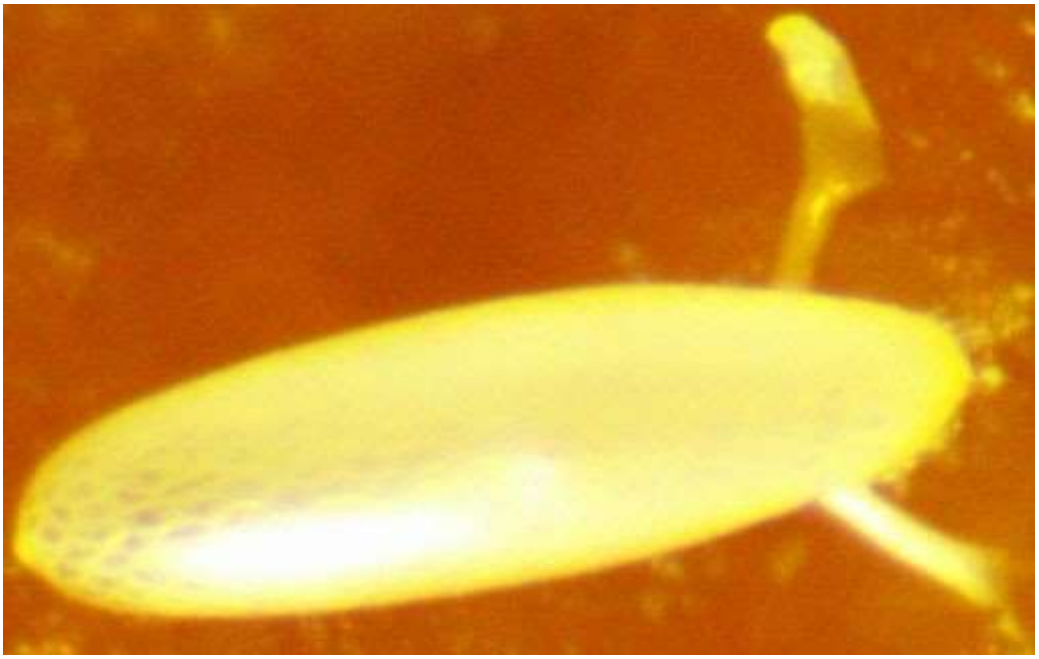

Figure 2: A single egg structure of *Drosophila melanogaster.*

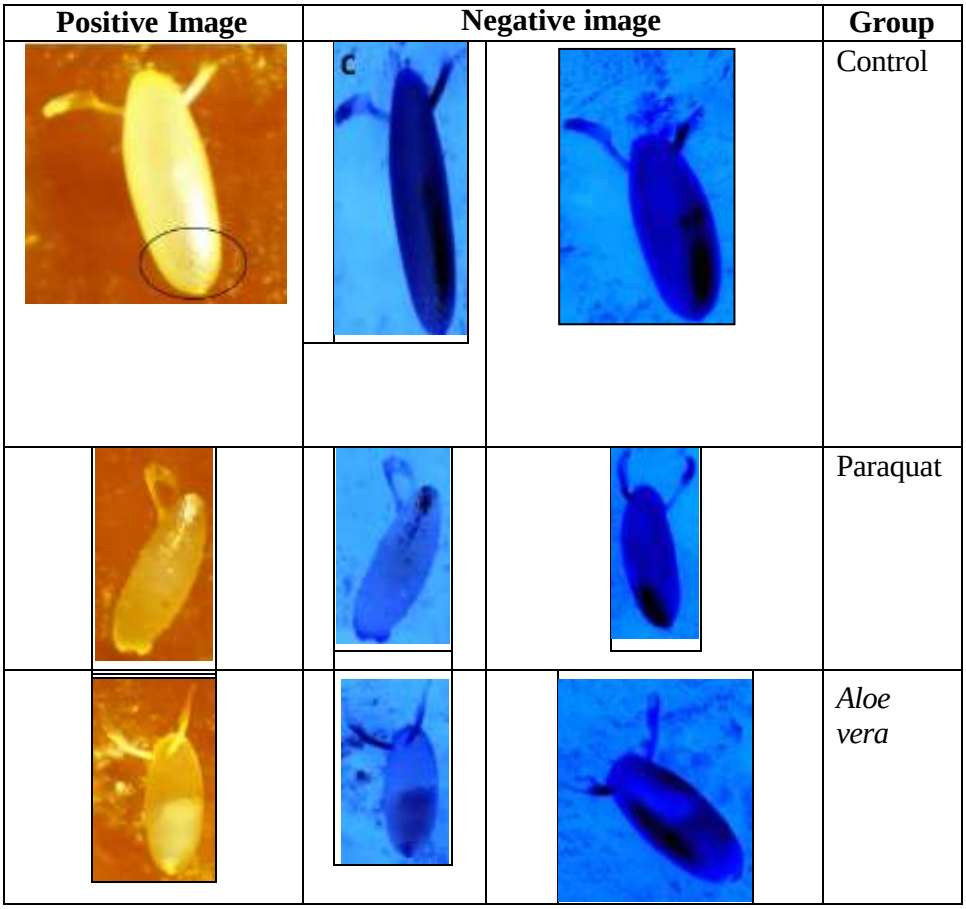

Figure 3: Positive and Negative Images of Control, Paraquat-Exposed, and *Aloe* Vera-Supplemented Eggs of *Drosophila melanogaster* Individuals

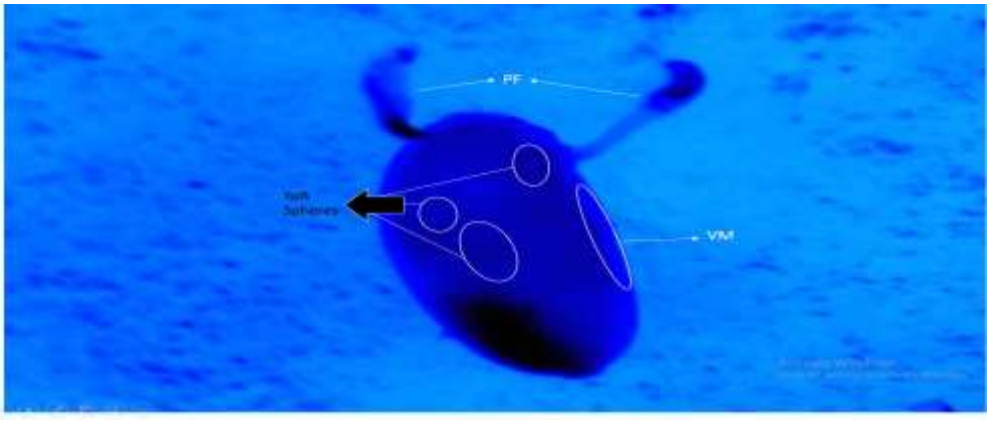

Figure 4: Negative image of an egg from a control *Drosophila* fly showing yolk spheres, vitelline membrane, and PF.

PQ's effect on the developmental morphology of wings:

A morphometric study was performed to analyze the PQ's effect on the development of wings. Wings from the CTR flies showed a regular appearance in their size and development (**Figure 5**), whereas wings from the flies exposed to PQ showed an abnormal appearance in their size and development (**Figure 6**). PQ-treated and AVG+PQ co-treated group improved their deformed wings (**Figure 7**).

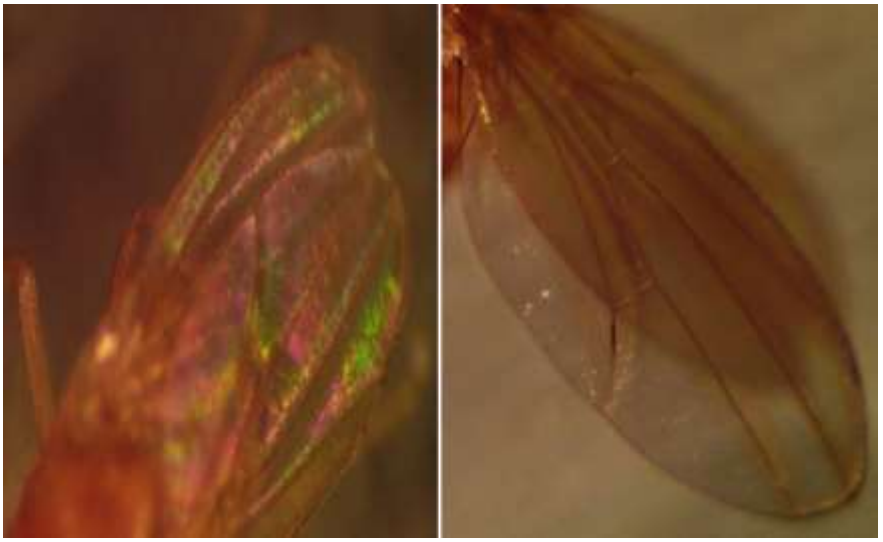

Figure 5: *Drosophila* wings from CTR flies.

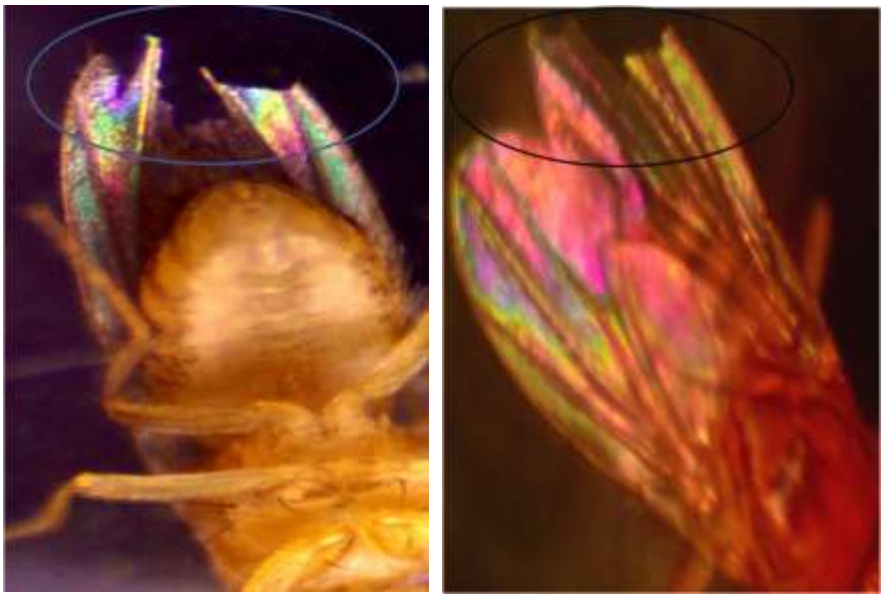

Figure 6: *Drosophila* wings from PQ-treated flies.

Figure 7: *Drosophila* wings from PQ-treated and Abmg-5-supplemented flies.

The female reproductive system of Dm was severely affected by PQ exposure. Females who received PQ treatment for their ovaries showed flaccid ovaries and tiny, underdeveloped follicles. The deposited eggs had smaller chorionic appendages or none at all and an uneven surface pattern imprinted on the egg during oogenesis by the follicular cells around it. Only a small percentage of eggs hatch into larvae, and many develop melanotic tumors or pass away later in life. The tiny number of adult survivors exhibited tumors and severe malformations, such as aberrant bristles, eyes, and legs, eerily identical to those deformities reported in anti-folate-treated mammals (Schardein, 2000).

Given that PQ is employed to stop rapidly dividing cells, these results may not be entirely unexpected. The existence of antioxidants and their transcripts in the female reproductive system has been the subject of numerous investigations (Tapiwanashe et al., 2006; Fryxell, 1988; Das et al., 2011). According to earlier research, the equilibrium between ROS and antioxidants has a significant impact on the reproductive processes in female mammalian animals, including endometrial changes during various luteal phases, folliculogenesis, ovulation, fertilization, placental growth, embryogenesis, and implantation (Akilandeswari et al., 2010). However, compromised reproduction and fertility may be induced under OS conditions, including impaired ovarian functions, deteriorated oocyte quantity, embryonic development disorders, gynecological disease, and infertility (Harbourne, 1973; Trease &

Evans, 1989; Nada et al., 2008). In order to sustain normal ovarian function, antioxidants are crucial for preserving the redox balance in the ovaries. Their precise chemical mechanisms and functions, however, are still unclear. Prior research has mainly concentrated on the roles ROS plays in the ovaries. Therefore, in this context, it is necessary to have a systematic understanding of antioxidant expression, regulation, and molecular pathways related to ovarian function. In the present study, PQ exposure demonstrated its oxidative nature, which was observable as egg and wing degeneration of the PQ-treated group. In contrast, the ameliorative effect of Aloe vera gel due to its phytoconstituents was demonstrated by the AVG+PQ co-treated group, where flies showed improved egg and wing morphometry. However, future investigations of possible antioxidant supplementation are necessary to protect against various nutrition-related diseases.

ACKNOWLEDGEMENT:

Usha Rani is highly thankful to UGC for providing RGNF-JRF/SRF [F1-17.1/2017-18/RGNF-2017-18-SC-HAR-38467/ (SA-III/Website) dated 29.11.2017].

CONFLICT OF INTEREST:

There is no conflict of interest with the writers.

References

Akilandeswari S, Senthamarai R, Valarmathi R, Prema S (2010) Screening of Gastric Antiulcer Activity of *Sida acuta* Burm. Int J Pharm Tech Res 2: 585–587.

Baker, K. D., & Thummel, C. S. (2007). Diabetic larvae and obese flies-emerging studies of metabolism in *Drosophila*. *Cell metabolism*, *6*(4), 257–266. https://doi.org/10.1016/j.cmet.2007.09.002

Brookheart, R. T., & Duncan, J. G. (2016). Modeling dietary influences on offspring metabolic programming in *Drosophila melanogaster*. *Reproduction (Cambridge, England)*, *152*(3), R79–R90. https://doi.org/10.1530/REP-15-0595

Cordier, A. G., Léveillé, P., Dupont, C., Tarrade, A., Picone, O., Larcher, T., Dahirel, M., Poumerol, E., Mandon-Pepin, B., Lévy, R., & Chavatte-Palmer, P. (2013). Dietary lipid and cholesterol induce ovarian dysfunction and abnormal LH response to stimulation in rabbits. *PloS one*, *8*(5), e63101. https://doi.org/10.1371/journal.pone.0063101

Das N, Achari B, Harigay Y, Dinda B (2011) A new flavonol glucoside from the aerial parts of Sida glutinosa. J Asian Nat Prod Res 13(10): 965-997.

Drummond-Barbosa, D., & Spradling, A. C. (2001). Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Developmental biology*, *231*(1), 265–278. https://doi.org/10.1006/dbio.2000.0135

Finkel T, Holbrook NJ (2000). Oxidants, oxidative stress and the biology of ageing. Nature, 408(6809): 239-247.

Fryxell, P.A. (1988) Malvaceae of Mexico. Systematic Botany Monographs, 25, 1-522. https://doi.org/10.2307/25027717

Harbourne (1973) Methods of extraction & isolation. In: Phytochemical methods Chapman & Hill, London.

Hohos, N. M., & Skaznik-Wikiel, M. E. (2017). High-Fat Diet and Female

Fertility. *Endocrinology*, *158*(8), 2407–2419. https://doi.org/10.1210/en.2017-00371

Nada MA, Hamdi MH, Nadia GK, Omar AM (2008). Molecules 13: 1501- 1517.

Rajan, A., & Perrimon, N. (2013). Of flies and men: insights on organismal metabolism from fruit flies. *BMC Biology*, *11*, 38. https://doi.org/10.1186/1741-7007-11-38

Ravindra PS, Shashwat S, Suman K (2004). Free Radicals and Oxidative Stress in Neurodegenerative Diseases: Relevance of Dietary Antioxidants. JIACM 5(3): 218-225

Schardein RL (2000). *Chemically induced birth defects.* New York Basel: Marcel Dekker Inc.

Selesniemi, K., Lee, H. J., & Tilly, J. L. (2008). Moderate caloric restriction initiated in rodents during adulthood sustains function of the female reproductive axis into advanced chronological age. *Aging cell*, *7*(5), 622–629. https://doi.org/10.1111/j.1474-9726.2008.00409.x

Sittipunt C. (2005). Paraquat poisoning. *Respiratory care*, *50*(3), 383–385.

Smith LL, Rose, M S, Wyatt I (1978). The pathology and biochemistry of paraquat. Ciba Found Symp 65: pp. 321–341.

Sohrabi, M., Roushandeh, A. M., Alizadeh, Z., Vahidinia, A., Vahabian, M., & Hosseini, M. (2015). Effect of a high fat diet on ovary morphology, in vitro development, in vitro fertilisation rate and oocyte quality in mice. *Singapore Medical Journal*, *56*(10), 573–579. https://doi.org/10.11622/smedj.2015085

Solon-Biet, S. M., Walters, K. A., Simanainen, U. K., McMahon, A. C., Ruohonen, K., Ballard, J. W., Raubenheimer, D., Handelsman, D. J., Le Couteur, D. G., & Simpson, S. J. (2015). Macronutrient balance, reproductive function, and lifespan in aging mice. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(11), 3481–3486. https://doi.org/10.1073/pnas.1422041112

Suntres ZE (2002). Role of antioxidants in paraquat toxicity. Toxicol 180(1): 65–77.

Tapiwanashe M, Melanie W, Kumars R, Michael PM, Robin AJ (2006) Mech Age Devpt 127: 356-370.

Thannickal, V. J., & Fanburg, B. L. (2000). Reactive oxygen species in cell signaling. *American journal of physiology. Lung cellular and molecular physiology*, *279*(6), L1005–L1028. https://doi.org/10.1152/ajplung.2000.279.6.L1005

Trease GE, Evans WC (1989). Comparative Phytochemical and Nutritional Composition of *Trichosanthes cucumerina* (*L.*) and Some *Solanum lycopersicum* (*L.*) Cultivars in Nigeria Pharmacognosy 13th Ed Bailliene Tindall Ltd London.

Trinh, I., & Boulianne, G. L. (2013). Modeling obesity and its associated disorders in *Drosophila*. *Physiology (Bethesda, Md.)*, *28*(2), 117–124. https://doi.org/10.1152/physiol.00025.2012