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Antioxidative potential of alpha-mangostin against hydrogen peroxide induced oxidative stress in human sperm

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ABSTRACT: Oxidative stress has detrimental effects on sperm viability and fertility, and antioxidant supplementation can improve sperm quality and function. Alpha-mangostin (α-MG) has high potential antioxidants, but its effects on human sperm quality have not been thoroughly explored. This study aimed to investigate the effects of α-MG on sperm motility, acrosome reaction after ionophore challenge (ARIC), enzymatic antioxidative activities, and malondialdehyde (MDA) levels in human sperm. Semen samples from 30 men were subjected to 150 μM of H_2O_2 (to induce oxidative stress) and various combinations of α-MG (25 μM and 50 μM). The results indicated that supplementing sperm with α-MG at 25 μM and 50 μM significantly increased the percentage of progressive sperm motility. Both α-MG concentrations resulted in significant reduction in the ARIC index. MDA levels in samples supplemented with α-MG were lower than in samples without α-MG. The concentration of α-MG at 50 μM substantially improved the catalase activity, and at 25 μM, substantially enhanced the glutathione peroxidase (GPx) activity. In summary, supplementation of α-MG optimized the motility and acrosome reaction index of human sperm exposed to oxidative stress.

KEYWORDS: acrosome reaction, alpha-mangostin, human sperm, malondialdehyde, oxidative stress

INTRODUCTION

Infertility is an important reproductive health issue affecting 10%–15% of couples, with approximately half of these issues being related to the male factor, particularly the production of healthy sperms. A semen analysis is a basic infertility test, which measures sperm concentration, motility, and morphology [1, 2]. However, the standard semen analysis does not assess acrosome reaction, a process that plays an important role in fertilization [3]. The acrosome reaction after ionophore challenge (ARIC) test demonstrated a positive correlation between acrosome reaction and pregnancy rate, which indicates that the ARIC index may be used as an alternative test for sperm fertilizing ability [4, 5].

Intrauterine insemination (IUI) and *in vitro* fertilization (IVF) are frequently used in the treatment of infertility in couples suffering from sperm dysfunction or unexplained male infertility. Even with recent technological advancements, the success rate of IUI and IVF remains between 13%–30% [6, 7]. Numerous biological and technical factors contribute to the success of IUI and IVF, including the quality of acrosomal activity. However, fragmentation of sperm DNA may impair fertilization leading to unfavorable IUI and IVF outcomes [8]. A possible cause of sperm damage is an attack by reactive oxygen species (ROS) during the sperm preparation process. Excessive amounts of ROS have pathological effects on spermatozoa, including decreasing sperm motility, promoting lipid peroxidation, DNA fragmentation, and premature acrosome reaction, which impair the spermatozoa's ability to penetrate the zona pellucida. These conditions may have an effect on fertilization and pregnancy rates [9–12].

Mangosteen (*Garcinia mangostana* L, Guttiferae) is a tropical tree native to Southeast Asia and extensively distributed across Thailand, Malaysia, and India. Xanthone α-MG is a major bioactive compound found in mangosteen [13, 14] which has been confirmed to have both antioxidative [15–17] and anti-inflammatory properties in several cell types [18–20].

Recent study showed that supplementation of α-MG at 25 μM and 50 μM could prevent retinal cell death after oxidative stress induction by H_2O_2 in both *in vivo* and *in vitro* studies [20]. Moreover, Nelli et al [21] reported that rats with streptozotocin (STZ) induced diabetes, when treated with α-MG, showed a significant increase in spermatozoa count, motility, and viability and a decrease in abnormal sperm morphology when compared with the control group. The α-MG decreased the MDA levels in testis when compared with the untreated control group. Fur-

thermore, when treated rats were compared with the control group, antioxidant levels such as superoxide dismutase (SOD), catalase, and GPx were considerably improved. The purpose of this study was to determine the effect of α-MG supplementation on the quality of sperm, specifically motility, viability, the ARIC index, MDA levels, and antioxidative enzyme activity in human sperm in the environment of oxidative stress.

MATERIALS AND METHODS

Collection of semen samples

Semen samples were collected from healthy volunteers ($n = 30$) who presented for semen analysis at the Infertility Center of Naresuan University Hospital. The protocol was approved by the Naresuan University Institutional Review Board (Ethics number: 532/59) and all participants signed an informed consent form before participating. Semen was collected via masturbation and placed in a sterile container for 30 min at 37 °C to liquefy. Only semen samples exhibiting normal parameters, according to the 5th edition of World Health Organization (WHO) guideline [1], were recruited.

Semen sample preparation

Motile sperm cells were separated by two density gradients, using Sil-Selected Plus® (FertiPro N.V., Beernem, Belgium). To separate immotile sperm cells and seminal plasma from motile sperm cells, liquefied semen samples were placed on top of two layers of Sil-Selected Plus® gradients (45% and 90%) and centrifuged at $360 \times g$ for 15 min. The separated motile sperm cells were centrifuged twice with Ferticult™ Flushing medium at $300 \times g$ for 5 min.

Induction of *in vitro* oxidative stress in human sperm and α-MG supplementation

To evaluate the *in vitro* effect of α-MG following H_2O_2-induced oxidative stress, motile sperms were resuspended and then divided into four groups: Group 1, sperm cells incubated with Ferticult™ medium for the control; Group 2, sperm cells incubated with Ferticult™ medium and 150 μM of H_2O_2 (H_2O_2); Group 3, sperm cells incubated with Ferticult™ medium, 150 μM of H_2O_2, and 25 μM of α-MG simultaneously (25 α-MG); and Group 4, sperm cells incubated with Ferticult™ medium, 150 μM of H_2O_2, and 50 μM of α-MG simultaneously (50 α-MG). All samples were incubated for 3 h at 37 °C and then evaluated for motility, viability, ARIC index, lipid peroxidation, and antioxidant enzymatic activities.

Sperm motility and viability

Sperm motility was assessed under a light microscope following the 5th edition WHO criteria. The percentage of motile sperm was determined by placing a 10 μl drop of sperms in a Makler® counting chamber. Sperm viability was determined using eosin-nigrosin staining, in which 20 μl of 1% eosin and 20 μl of 10% nigrosin were mixed, and then smeared on a glass slide. Each slide was observed at a magnification of 400 ×. Two hundred sperm cells were counted twice, to determine the live (unstained) and the dead (stained) cells. The percentage of live sperm was used to calculate sperm viability.

Acrosome reaction after ionophore challenge (ARIC) test

The ARIC index was determined in our study using the procedure described previously by Cummins et al [4]. Each of the four incubated sperm cell groups was divided into two equal aliquots. One of the aliquots was filled with 10 μM calcium ionophore A23187 (induced acrosome reaction), while the other was allowed to undergo spontaneous acrosome reaction. After 30 min, a sample from each aliquot was smeared on glass slides, allowed to air dry, and then fixed in 95% ice cold ethanol for 30 min to permeabilize the sperm membrane, then stained with fluorescein isothiocyanate conjugated *Pisum sativum agglutinin* (FITC-PSA) for 15 min in a dark environment. The slides were gently washed with phosphate buffer saline (PBS) to remove any remaining residue and observed at 400 × magnifications for acrosome reaction. The sperm cells were counted twice, wherein, 200 sperm cells were exhibiting acrosome reaction. In intact acrosomes, the acrosome region of the sperm head was bright and uniformly fluorescent; whereas in the acrosome reactive sperm cells, only the equatorial segment part of the acrosome was stained. The ARIC index was calculated as the difference between percentage of the induced acrosome-reacted cells and the spontaneous acrosome-reacted cells.

Measurement of lipid peroxidation

Lipid peroxidation in sperm cells was determined by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). After incubation, a sperm pellet containing 1×10^6 sperm cells was sonicated and centrifuged at $12\,000 \times g$ for 15 min at 4 °C, and the supernatant was added to a mixture containing 1.5 ml of 35% acetic acid, 200 μl of 8.1% sodium dodecyl sulfate, and 1.5 ml of 0.8% TBA. The mixture was incubated at 90 °C for 1 h, and then assessed using microplate reader at 532 nm. The results are expressed as nmol/10^6 cells/ml.

Measurement of catalase activity

Catalase activity was determined by measuring the decrease in H_2O_2 at 240 nm, as described by Beers and Sizer [22]. A sperm pellet containing 5×10^5 sperm cells was sonicated and centrifuged at $12\,000 \times g$ for 15 min at 4 °C, and the supernatant was mixed with 1.9 ml of catalase buffer (0.05 M PBS, pH 7). The rate

of extinction was recorded at 240 nm at 1-min intervals for 5 min immediately following the addition of 1 ml of 30 mM H_2O_2 solution into the mixture. Values of catalase activity are presented as μmol H_2O_2/min/10^5 cells/ml.

Measurement of GPx activity

The GPx activity was evaluated using a commercial enzyme assay kit (Cayman Chemical, Ann Arbor, MI), following the instructions provided by the manufacturer. A 20 μl supernatant of the sample was added into the working reagent, followed by 20 μl addition of enzyme solution. GPx activity was measured indirectly via a coupled reaction with glutathione reductase (Gr). Oxidized glutathione (GSSG) was produced from the reduction of H_2O_2 during GPx activity and subsequently converted to Glutathione (GSH) with a concomitant oxidation of NADPH to $NADP^+$. The rate of extinction was recorded every 1 min for 5 min at 340 nm. GPx activity is expressed as nmol/min/ml.

Statistical analysis

Data are expressed as the mean of the number of determinations and the standard error of the mean (S.E.M). Statistical significance was determined using the analysis of variance (ANOVA), followed by a Tukey's multiple comparisons test to identify individual differences between means. All analyses were carried out using the GraphPad Prism version 7.04 (GraphPad Software, CA, USA).

RESULTS

Effects of α-MG on sperm motility and viability

The three hour incubation of spermatozoa with 150 μM H_2O_2 caused a substantial reduction in progressive sperm motility compared with the control ($p < 0.05$). This reduction was completely reversed in a dose-dependent manner when 25 μM and 50 μM of α-MG ($p < 0.05$) were introduced. However, no significant change in the percentage of viable sperm cells was observed across all groups (Table 1).

Effect of α-MG on acrosome reaction after ionophore challenge

The acrosome reaction of spermatozoa incubated with H_2O_2 and supplemented with 25 μM and 50 μM of α-MG was measured following a 3-h incubation. In comparison to the control group, the ARIC index in the H_2O_2 group indicated a significantly increased rate of premature acrosome reaction (24.72 ± 1.50 versus 16.46 ± 0.74) ($p < 0.05$). The ARIC indices in the groups where specimens triggered with H_2O_2 and then supplemented with 25 μM and 50 μM of α-MG were 14.73 ± 0.72 and 14.85 ± 0.64, respectively; the values were considerably lower than in the H_2O_2 group (Fig. 1).

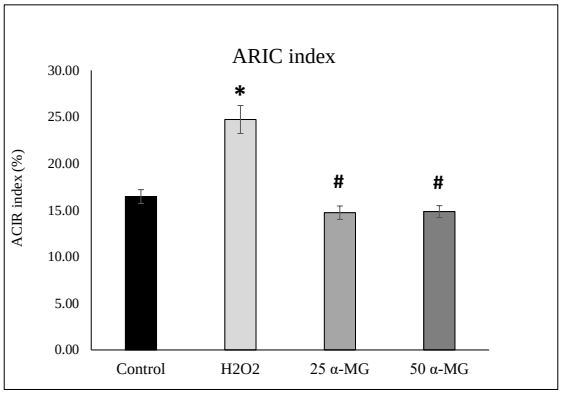

Fig. 1 Effect of H_2O_2 and α-MG at 25 μM and 50 μM on ARIC index. Values are expressed as mean ± S.E.M; * $p < 0.05$ vs. control group; # $p < 0.05$ vs. H_2O_2 group.

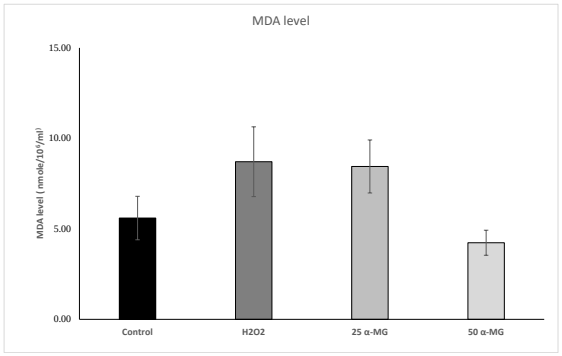

Fig. 2 Effect of H_2O_2 and α-MG at 25 μM and 50 μM on Malondialdehyde (MDA) levels. Values are expressed as mean ± S.E.M.

Effect of α-MG on sperm lipid peroxidation

When human spermatozoa were exposed to H_2O_2 150 μM for 3 h to induce oxidative stress, MDA levels increased in comparison to the control (9.06 ± 1.93 and 5.60 ± 1.98 nmol/10^6 cells/ml, respectively). In contrast, MDA levels decreased in the H_2O_2 groups supplemented with 25 μM of α-MG (6.45 ± 1.62 nmol/10^6 cells/ml) and 50 μM of α-MG (4.23 ± 1.73 nmol/10^6 cells/ml). However, these results were not statistically significant at $p < 0.05$ when compared with the H_2O_2 group (Fig. 2).

Effect of α-MG on sperm catalase activity

Induction of 150 μM of H_2O_2 compound resulted in a significant reduction in catalase activity when compared with the control group (0.025 ± 0.005 versus 0.069 ± 0.008 μmol H_2O_2/min/10^5 cells/ml) ($p < 0.05$). On the other hand, supplementation with α-MG 50 μM substantially enhanced catalase activity compared with that of the H_2O_2 group (0.083 ± 0.009

Table 1 Mean ± S.E.M. of progressive motility and viability of human sperm cells after three-hour incubation with α-MG at 25 μM and 50 μM.

	Control	H_2O_2	25 α-MG	50 α-MG
Progressive motility (%)	81.48 ± 2.75	63.42 ± 3.83*	76.02 ± 2.22**	76.11 ± 1.70**
Viability (%)	88.90 ± 1.85	85.82 ± 2.01	89.18 ± 1.48	90.84 ± 1.16

* $p < 0.05$ vs. control; ** $p < 0.05$ vs. H_2O_2.

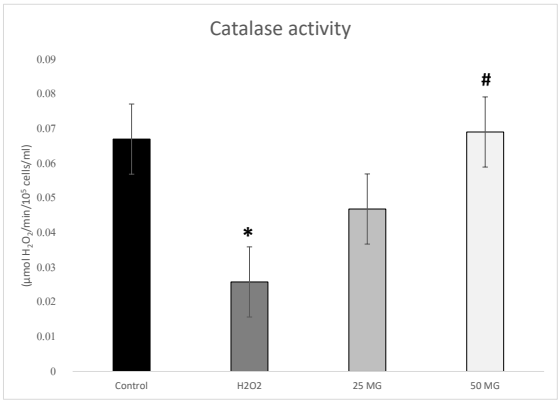

Fig. 3 Effect of H_2O_2 and α-MG at 25 μM and 50 μM on catalase activity. Values are expressed as mean ± S.E.M; * $p < 0.05$ vs. control group; # $p < 0.05$ vs. H_2O_2 group.

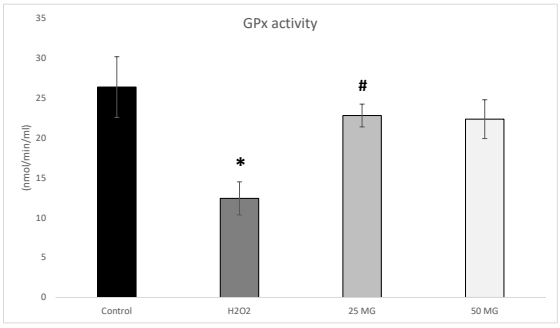

Fig. 4 Effect of H_2O_2 and α-MG at 25 μM and 50 μM on glutathione peroxidase (GPx) activity. Values are expressed as mean ± S.E.M; * $p < 0.05$ vs. control group; # $p < 0.05$ vs. H_2O_2 group.

versus 0.025 ± 0.005 μmol H_2O_2/min/10^5 cells/ml) ($p < 0.05$). Although supplementation with α-MG 25 μM increased the catalase activity, this was not statistically significant when evaluated against the H_2O_2 group (Fig. 3).

Effect of α-MG on sperm glutathione peroxidase activity

Sperm cells exposed to H_2O_2 (12.41 ± 2.07 nmol/min/ml) showed a significant decrease in GPx activity when compared with the control group (26.34 ± 3.80 nmol/min/ml) ($p < 0.05$). When α-MG at a concentration of 25 μM was added, a significant difference in GPx activity (22.78 ± 1.43 nmol/min/ml) was observed when compared with the H_2O_2 group (12.40 ± 2.07 nmol/min/ml) ($p < 0.05$). Supplementing 50 μM of α-MG resulted in an increase in GPx activity (22.32 ± 2.43 nmol/min/ml). However, this result was not statistically significant ($p < 0.05$) when compared with the H_2O_2 treated group (Fig. 4).

DISCUSSION

The results of this study demonstrated that α-MG supplementation improved human sperm motility and ARIC index, reduced MDA levels, and increased catalase and GPx activities. These results suggest that supplementation with α-MG can protect human sperm against H_2O_2-induced oxidative stress. This protective effect may be mediated through the scavenging activity of α-MG and the ability to maintain a balance between ROS and antioxidant activity.

Although the technology in IUI and IVF procedures has advanced, their success rates remain between 13%–30% [6, 7]. Unfortunately, high levels of ROS are produced during the sperm manipulation in both IUI and IVF process. ROS is related to a decrease in the human sperm quality which, subsequently, reduces the possibility of fertilization and pregnancy [23]. When ROS, particularly H_2O_2, are produced in excess, they activate the lipid peroxidation cascade on the polyunsaturated fatty acid (PUFA) of the human sperm membrane, resulting in the loss of membrane fluidity, integrity, and function [24, 25]. Oxidative stress has been shown to cause axonemal damage and increase mid-piece sperm morphological defect [26]. H_2O_2 supplementation decreases progressive sperm motility by directly inhibiting GPx activity and increasing the concentration of lipid peroxidation [27]. These factors have the potential to damage sperm motility. Additionally, H_2O_2 stimulates a premature acrosome reaction in rat sperm, minimizing sperm-oocyte penetration capacity [10].

MDA levels above the normal range in infertile men are associated with abnormal acrosomal structures [28] and may also affect the level of oxidative stress, leading to low sperm motility and inducing abnormal acrosome reaction. Therefore, to prevent such ROS-induced damage, sperm manipulation combined with an exogenous antioxidant factor may protect

sperm quality and increase success rate of IUI and IVF.

α-MG has been shown to possess antioxidant properties in a variety of other cell types including neuronal and cardiac cells [29, 30]. Several studies have revealed that the antioxidant potential of α-MG may be due to its ability to scavenge OOH, prevent H_2O_2-induced cell damage in neuronal and retinal cells [31, 32], and modulate the activity of antioxidative enzymes [33, 34]. Nelli et al [21] previously demonstrated that α-MG had a positive effect on sperm quality and antioxidant activity in diabetic male rats. Our findings also show that α-MG supplementation increases both progressive sperm motility and the activity of GPx and catalase in human sperm exposed to H_2O_2-induced oxidative stress. The antioxidative effect of α-MG may protect axonemal damage and mid-piece defect via the potential pathway of GPx and catalase activity. However, further study should be performed.

Acrosome reaction is an important process for a successful fertilization [4]. A previous study reported the negative effect of H_2O_2 on premature acrosome reaction in rat sperm, which has been associated with decreased sperm penetration and fertilization ability [35]. Previous literatures have demonstrated that the ARIC index can be used to determine the correlation between the quality of acrosome reaction and the pregnancy rate. From our experiment, oxidative stress-induced model may increase the rate of premature acrosome reaction. The acrosome reaction could be detected, but it could not be definitely known whether it was premature acrosome reaction. Therefore, the high ARIC index of H_2O_2 group in our experiment may represent premature acrosome reaction which relates to decreased sperm penetration and fertilizing ability. Conversely, supplementation with α-MG, at both 25 μM and 50 μM concentrations, can mitigate the negative effects of H_2O_2 by lowering the ARIC index to a level comparable to the control group. As a result, this may enhance the fertilization capacity of sperm. Unfortunately, we were unable to validate this result due to the fact that *in vitro* fertilization using oocytes was not performed. Further investigation into the rate of fertilization should be carried out in animal models prior to moving forward with a clinical trial.

CONCLUSION

Supplementation of α-MG during sperm preparation process can attenuate the effects of H_2O_2-induced oxidative stress, as evidenced by an enhancement in sperm motility and a reduction in the ARIC index. The α-MG supplementation also has positive effects on MDA levels and the activity of antioxidative enzymes. Our findings provide substantial evidence that will contribute to an increase in the success rate of male infertility treatment.

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REFERENCES

1. WHO (2010) *Laboratory Manual of the Examination and Processing of Human Semen*, World Health Organization, Geneva.
2. Taylor H, Pal L, Seli E (2020) *Clinical Gynecologic Endocrinology and Infertility*, 9th edn, Wolters Kluwer, Philadelphia, USA.
3. Aktan G, Doğru-Abbasoğlu S, Küçükgergin C, Kadıoğlu A, Ozdemirler-Erata G, Koçak-Toker N (2013) Mystery of idiopathic male infertility: is oxidative stress an actual risk? *Fertil Steril* **99**, 1211–1215.
4. Cummins JM, Pember SM, Jequier AM, Yovich JL, Hartmann PE (1991) A test of the human sperm acrosome reaction following ionophore challenge. Relationship to fertility and other seminal parameters. *J Androl* **12**, 98–103.
5. Makkar G, Ng EH, Yeung WS, Ho PC (2003) The significance of the ionophore-challenged acrosome reaction in the prediction of successful outcome of controlled ovarian stimulation and intrauterine insemination. *Human Reprod* **18**, 534–539.
6. Ombelet W (2017) The revival of intrauterine insemination: evidence-based data have changed the picture. *Facts Views Vis Obgyn* **9**, 131–132.
7. Medical Advisory Secretariat (2006) *In vitro* fertilization and multiple pregnancies: An evidence-based analysis. *Ont Health Technol Assess Ser* **6**, 1–63.
8. Zhang Z, Zhu LL, Jiang HS, Chen H, Chen Y, Dai YT (2016) Predictors of pregnancy outcome for infertile couples attending IVF and ICSI programmes. *Andrologia* **48**, 962–969.
9. Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jennings Z, Irvine D (1998) Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biol Reprod* **59**, 1037–1046.
10. Hsu PC, Hsu CC, Guo YL (1999) Hydrogen peroxide induces premature acrosome reaction in rat sperm and reduces their penetration of the zona pellucida. *Toxicology* **139**, 93–101.
11. Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ, Agarwal A (2000) Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertil Steril* **73**, 459–464.
12. Aitken RJ, Smith TB, Jobling MS, Baker MA, De Iuliis GN (2014) Oxidative stress and male reproductive health. *Asian J Androl* **16**, 31–38.
13. Chairungsrilerd N, Takeuchi K, Ohizumi Y, Nozoe S, Ohta T (1996) Mangostanol, a prenyl xanthone from *Garcinia mangostana*. *Phytochemistry* **43**, 1099–1102.
14. Walker EB (2007) HPLC analysis of selected xanthones in mangosteen fruit. *J Sep Sci* **30**, 1229–1234.
15. Ibrahim MY, Hashim NM, Mariod AA, Mohan S, Abdulla MA, Abdelwahab SI, Arbab IA (2016) α-mangostin from *Garcinia mangostana* Linn: An updated review of its pharmacological properties. *Arab J Chem* **9**, 317–329.
16. Sánchez-Pérez Y, Morales-Bárcenas R, García-Cuellar CM, López-Marure R, Calderon-Oliver M, Pedraza-Chaverri J, Chirino YI (2010) The alpha-mangostin prevention on cisplatin-induced apoptotic death in LLC-PK1 cells is associated to an inhibition of ROS production and

p53 induction. *Chem Biol Interact* **188**, 144–150.
17. Gupta SC, Tyagi AK, Deshmukh-Taskar P, Hinojosa M, Prasad S, Aggarwal BB (2014) Downregulation of tumor necrosis factor and other proinflammatory biomarkers by polyphenols. *Arch Biochem Biophys* **559**, 91–99.
18. Gutierrez-Orozco F, Chitchumroonchokchai C, Lesinski GB, Suksamrarn S, Failla ML (2013) alpha-Mangostin: anti-inflammatory activity and metabolism by human cells. *J Agric Food Chem* **61**, 3891–3900.
19. Upegui Y, Robledo SM, Gil Romero JF, Quinones W, Archbold R, Torres F, Escobar G, Narino B, et al (2015) *In vivo* antimalarial activity of alpha-mangostin and the new xanthone delta-mangostin. *Phytother Res* **29**, 1195–1201.
20. Fang Y, Su T, Qiu X, Mao P, Xu Y, Hu Z, Zhang Y, Zheng X, et al (2016) Protective effect of alpha-mangostin against oxidative stress induced-retinal cell death. *Sci Rep* **6**, 210–218.
21. Nelli GB, K AS, Kilari EK (2013) Antidiabetic effect of alpha-mangostin and its protective role in sexual dysfunction of streptozotocin induced diabetic male rats. *Syst Biol Reprod Med* **59**, 319–328.
22. Beers RF Jr., Sizer IW (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol* **195**, 133–140.
23. Agarwal A, Said TM, Bedaiwy MA, Banerjee J, Alvarez JG (2006) Oxidative stress in an assisted reproductive technique setting. *Fertil Steril* **86**, 503–512.
24. Allamaneni SS, Agarwal A, Nallella KP, Sharma RK, Thomas Jr AJ, Sikka SC (2005) Characterization of oxidative stress status by evaluation of reactive oxygen species levels in whole semen and isolated spermatozoa. *Fertil Steril* **83**, 800–803.
25. Aitken RJ, Jones KT, Robertson SA (2012) Reactive oxygen species and sperm function–in sickness and in health. *J Androl* **33**, 1096–1106.
26. Agarwal A, Virk G, Ong C, du Plessis SS (2014) Effect of oxidative stress on male reproduction. *World J Mens Health* **32**, 1–17.
27. Griveau JF, Dumont E, Renard P, Callegari JP, Le Lannou D (1995) Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. *J Reprod Fertil* **103**, 17–26.
28. El-Taieb MA, Ali MA, Nada EA (2015) Oxidative stress and acrosomal morphology: A cause of infertility in patients with normal semen parameters. *Middle East Fertil Soc J* **20**, 79–85.
29. Pedraza-Chaverri J, Reyes-Fermin LM, Nolasco-Amaya EG, Orozco-Ibarra M, Medina-Campos ON, Gonzalez-Cuahutencos O, Rivero-Cruz I, Mata R (2009) ROS scavenging capacity and neuroprotective effect of alpha-mangostin against 3-nitropropionic acid in cerebellar granule neurons. *Exp Toxicol Pathol* **61**, 491–501.
30. Devi Sampath P, Vijayaraghavan K (2007) Cardioprotective effect of alpha-mangostin, a xanthone derivative from mangosteen on tissue defense system against isoproterenol-induced myocardial infarction in rats. *J Biochem Mol Toxicol* **21**, 336–339.
31. Weecharangsan W, Opanasopit P, Sukma M, Ngawhirunpat T, Sotanaphun U, Siripong P (2006) Antioxidative and neuroprotective activities of extracts from the fruit hull of mangosteen (*Garcinia mangostana* Linn.). *Med Princ* **15**, 281–287.
32. Martínez A, Galano A, Vargas R (2011) Free radical scavenger properties of α-Mangostin: thermodynamics and kinetics of HAT and RAF mechanisms. *J Phys Chem* **115**, 12591–12598.
33. Guzman-Beltran S, Orozco-Ibarra M, Gonzalez-Cuahutencos O, Victoria-Mares S, Merchand Reyes G, Medina-Campos O, Pedraza-Chaverri J (2008) Neuroprotective effect and reactive oxygen species scavenging capacity of mangosteen pericarp extract in cultured neurons. *Curr Top Nutraceut R* **6**, 149–157.
34. Ngawhirunpat T, Opanasopi P, Sukma M, Sittisombut C, Kat A, Adachi I (2010) Antioxidant, free radical-scavenging activity and cytotoxicity of different solvent extracts and their phenolic constituents from the fruit hull of mangosteen (*Garcinia mangostana*). *Pharm Biol* **48**, 55–62.
35. Johansson L (1989) Premature acrosome reaction in spermatozoa from lead-exposed mice. *Toxicology* **54**, 151–162.