EFFECTS OF *Carica papaya* AND *Punica granatum* JUICE ON SPERM'S QUALITY AGAINST HIGH FAT DIET INDUCED OBESITY IN RATS

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Abstract
Malaysia, known as Asia's fattest country, recorded an increase in obesity rate, with the latest statistics in 2015 showing that more than half Malaysian population was diagnosed as obese and overweight. Thus, this study were conducted to determine anti obesity effects of *Punica granatum* and *Carica papaya* fruit juice and its effects on the sperm quality. Twenty Sprague Dawley rats were divided into four groups (n =5); normal group (NC), obese group (OG), *P. granatum* treatment group (PG) and *C. papaya* treatment group (CG). Rats in PG,CG and OG were induced to obesity using food pellets covered with butter. Fruit juices were given to PG and CG groups respectively, for 21 days (1ml/100g of b.wt). Sperm from epididymis were collected for the assessment of sperm count, morphology, motility, and viability. Relative body weight gain showed significant decreased in PG group on 7–14 days and 14–21 days ($p \leq 0.05$). For sperm quality parameters, it showed non-significant results for all groups but positive trend was observed in PG with higher sperm count and viability compared to CG.The results suggested that consumption of fruit juices especially *P. granatum* did help in reducing body weight and increasing sperm quality in obese male rats.

Keyword: Anti-obesity, sperm quality, *Punica granatum*, *Carica papaya*

Introduction
Obesity is one of major dietary problems in developing countries, where it leads to substantial increase in morbidity and mortality. It also directly affects reproduction system by decreasing sperm quality, by altering the physical and molecular structure of germ cells in the testes (Chavarro et al., 2010). Male hormonal profiles were also affected where it reduced inhibin B and androgen (testosterone) levels accompanied by elevated estrogen levels and scrotal temperature (Du Plessis et al., 2010). Management of obesity could be use as a way to improve sperm quality and erectile dysfunction (Türk et al., 2008; Forest et al., 2007). One of the obesity management is by using the antioxidant, where it will react by suppressing continuous formation of excessive free radicals. Studies has reported that few antioxidant, mainly flavonoids, alkaloids and tannins in fruits showed hypolipidemic effects that will lead to the changes in total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (Bray, 2004). *P. granatum* and *C. papaya* juice could be beneficial to obese patient due to the existance of high antioxidant level in the fruit. Antioxidants in *P. granatum* are from polyphenolic class that includes tannins, anthocynins, flavonoids, punicalagin, ellagic and gallic acid (Al-Moraie et al., 2013; Dkhil et al., 2013). *P. granatum* also have antibacterial properties and have the potential as therapy for prostate cancer, erectile dysfunction, Alzheimer's and also reducing obesity since they have both ellagic acid and punicalagin content in their fruit (Forest et al., 2007; Miguel et al., 2010). Leaves, seeds, and fruit of *C.*

papaya possess antioxidant compound such as alkaloids, saponin, flavonoids and cardiac glycosides whereas it's juice has the ability to improve the cross section of rat's epididymis (site of sperm maturation) (El Ghazzawy et al., 2011). Study also reported that the decreasing of epididymis and testes weight occurred after consumption of the juice due to antioxidant activity in those organs in obese rats (Chitra et al.,2002). High dietary fibers found in *C. papaya* fruit are beneficial in digestive system where it could solve constipation and rotting waste problems. Eventhough the fruit of *C. papaya* and *P. granatum* are used for various medicinal treatment, to our knowledge, there were limited literature available on its effects on the male reproductive system especially for papaya fruit juices. Therefore, this study was designed to investigate the effects of *P. granatum* and C. *papaya* juice on weight reduction and sperm quality of obese induced rats.

Materials and Methods

Animal test preparation

The experiment was performed on twenty healthy adult male rats (Sprague Dawley) of eight weeks old, with body weight of 140g - 160g. The rats were divided into four groups, where each groups contain five rats (n=5). The groups were obese group (OG), *P. granatum* treated group (PG), *C. papaya* treated group (CG) and normal group (NC). The rats were fed with standard laboratory diets, given water *ad libitum* and maintained under laboratory condition of temperature 22°C (±3°C), with 12 h dark and 12 h light cycle. Rats were acclimatized with lab conditions for at least one week. The obese and treatment groups (PG and CG) were given food pellet coated with butter and water *ad libitum* for the induction of obesity. PG and CG group were also fed with *P. granatum* and *C. papaya* juice daily (1 ml/100 g) after obese body weight achieved (>160 gram). Body weight, food, and water consumption were monitored at Day 0, 7, 14, and 21.

Preparation of *P. granatum* and *C. papaya* juice

Two kilograms (kg) of fresh *P. granatum* and *C. papaya* were washed and manually peeled, with seeds for *P. granatum* and without seeds for *C. papaya*. Juices were obtained using a commercial blender (Panasonic, Japan), filtered with a filter paper and diluted with 500 ml of distilled water in 1:4 ratio (Al-Moraie et al., 2013). The juices were kept chilled and tightly capped.

Sample collection

The rats were sacrificed using excess anaesthesia on day 22 (Padmalatha & Vijayalaxmi, 2001). Prior to sacrifice, the rats were fasted for 12 hours. The epididymis and testes were obtained for gross necropsy findings and weighted before proceeding to sperm evaluation.

Evaluation of sperm quality

All the procedure for sperm evaluation followed WHO standard guidelines (World Health Organization, 2010). Sperm suspension were collected by minced the right epididymis using scarpel in 1 mL saline solution in the petri dish.

Sperm count

About 10 µl of sperm suspension was taken to the haemocytometer and counted under 400x magnification of light microscope.

Sperm motility

Three drops of diluted sperms was taken from the petri dish and put onto the slide, immediately after euthanization and sperm collection. Sperm motility was observed under the microscope using 400x magnification, where it was evaluated according to the WHO classification of motile sperm and expressed as a percentage (World Health Organization, 2010).

Sperm morphology and viability

Sperm suspension was stained with Eosin Y to perform motility, morphology and viability test. The stain was prepared by dissolving 0.9 g of sodium chloride (NaCl) in 100 ml distilled water. The diluted NaCl was then used to dissolve 0.5 g of Eosin Y (World Health Organization, 2010). Classification of abnormal sperm morphology was according to (Padmalatha & Vijayalaxmi, 2001); whether it is hookless, banana-shaped, amorphous, fused-head and double-head, bent-tail or two-tailed. For sperm viability, non-viable sperm have dark-pink heads while viable sperm have white or faintly-pink head (Franken et al., 2010).

Statistical analysis

Relative body weight changes, sperm count, motility and viability for both treated and control rats were analysed using One-Way Analysis of Variance (ANOVA). A *p*-value of 0.05 or less ($p \leq 0.05$) was considered to be significant. All data were expressed as means ± standard error of means (SEM). The data were analysed using SPSS version 21.0.

Results and Discussions

Relative body weight gain

Weight gain was defined as having extra or excessive fats, muscles or body fluid such as water that lead to an increase in body weight (Dakin et al.,2004). The relative body weight did not measure the total body adiposity but it is correlated moderately with body fatness (Kuczmarski & Flegal, 2000; Monasta, 2011). Decreased body weight gain was observed in PG group from 35.98 ±2.29 grams (day 0-7) to 18.32 ±1.24 grams (day 14-21) ($p<0.05$) as shown in Figure 1. Studies by Al-Muammar & Khan (2012) proposed two mechanism on how antioxidants properties of *P.granatum* juice gives out anti obesity effects. First mechanism was the inhibition of lipase activity while the second mechanism was the supression of energy intake. In the same study, it stated that *P. granatum* juice effect on the supression of energy intake work similarly as a medicine called orlistat. The antioxidant compunds, namely ellagic acid, and tannic acid are proven to increase fecal fat excretion in rats, by using proposed two mechanism as stated (Al-Muammar & Khan, 2012). Throughout the experiment, soft texture of feces was observed in PG compared to NC and OG groups (data not recorded). Body weight gain for CG and NC groups was slightly decreased, week by week ($p >0.05$). In reverse with OG group, the body weight gain was slightly increased for three consecutive weeks ($p>0.05$). This fact had verified that *P. granatum* juice was able to decrease body weight in mammals thus helps in healthy weight management.

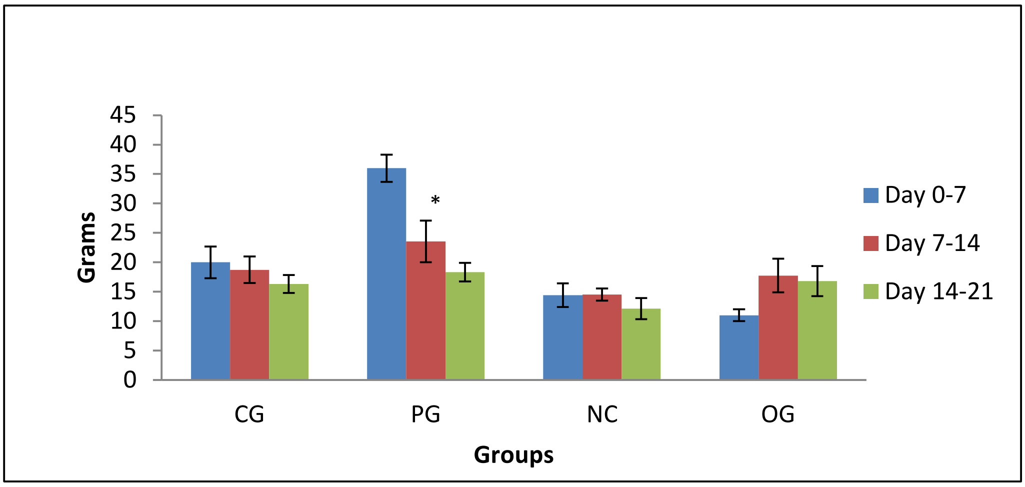

Figure 1 Relative body weight gain for 21 days treatment *, showed significant difference at $p \leq 0.05$

Sperm quality parameters

Sperm morphology is divided into normal and abnormal sperm. A normal rodent sperm has a hook-shaped head while an abnormal sperm can be in various forms (Figure 2). From this experiment, lowest morphologically normal sperm (47.0±8.91%) was observed in CG with the highest abnormal sperms (53.0±11.47%) compared to other treatment groups with p>0.05. This may be because the CG and PG group rats were induced to obesity before subjected to the treatment juice. Study by Nwaehujor et al., (2014) stated that being obese had lower the testosterone level in male rats thus defecting the morphology of the sperm. *C. papaya* fruits regardless of their ripeness contained an enzyme called papain. This enzyme will hydrolyze the semen protein, making it unavailable for the sperm cell and lead to defect. Male semen has about 50% of protein inside it which functioned in the nourishment of sperm cells and promoting sperm capacitation. Thus, without this protein, sperms were unable to undergo physiological changes in order to penetrate and fertilize the egg (Visconti et al.,2011). Even though the level of papain enzyme may not so high in ripe *C. papaya* compare to unripe fruit, continuous intake of this fruit in large amount might slowly affect the reproductive health.

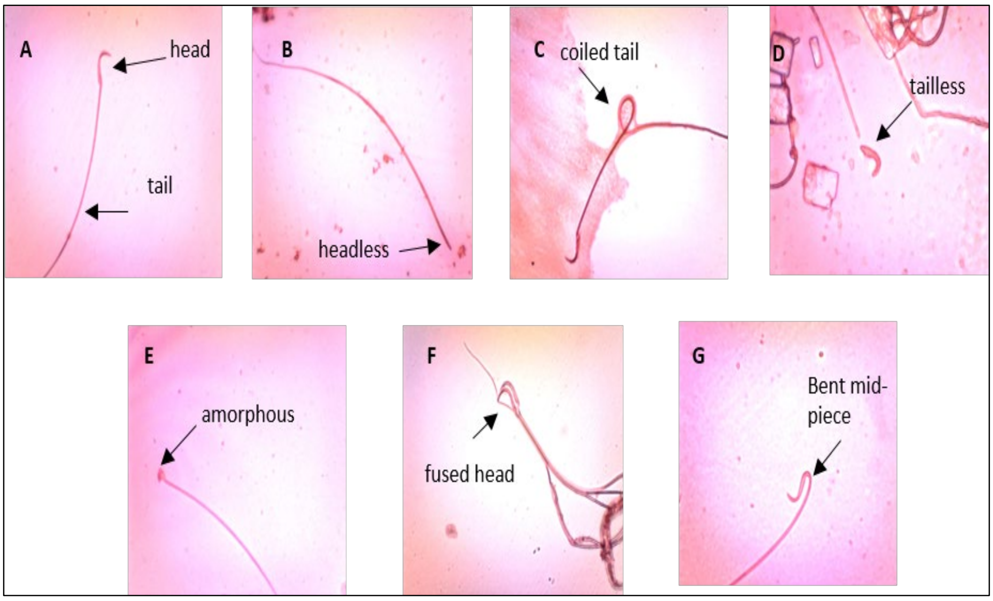

Figure 2 Sperm abnormalities. (A) Normal sperm, (B) Sperms with headless tail, (C) Sperm with coiled tail, (D) Sperm with no tail, (E) Sperm with amorphous head, (F) Sperm fused head and (G) sperm with bent mid-piece

However, by looking at the result (Table 1) *P. granatum* juice had played its role in lowering the number of morphologically defects sperm in PG when compared to CG even though was not significant. Present of ellagitannin in *P. granatum* juice protected the sperm from peroxidative damage by converting into ellagic acid (EA) and this compound is responsible for the antioxidant activities (Atilgan et al., 2014). Sperm is prone to oxidative damage because they contain a high level of polyunsaturated fatty acids (PUFA) and a specific lipidic composition. These lipids are known as the main substrates for peroxidation that later may provoke functional disorder of sperm (Sanocka & Kurpisz, 2004). Furthermore, studies had stated that sperm morphology is important information in classifying fertile and infertile male because the normality of the sperm structure increases the chance for them to fertilize (Gollenberg et al., 2010; Udoh, 2005)

Table 1 Analysis of sperm parameter for treatment group, obesity group, and normal group.

Type of sperm parameter	PG	CG	OG	NC
Sperm Motility	**(%)**	**(%)**	**(%)**	**(%)**
Fast-progressive	0.0 ± 0.0	5.48 ± 0.80	3.29 ± 0.74	4.4 ± 0.74
Slow-progressive	3.0 ± 0.76	1.00 ± 0.20	11.2 ± 0.76	8.81 ± 0.76
Non-progressive	21.0 ± 1.64	5.00 ± 0.80	7.61 ± 1.64	16.27 ± 1.64
Immotile	75.0 ± 5.18	67.00 ± 5.17	80.7 ± 5.18	82.7 ± 5.18
Sperm Viability	(%)	(%)	(%)	(%)
Viable	85.2 ± 5.97	74.40 ± 5.89	81.1 ± 1.09	74.8 ± 4.3
Non-viable	14.8 ± 0.7	25.60 ± 1.81	18.9 ± 2.12	25.2 ± 1.49
Sperm Morphology	(%)	(%)	(%)	(%)
Normal	53.1 ± 4.8	47 ± 8.91	56.6 ± 6.03	55.7 ± 5.82
Abnormal	46.9 ± 10.48	53 ± 11.47	43.4 ± 6.02	44.3 ± 5.82
Sperm Count	10^6/ml	10^6/ml	10^6/ml	10^6/ml
Concentration	9.8 ± 5.86	6.60 ± 4.21	8.2 ± 5.86	6.6 ± 5.86

Nevertheless, for sperm's motility, fast progressive sperm were absent in PG while for the CP, OG and NC was 5.48 ± 0.80 %, 3.29 ± 0.74 % and 4.40 ± 0.74 %, respectively ($p>0.05$). In the meantime, highest percentage of non-progressive sperm was observed in PG with 21.0± 1.64% ($p>0.05$). This type of sperm is still able to move their tail, locally where they do not change the location (Jalali et al., 2012). These sperms are called as the dancing sperm and it is suggested that this sperm are hyperactivated (Sapiro et al., 2002). Lower percentage of immotile sperm with 67.00 ± 5.17 was observed in CG compared to other groups ($p>0.05$). This finding suggested that *C. papaya* may have the ability to improve rat's sperm motility which in parallel with studies from Guthrie & Welch, 2012); Norshazila et al.,2010; Suleiman, 1996. Moreover, present of antioxidant component in ripe *C. papaya* such as vitamin E, vitamin C and carotenoid protect the sperm from the endogenous oxidative stress and membrane damage although it only showed little effect. The level of carotenoid and vitamin C will increase with the increasing of ripening stages (Sancho et al., 2011). This means that the ripening of the fruit may have an impact towards the amount of antioxidants components in the fruit.

Higher percentage of viability was observed in PG with 85.2 ± 5.97 %, followed by OG 81.1 ± 1.09% and NC 74.8 ± 4.3% ($p>0.05$). Viable sperms include the immotile and non-progressive sperm where some of the sperms that falls into these two category are still categorized as alive sperm when stained (Padmalatha & Vijayalaxmi, 2001). Study by Al-Mossawi, (2014) stated that the phenolic compound found in *P. granatum* juice help in the inhibition process of lipid oxidation and in scavenging free radicals and reactive oxidant species (ROS) from the sperm itself.

Higher sperm count was also observed in PG with 9.8 ± 5.86 million/ml followed by OG with 8.2 million/ml and NC and CG with 6.6 million/ml even though the results were not significant. This finding was supported by the study of Dkhil et al., (2013) where the testis weight of rats that were given *P. granatum* juice increase slightly compared to the groups without treatment. The structural protection of the seminiferous tubules in the testis was provided by antioxidants in the *P. granatum* juice and this also increased the diameter of seminiferous tubules that lead to the uprising of sperm concentration (Bhowmik et al., 2013)

Conclusion

Sperm quality were improved mostly in sperm morphology, viability and sperm concenrations upon consumption of *P.granatum* juice. Moreover, *P.granatum* juice also displays its antiobesity properties by showing decreasing pattern of relative body weight of rats in three weeks. In a meanwhile, sperm evaluation after consumption of *C. papaya* juice mostly showed nonsignificant result, suggesting it does not cause beneficial effect on male reproductive system in 21 days of treatment. As for anti-obesity properties, positive effect was observed in decreasing the total body weight, eventhough the results was not as significant as the *P. granatum* juice. Perhaps by increasing duration of study, it will help more in revealling the antiobesity potential of this fruit.

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Conflict of interest

The author(s) declare(s) that there is no conflict of interest.

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