

The Effects of Methanolic Extract of *Syzygium aromaticum* (Clove bud) on the Histology of Testis in Adult Male Wistar Rats

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Abstract

Syzygium aromaticum (clove bud) is a highly recognized spice all over the world for its medicinal and culinary qualities. This experimental study evaluates the effect of a methanolic extract of this *Syzygium aromaticum* on the histology of the testis and sperm parameters of adult male wistar rats in varying doses. Sixteen adult male wistar rats of close weight range were assigned into four groups 1, 2, 3 and 4 of four animals each. Groups 2, 3 and 4 were treated with 50mg/ml, 100mg/ml and 200mg/ml of the extract respectively while the Group 1 served as the control group within an experimental period of twenty-one days. Twenty-four hours after the last administration, the animals were sacrificed for the tissue harvesting and proper investigation. The sperm parameters evaluation revealed that the mean sperm count of the control group is lower than those of the other groups while the level of sperm count and sperm viability of treated animals increased in a dose dependent fashion. The histological findings revealed tissues of Group 4 had shrinkage, fibrosis and degeneration of the testicular tissues when compared to other groups. This study thereby suggests that moderate intake of *Syzygium aromaticum* (clove bud) could influence the sperm count by increasing it and yet preserve the testicular tissues but excessive intake of it could cause damages to the testicular tissues and so it should be advised against before it could result to infertility.

Keywords: Clove bud, Methanolic, *Syzygium aromaticum*, Testis, Wistar rats.

INTRODUCTION

Syzygium aromaticum commonly known as clove bud is one of the highly priced spices widely recognized all over the world for its medicinal and culinary qualities. The clove tree is an evergreen growing to 8-12m tall, having large leaves and sanguine flowers grouped in terminal clusters. The buds begin as pale hue before gradually becoming green, then transitioning to a bright red colour and are ready for collection. Cloves are harvested when 1.5-2cm long. In Nigeria, it is commonly called “Kanufari” and “Kanufuru” by the Hausas and Yorubas respectively, and used as a health spice in food and drinks such as *yaji* (suya meat sauce), *kunu zaki* (millet drink), and *jedijedi* (yoruba concoction).

Clove bud contains a good amount of vitamin A (retinol) and beta carotene levels which are known to have antioxidant properties and vitamin A is also required by the body for maintaining healthy mucus membranes and skin in addition to being essential for vision (Dorman *et al* 2000). This spice is a good source of vitamin K (Phylloquinone: menaquinones) for blood clotting, vitamin B6 (pyridoxine), vitamin B1 (thiamine) and vitamin C (ascorbic acid) which helps the body develop resistance against infectious agents and scavenge harmful oxygen free radicals (Nadkarni 2000). Eugenol is the main compound most responsible for the cloves' aroma and comprises 72-90% of the essential oil extracted from cloves. Other important essential oil constituents of clove oil include acetyl eugenol, beta-caryophyllene and vanillin, crategolic acid, tannins such as bicornin (Li-Ming *et al* 2012), gallotannic acid, methyl salicylate (painkiller), the flavonoids eugenin, kaempferol, rhamnetin, and eugenitin, triterpenoids like oleanolic acid, stigmasterol and campesterol and several sesquiterpenes (Dan *et al* 2004). Eugenol can also be toxic in relatively small quantities as low as 5ml (Hartnoll *et al* 1993).

The clove are said to be a natural anthelmintic, it can be used as carminative, to increase hydrochloric acid in the stomach and to improve peristalsis (Balch and Balch 2000). It can also be used for tonic application and cream combined with other aromatic drug against inflammation-pain in the toes and leg, it is also used in many therapeutic fields such as the treatment of kidney and intestinal diseases, against impotence and genital pain and is reported as stomachic and has smooth muscle relaxant property (Damiani *et al* 2003). Topical application of clove oil over the stomach or abdomen are said to warm the digestive tract. When applied to a cavity in a decayed tooth, it also relieves toothache (Alqareer *et al* 2012), and it plays a significant role in functional maturation of spermatozoa (Prashar *et al* 2006).

Extract of clove has been shown to enhance the sexual behaviour. The results of the study resulted in a significant and sustained increase in the sexual activity of normal male rats, without any adverse effects. The results seem to support the claims for its traditional usage as an aphrodisiac (Baberjee *et al* 2006). The

compound eugenol from cloves has been found to be a potent platelet inhibitor (prevents blood clots) (Agbaje *et al* 2009).

Preliminary study of effect of 50% ethanolic extraction of *Syzygium aromaticum* (clove) on sexual behavior of normal male rats, hydro alcoholic extract (50%) of clove using only mounting frequency and mating performance as the marker for sexual function in normal male mice, the results from the study demonstrated the aphrodisiac activity and safety from short term toxicity of the test drug indicating that the 50% ethanol extract of clove possesses potent aphrodisiac activity in normal male albino rat without any gastric ulceration and adverse effects particularly at the dose of 500mg/kg. The result shows that oral administration of the extract significantly increased the mounting frequency, intromission frequency, erections, quick flips, long flip as well as aggregate of penile reflexes and causes significant reduction in the mounting latency and post ejaculatory interval (Tajuddin *et al* 2003).

A study on the effects of the aqueous extract of clove (*Syzygium aromaticum*) on wistar albino rats, showed that impairment of growth and hepatonephrotoxicity were observed in the rats of all groups. The changes were correlated with alterations in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities and total protein, cholesterol and urea concentration in the liver of 200mg/kg of aqueous extract animals, also fatty cytoplasmic vacuolation of the center lobular hepatocytes and hemorrhage in the liver of 400mg/kg of aqueous extract animals, packing of the glomerular tubules, dilatation and necrosis of the renal tubules in the kidney of 400 mg/kg aqueous extract, infiltration of lymphocytes and disquamation of the intestinal epithelium in the intestine of 800 mg/kg aqueous extract animals (Sharma *et al* 2013).

In another study, it was observed that treatment with lower dose, 15mg of *Syzygium aromaticum* increased the motility of sperm and stimulated the secretory activities of epididymis and seminal vesicle, while higher doses of 30 and 60mg had adverse effects on sperm dynamics of cauda epididymis, also on the secretory activities of epididymis and seminal vesicle. Libido was not affected in treated males; however, a significant decrease in litter in females sired by males treated with higher doses of *Syzygium aromaticum* was recorded (Mishra and Singh 2013).

The testis is the male gonad, they are paired ovoid reproductive glands that produce sperms (spermatozoa) and male hormones primarily testosterone. It is suspended in the scrotum by the spermatic cords (Keith *et al* 2010). During gestational life in humans, the testis begins to develop by the 8th week, reaches the inguinal canal by approximately 12th week and the scrotum by the 33rd week (Sadler 2012).

MATERIALS AND METHOD

Breeding of Animals

Sixteen adult male wistar rats were randomly allocated into four groups of four animals each, with those of close range body weights in a group. Each group was housed separately in wooden cages with sawdust as bedding material. Body weight and general health of the animals were monitored regularly throughout the experimental period. Animals were maintained according to the guidelines of Institutional Animal Ethics Committee.

Experimental Protocols

The sixteen adult wistar rats were allocated into four groups (1, 2, 3 and 4) of four animals each, after which they were acclimatized for two weeks during which they were fed *ad libitum* with growers mesh and water before commencement of the experiment proper. During the experiment, Group 1 served as the control group receiving no extract while Groups 2, 3 and 4 each received 50mg/ml, 100mg/ml and 200mg/ml each day respectively for a period of 21 days by oral administration.

Preparation of Extract

The sun-dried unopened clove buds were procured from Minna market, Niger state in Nigeria and identified at the Department of Pharmacognosy of Madonna University, Elele, Rivers state of Nigeria. The dry clove buds were milled to obtain a fine dry coarse powder and then was sieved through No. 20 mesh size before weighing with a single pan electronic weighing balance. The clove extract was obtained by maceration process where the powder was soaked in 80 % methanol in a 250ml Edenmeyer flask for 48 hours at room temperature with intermittent shaking. The flask was closed with cotton plug and aluminum foil to prevent evaporation of methanol. The mixture was centrifuged at 3500xg for 20 minute and finally filtered through Whatmann filter paper No 1. The pallet was discarded and the supernatant was collected in form of dark brown viscous oil and concentrated under reduced pressure in a rotary vacuum evaporator until semi solid substance was obtained. This was dried inside the crucible under a controlled temperature of 45°C to obtain solid powder. This process of extraction is repeated until the weight of 500mg was obtained. The yield of extract was 10.40% w/w in terms of dried starting material. The extract was preserved in a refrigerator. 1g of the extract was measured using a weighing balance and then dissolved in 10ml of distilled water to get a stock solution of 100mg/ml before giving to the animals.

Tissue Processing

The animals were sacrificed and then the testes were collected from Group 1, 2, 3 and 4, then, fixed with 10% formal saline. The tissue sample were dehydrated in alcohol by first putting the tissue in ascending grades of alcohol (50%, 70%, 90%, and 95%) for 1-2 hour and lastly in absolute alcohol (100%) 1-2 hours for three times. Immediately, the tissues were cleared with xylene twice each for 1-2 hours before being placed in molten paraffin wax at a constant temperature of 56-60°C, the paraffin was changed twice for 2 hours each. The tissues were allowed to solidified and then attached to a wood for sectioning using a rotatory microtome of about 10 microns thin. The tissues were stained in haematoxylin and eosin dye (H&E) before mounting, and then viewed under microscope.

Sperm Analysis

For proper analysis of the sperm, the animals were anaesthetized with diethyl ether, scrotal incision was made to exteriorize the testis and epididymis. The epididymis were carefully dissected out of the testis and blotted free of blood. To prepare sperm suspension, epididymal sperm were obtained by mincing cauda epididymis of each animal in prewarmed beaker containing 2ml of physiological saline (maintained at 37°C) and the following parameters were determined:

Sperm count: It was done using the improved Neubauer's haemocytometer under the light microscope at ×400 magnification. The count was expressed as million/ml of suspension.

Sperm viability: It was assessed using eosin-nigrosin test. The percentages of unstained which are viable (live) and stained which are nonviable (dead) spermatozoa were calculated by counting 200 spermatozoa per sample.

Sperm morphology: Morphological appearance of normal and abnormal spermatozoa was determined by examining stained smears under the oil immersion of ×100. Each sample was examined at ×400 magnification and at least 200 spermatozoa were observed for the calculation of percentage of the total numbers of sperm. The percentage of the abnormal sperm morphology was calculated from the following formula:

$$\% = \frac{\text{Abnormal sperm}}{\text{Total sperm}} \times 100$$

The sperm cells were categorized based on the presence of one or more abnormal features such as tail defect (short, irregular, coiled or multiple tails) neck and middle piece defect (distended irregular piece), and head defects (round, small, large, double or detached heads).

Statistical Analysis

The mean ± SEM values were calculated for each group to determine the significant of intergroup difference. Differences were considered significant at $P < 0.05$.

RESULTS

Physical Analysis

It was observed that the animals treated with *Syzygium aromaticum* were generally normal as daily cage-side observations did not reveal any physical changes in the skin, fur, eyes, respiratory system and general behavioural patterns. The only toxic manifestation however was abdominal writhing and so a few animals died before the end of the study.

Sperm Parameter Analysis

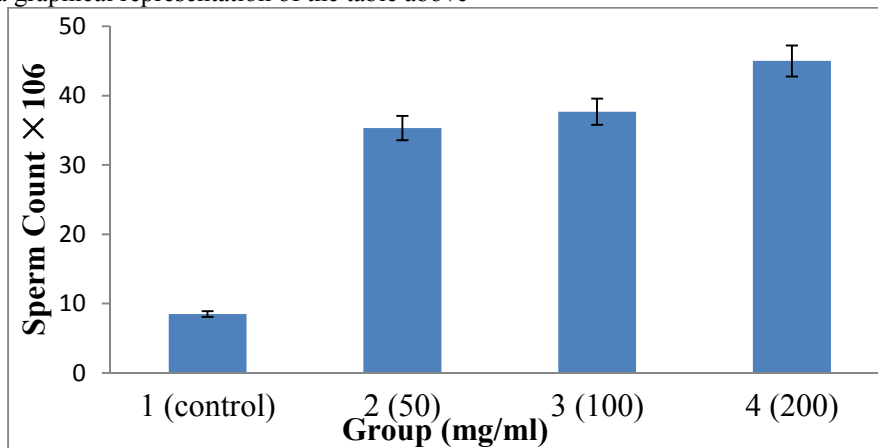
It was found that the mean sperm count of untreated *Syzygium aromaticum* rats is lower than that of the treated rats. The level of sperm count of treated animals increased with the quantity of *Syzygium aromaticum* doses administered to the rats, thus, the mean difference is statistically significant ($P < 0.05$) while the viability of the sperm increased in a dose dependent manner.

Table 1: Shows the analysis of the sperm parameters

GROUP (mg/ml)	SPERM COUNT (×10 ⁶)	MORPHOLOGY (%)		VIABILITY (%)	
		NORMAL	ABNORMAL	VIABLE	NONVIABLE
1 (control)	8.50±7.78	85.00±7.10	15.00±7.10	85.00±7.10	15.00±7.10
2 (50)	35.33±17.61	80.00±10.00	20.00±10.00	90.67±9.29	9.33±9.29
3 (100)	37.67±6.11	97.33±2.00	82.67±2.08	93.00±1.00	7.00±1.00
4 (200)	45.00±18.58	94.25±3.80	65.75±3.86	93.25±2.70	56.75±2.75

Mean value±SD of effect of *Syzygium aromaticum* on sperm of the animals

Fig 1: Shows a graphical representation of the table above



Histological Findings

Group 1 (Control):

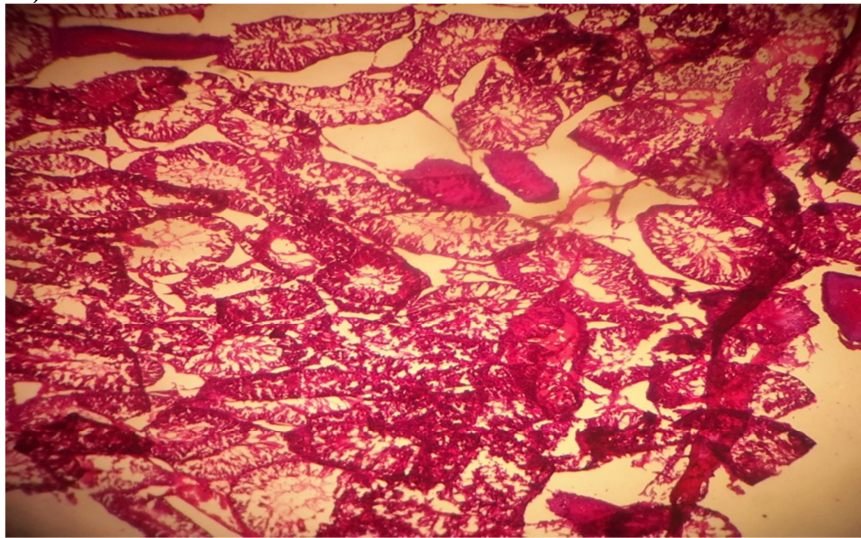


Fig 1: Photomicrograph shows visible seminiferous tubules, spermatozoa lie freely in the lumen and the interstitial cells of Leydig are clearly seen ×400.

Group 2 (50mg/ml):

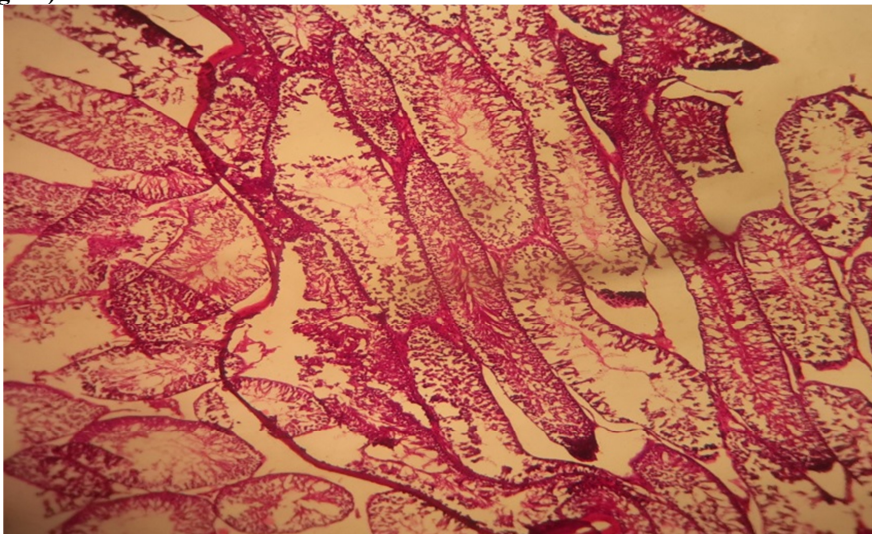


Fig 2: Photomicrograph shows normal seminiferous tubule, spermatozoa containing lumen and interstitial cells of Leydig ×400.

Group 3 (100mg/ml):

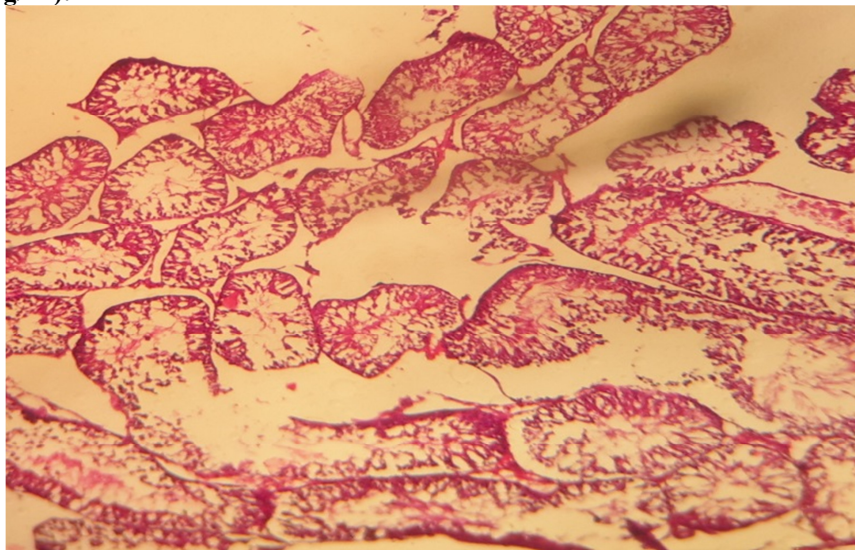


Fig 3: Photomicrograph shows normal seminiferous tubules, spermatozoa containing lumen and interspersed interstitial cells of Leydig $\times 400$.

Group 4 (200mg/ml):

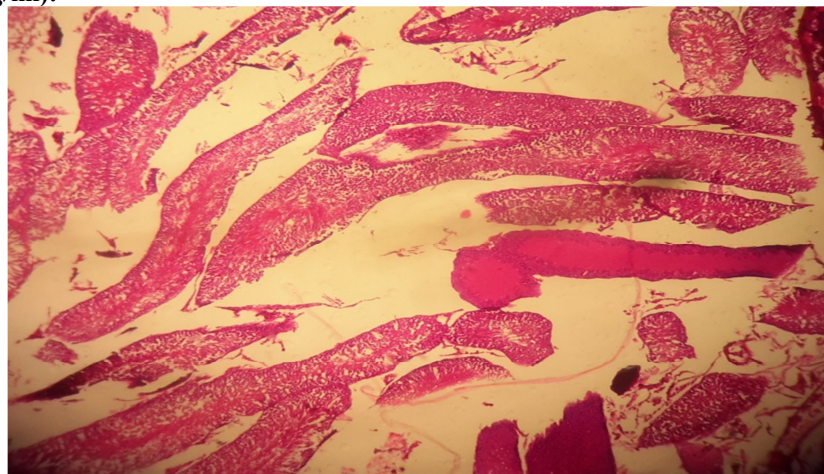


Plate 4: Photomicrograph shows fibrosis of the testis, shrinkage of the seminiferous tubule and degeneration of connective tissues $\times 400$.

DISCUSSION

In this current study, clove extracts increased sperm count in the adult male wistar rats in a dose dependent fashion. The sperm morphology of the treated animals was noticed to have some abnormal sperm morphology although it is lower in the rats treated with 50mg/ml extract and higher in those treated with 100mg/ml extract when compared to those of Group 4 (200mg/ml). Also there was increased in sperm viability in a dose dependent fashion.

The histology of the testis of the animals treated with 50mg/ml and 100mg/ml is normal as can be seen in the control group but those treated with 200mg/ml showed fibrosis of the testis, shrinkage of the seminiferous tubules and degeneration of the connective tissues in the testis.

CONCLUSION

In conclusion, *Syzygium aromaticum* can increase the sperm quality in low doses and serve as aphrodisiac but high doses of it can be toxic and cause deterioration of the testis. Also in this study, it increases spermatogenesis to an extent up to a high dose of 200mg/kg and then starts to destroy the seminiferous tubules.

Syzygium aromaticum consists of important compounds which aid treatment of diverse ailments. Individuals with sexual disorders can go for clove supplement to improve their sexual health which should be taken at moderate dose to prevent testicular problems as can be seen in this study. Also, researchers should work more on clove bud to understand the mechanism on how clove bud works in the system.

REFERENCES

1. Dorman H.J.D., Figueiredo A.C., Barroso J.G and Deans S.G (2000). In Vitro Evaluation of Antioxidant Activity of Essential Oils and Their Components. *Flavour and Fragrance Journal*, 15: 12–16.
2. Nadkarni K.M (2000). *Myrtuscaryophyllus*. In: Nadkarni K.M (Ed.), *Materia Medica*. Popular Prakashan Pvt. Ltd., Mumbai, India, Pp: 835-836.
3. Li-Ming B., Eerdunbayaer A.N., Eizo T., Keinosuke O., Hideyuki I., Tsutomu Hatano (2012). Hydrolysable Tannins Isolated from *Syzygium aromaticum*: Structure of a New C-Glucosidic Ellagitannin and Spectral Features of Tannins with a Tergalloyl Group. *Heterocycles*, 85 (2): 365–381.
4. Dan B., Steven C., Erich S., Andrew G. (2004). *Chinese Herbal Medicine: MateriaMedica*, Third Edition.
5. Hartnoll G., Moore D., Douek D. (1993). Near Fatal Ingestion of Oil of Cloves. *Archives of Disease in Childhood*, 69 (3): 392–393.
6. Balch P., Balch J. (2000). *Prescription for Nutritional Healing*, 3rd Edition. Garden City Park, N.Y Avery. ISBN 158330771.
7. Damiani C.E., Rossoni L.V., Vassallo D.V (2003). Vasorelaxant Effect of Eugenol on Rat Thoracic Aorta. *Vasc. Pharmacol.*, 40: 59-66.
8. Alqareer A., Asma A., Lars A. (2012). The Effects of Clove and Benzocaine versus Placebo as Topical Anaesthetics. *Journal of Dentistry*, 34 (10): 747-750.
9. Prashar A., Locke I.C., Evans C.S (2006). Cytotoxicity of Clove (*Syzygium aromaticum*) Oil and Its Major Components to Human Skin cells. *Cell Prolif.*, 39: 241-248.
10. Baberjee S., Panda C.K., Das S. (2006). Clove (*Syzygium aromaticum* L.), a Potential Chemopreventive Agent for Lung Cancer. *Carcinogenesis*, 27 (8): 1645-1654.
11. Agbaje E.O., Adeneye A.A., Daramola A.O. (2009). Biochemical and Toxicological Studies of Aqueous Extract of *Syzygium aromaticum* (L.) Merry and Perry (Myrtaceae) in Rodents. *Afr. J. Tradit. Complement Altern. Med.* 6 (3): 241-254.
12. Tajuddin S., Ahmad A. L., Qasmi I.A. (2003). Aphrodisiac Activity of 50% Ethanol Extract of *Myristicafragrans* Houtt (nutmeg) and *Syzygium aromaticum* (L.) Merry and Perry (Clove) in Male Mice: A Comparative Study. *BMC Complementary and Alternative Medicine*, 3: 6.
13. Sharma I.Y.A., Suha. B. M., Warda S.A. (2013). Effects of the Aqueous Extract of Clove (*Syzygium aromaticum*) on Wistar Rats. *British Journal of Pharmacology and Toxicology* 4 (6): 262-266.
14. Mishra R.K., Singh S.K. (2013). Reproductive Effects of Lipid Soluble Components of *Syzygium aromaticum* Flower Bud in Male Mice. *J Ayurveda Integr Med.*, 4 (2): 94-98.
15. Keith L.M., Arthur F.D., Anne M.R.A. (2010). *Clinically Oriented Anatomy*, 6th Edition. Wolters Kluwer|Lippincott Williams & Wilkins, USA. Chapter 2, Pg 209.
16. Sadler T.W. (2012). *Langman's Medical Embryology*, 12th Edition. Wolters Kluwer|Lippincott Williams & Wilkins, China. Chapter 16, Pp 252 and 256.

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