

Full Length Research Paper

# Antagonistic salubrious effects of Tahitian Noni in *Momordica charantia*-induced cytoarchitectural alterations in rat testes: Parallel light microscopic findings

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Drug interaction is a situation in which a substance affect the activity of another to either increased or decreased its outcome. It could also produce an entirely new effect. To determine if addition of Tahitian Noni dietary supplement can alleviate *Momordica charantia*-induced testicular alterations in Sprague-Dawley rats. Thirty adult male 16 - 20 weeks' old Sprague-Dawley rats randomized into 6 groups (n=5) were used. Group I (DW<sub>8wk</sub>) the control: administered distilled water for 8 weeks. Group II (MC<sub>50 (8wk)</sub>): received 50 mg/100 g b.w. of *M. charantia* seed extracts for 8 weeks. Group III (MC<sub>50 (4wk)</sub>-N<sub>5 (4wk)</sub>): gavaged 50 mg/100 g b.w. of *M. charantia* seed extracts for 4 weeks thereafter 5 ml/kg of Noni for another 4 weeks. Group IV (MC<sub>50 (8wk)</sub>+N<sub>5 (8wk)</sub>): received 50 mg/100 g b.w. of *M. charantia* seed extracts and 5 ml/kg of Noni concurrently for 8 weeks. Group V (N<sub>5 (8wk)</sub>): received 5 ml/kg of Noni alone for 8 weeks. Group VI (N<sub>5 (4wk)</sub>-MC<sub>50 (4wk)</sub>): treated with 5 ml/kg of Noni for 4 weeks and 50 mg/100 g b.w. of *M. charantia* seed extracts for another 4 weeks. The animals were sacrificed on day 57. The testes harvested cleared off surrounding connective tissues and processed for histology. Comparing the slides from treated rats to control, histology showed depletion of spermatogenic cells lines in the seminiferous tubular lumen in MC<sub>50 (8wk)</sub>. This indicates a harmful effect of the extract. Those exposed to Noni, concurrently or otherwise (N<sub>5 (8wk)</sub>, MC<sub>50 (4wk)</sub>-N<sub>5 (4wk)</sub> and MC<sub>50 (8wk)</sub> +N<sub>5 (8wk)</sub>) tended towards the normal reference. Light microscopic evidence supports the ameliorative effect of Tahitian Noni dietary supplement on the testicular toxicity induced by high dose *M. charantia* extract.

**Key words:** *Momordica charantia*, Tahitian Noni sprague-dawley, histology, testes.

## INTRODUCTION

The use of plant product has increased significantly among adults over the past 15 years, with products such as Echinacea for colds or ginger for nausea now in common use (Teen prescription and OTC drug abuse, 2008). Plant products have enjoyed unparallel patronage either as supplement alternatives or out rightly replacing mainstream medications. Amongst such plants is *Momordica charantia* (MC), which is now being widely

publicized and utilized for its hypoglycemic effects. Its efficacy has continually received accolade and described in one study described to improved glucose tolerance to a degree similar to the oral hypoglycemic agent, tolbutamide (Alternative Medicine Review, 2007).

However, in some other cases healthy subjects have employed MC culinary usage simply for its bitter taste to season several menus. Herbal formations are known for being accidentally misused because they are often consumed without prescription (Teen prescription and OTC drug abuse, 2008). The lack of relevant knowledge about the exact quantity of the active ingredients in these substances makes 'frivolous abuse' inevitable. There is

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also the flawed conviction that medications from plant materials are usually or generally safe. This is not true as many abuses with resulting adverse effects have been well documented in literature (Vandana, 2005). Little is known of about the organo-toxicity effect of MC. A recent research work established a negative effect of MC seed extract on the testes of 'healthy' Sprague-Dawley (S-D) rats. It was observed to suppress sperm quotient in a dose dependent manner (Yama et al., 2011a). It also depressed the testicular weight/volume and extra-testicular testosterone concentrations (Yama et al., 2011a). In another study, a conclusive finding suggests the extract resulted in increased testicular oxidative stress amid inclusive compromise of fertility in the treated rats (Yama et al., 2011b).

Tahitian Noni dietary supplement is a liquid dietary supplement made up of 89% *Morinda citrifolia*. Noni, a common name for *M. citrifolia* is one of the most common plants used in herbal remedies (Basseyy et al., 2011). Apart from literature citations of the use of the fruit juice in the remedy of a plethora of illnesses (alternative medicine) and in the management of drug addiction (Wang et al., 2002), we have also confirmed qualitatively its ameliorative prospect (Basseyy et al., 2011).

Interactions is known to exist between drugs and foods (drug-food interactions), as well as drugs and herbs (drug-herb interactions) (Bushra et al., 2011). In this present study, we evaluated parallel seminiferous tubular (ST) variations in the interactive effect of Tahitian Noni on the testicular micro-anatomy of rats treated with high dose of MC extract.

## MATERIALS AND METHODS

### Tahitian Noni (*M. citrifolia*) dietary supplement

The regular bottled commercial form of Tahitian Noni dietary supplement produced by Morinda Inc., United States of America was obtained from a registered Tahitian Noni distributor. The producing company's bottle cap was observed intact before commencement of use.

### Plant procurement and processing of seed extract

Fresh fruits of *M. charantia* (from plants grown in Southern part of Nigeria) were acquired in a local market at Mushin, Lagos State Nigeria. The same was identified and authenticated by Professor J. Olowokudejo, a taxonomist in the Botany Department of the University of Lagos, where the voucher specimen was deposited (ascension number FHI 108422) in the herbarium.

The processes leading to the constitution of the appropriate formulation of 230 g of MC in 1000 ml of methanol was done in the Pharmacognosy Department CMUL. These included drying the fruits to get seeds which were then weighed and Soxhlet extraction done using alcohol (absolute methanol) as solvent (Yama et al., 2010). The percentage yield of the seed extract obtained was 23.0% w/w; from the dried extract (alcohol evaporated), 50 mg/100 g body weight (b.w.) dose was prepared in distilled water immediately before use.

## Animals

This study was conducted on thirty healthy adult male albino rats of Sprague-Dawley breed weighing between 105 - 200 g. The rats were allotted randomly into six groups: a control and five experimental groups. They were obtained from the Department of Biochemistry, College of medicine University of Lagos (CMUL). The rats were housed in well-ventilated metal cages under standard conditions (temperature: 28 - 31°C; light: approximately 12 h natural light alternating with 12 h darkness per day; humidity; 50 - 55%) in the Department of Anatomy, CMUL. They were allowed free access to standard laboratory food and water *ad libitum* throughout the experiment. The animals were kept for at least 2 weeks to acclimatize to the laboratory conditions before experimentation.

## Experimental procedure, necropsy schedule and animal ethics

The investigation protocol was approved by the Departmental Ethics Committee CMUL. The test solutions (MC, Noni and distilled water) were administered between the 7 - 9 h daily to the treated and control animals with a metal canula by gastric gavage. The total experimental duration was 56 days to ensure the test solutions effects were observed for one complete spermatogenic cycle (Jegou et al., 2002). The animals were treated with distilled water, extract and Noni as follows:

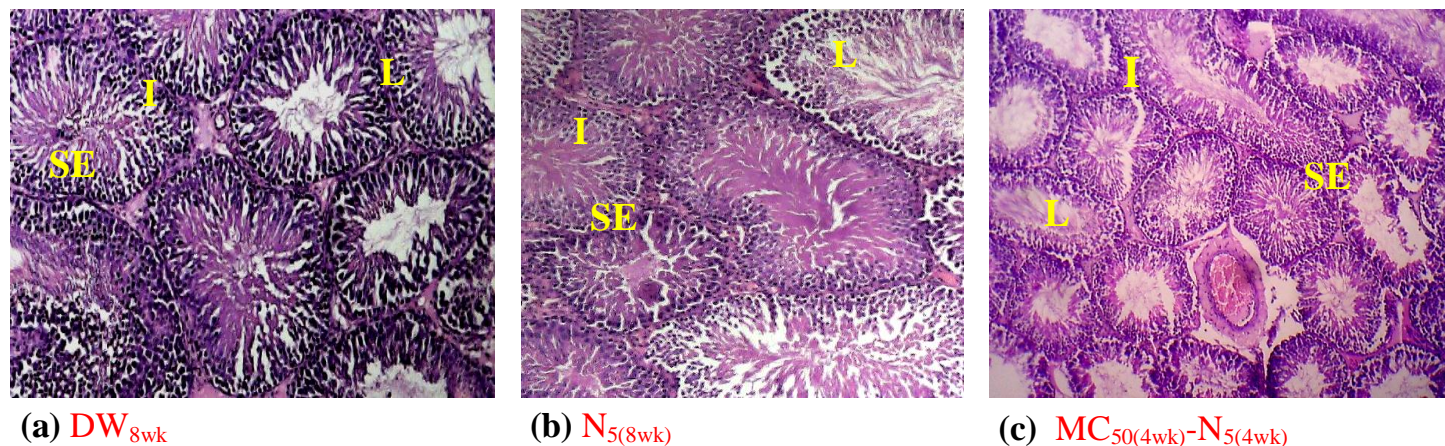
1. Group I (DW<sub>8wk</sub>), control: Distilled water (4 - 5 ml) for 8 weeks.
2. Group II (MC<sub>50(8wk)</sub>): MC seed extract (50 mg/100 g b.w.) for 8 weeks.
3. Group III (MC<sub>50(4wk)</sub>-N<sub>5(4wk)</sub>): MC seed extract (50 mg/100 g b.w.) for 4 weeks alternating with Noni (5 ml/kg) for another 4 weeks.
4. Group IV (MC<sub>50(8wk)</sub>+N<sub>5(8wk)</sub>): MC seed extract (50 mg/100 g b.w.) and Noni (5 ml/kg) concurrently for 8 weeks.
5. Group V (N<sub>5(8wk)</sub>): Noni (5 ml/kg) alone for 8 weeks.
6. Group VI (N<sub>5(4wk)</sub>-MC<sub>50(4wk)</sub>): Noni (5 ml/kg) for 4 weeks thereafter MC seed extract (50 mg/100 g b.w.) for another 4 weeks.

On the 57th day, the animals were made unconscious by cerebral dislocation which allowed the surgical procedure involving a ventral laparotomy to be carried out. The testes were delivered *per* abdomen.

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002) and were approved by the Departmental Committee on the Use and Care of Animals in conformity with international acceptable standards.

## Tissue processing for histological studies

The harvested testes were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were processed by the method described previously (Animal tissue techniques, 2009) with slight modification. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 h, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicular to the long axis of the testes. The sections were designated "vertical sections". Serial sections of 5 µm thick were obtained from a solid block of



**Figure 1.** Cross-section of the seminiferous tubules of rats treated with: (a) 4-5 ml distilled water:  $DW_{8wk}$ ; (b) 5 ml/kg of Noni:  $N_{5(8wk)}$ ; (c) 50 mg/100 g of *Momordica charantia* seed extract for 4 weeks and afterwards 5 ml/kg of Noni for 4 weeks:  $MC_{50(4wk)}-N_{5(4wk)}$ . Stains: Haematoxylin and Eosin. Mag.  $\times 100$ ; L = lumen of seminiferous tubule; SE = seminiferous epithelium; I = testicular interstitium.

tissue, fixed on clean slides to which Mayer's egg albumin had been coated to cement the sections to the slides properly and later stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene the sections were oven-dried between 35 and 40°C.

## RESULTS

### Histological assessment the testes

Histological sections from groups  $DW_{8wk}$ ,  $N_{5(8wk)}$  and  $MC_{50(4wk)}-N_{5(4wk)}$  were similar. The ST, contained germ cells at all levels of differentiation with maintenance of normal polarity of germ cells. The interstitium contained normal Leydig cells. However  $N_{5(8wk)}$  showed more densely populated ST (Figure 1).

Sections from  $MC_{50(8wk)}$  showed tubular hypocellularity, interstitium diminution and destruction of the spermatid layer with few to absent luminal spermatozoa (Figure 2).  $N_{5(4wk)}-MC_{50(4wk)}$  slides were similar only degree of alteration was lesser.

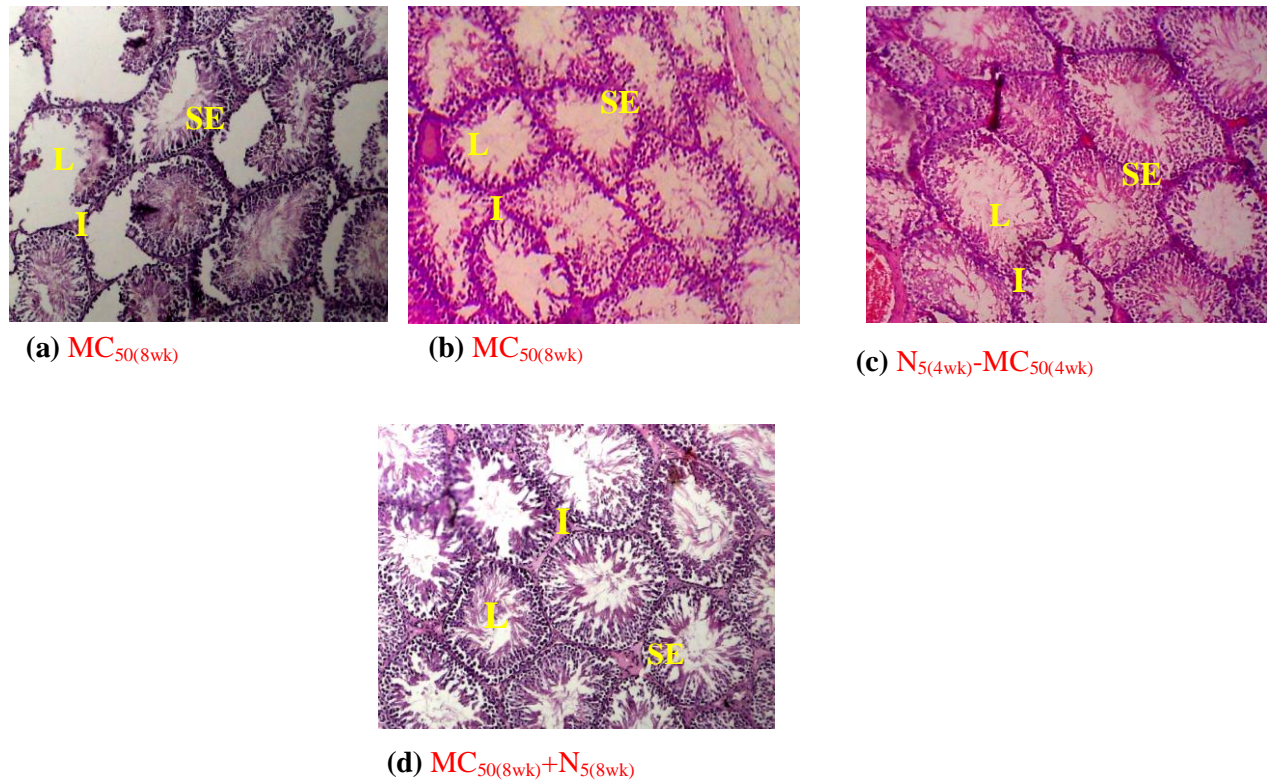
Finally, histological sections from  $MC_{50(8wk)}+N_{5(8wk)}$  rats revealed basal epithelium and the tubular lumen was fairly well populated with viable germ cells at various spermatogenic stages. The tubular cross sections showed profiles that were fairly regular, signifying some protective role.

## DISCUSSION

Just as the recreational use of over-the-counter and prescription drugs are on the rise, there is also a growing trend of use of herbal drugs. One reason for this shift to herbal drugs is that users tend to think of herbal products as natural and safe. But many of these products can be

dangerous and even deadly (Teen prescription and OTC drug abuse, 2008). Different herbal formulations act in different ways causing harmful/toxic effects to human physiology (Vandana, 2005). MC herb of ubiquitous importance and gaining popularity as alternative in the management of diabetes. It was recently shown to result in dose dependent alteration in the sperm production and androgen levels when administered for a duration equivalent to one spermatogenic cycle (Yama et al., 2011a). Failure of the pituitary gland to produce adequate amounts of gonadotrophins can lead to decreased sperm counts (Al-Daghistani and Abdel-Dayem, 2002) and might be the factor in the reduction of sperm counts. This correlates with features from our histological findings of rats fed high dose of MC. The extract showed it was capable of causing seminiferous testicular alteration. There was decreased tubular cellularity, as well as modification in the interstitium (Leydig cells); while in certain regions the epithelia showed focal degeneration with few to absent luminal spermatozoa. Conversely, rats treated pre-treated with MC before administering the dietary supplement recuperated because they had a comparable histoarchitecture as the normative control. Their germ cells well organised and stratified and all cells of the spermatogenic series represented. The tubular lumens observed to contain many viable germ cells.

The histological findings of the testes in the slides reviewed from the rats administered MC and Noni showed modulating effect and patterns similar to control. There were no marked disruptions in the basal epithelium and the tubular lumen was fairly well populated with viable germ cells at various spermatogenic stages. The tubular cross sections showed profiles that were fairly regular, signifying some protective role. The testes from rats inspected for a probable prophylactic effect showed sections similar to those treated with MC although



**Figure 2.** Cross-section of the seminiferous tubules of rats treated with: (a) and (b) 50 mg/100 g of *Momordica charantia* seed extract for 8 weeks:  $MC_{50(8wk)}$  (c) 5 ml/kg of Noni for 4 weeks afterwards 50 mg/100 g *Momordica charantia* seed extract for another 4 weeks:  $N_{5(4wk)}-MC_{50(4wk)}$ ; (d) concurrently with 50 mg/100 g *Momordica charantia* seed extract and 5 ml/kg Noni for 8 weeks:  $MC_{50(8wk)}+N_{5(8wk)}$ . Stains: Haematoxylin & Eosin. Mag. x 100; L = lumen of seminiferous tubule; SE = seminiferous epithelium; I = testicular interstitium.

there were moderately populated areas.

The exact mechanism by which Tahitian Noni brought about the effects is unknown the following pathways are speculative. It is known that Tahitian Noni acts as a powerful immune modulator (Bassey et al., 2011; Heinicke, 1985); hence it helps in keeping the endocrine system in a well balanced condition and so may help in cases of sterility. In a case of altered spermatogenic cell lines, Noni may have helped to improve this condition. Studies have shown that MC possesses antioxidant property (Technical data on *M. charantia*, 2002; Dhanasekar and Sorimuthu, 2005) which is expected to produce a protective effect on the testes. Researchers have shown that depending on the dose a substance may act either as a pro-oxidant or antioxidant (Tan et al., 2000). The MC extract acted as a pro-oxidant due to the prolonged daily exposure (4–8 weeks) and at a high concentration 50 mg/100 g (Yama et al., 2011b) the body could not adapt or overcome to its effect, generating free radicals. Thus if the observed histological alteration produced by MC is due to a high increase in free radicals' damage, Noni may have helped to improve this condition by its 'supreme' antioxidant properties. Also Noni is able to affect so many systems through its ability to promote new cellular growth

and to repair damaged cells (Bassey et al., 2011; Heinicke, 1985).

## Conclusion

Although more work needs to be done in identifying biochemical and cellular events such as apoptosis, necrosis and cellular proliferation to corroborate further the testicular histoarchitectural findings. This study has presented histological evidences which gives clue to the primary cells affected during the interaction of Tahitian Noni and MC. The dietary supplement may possibly be useful in antagonizing the effects of high dose of the extract by preventing or reversing testicular toxicity.

## REFERENCES

- Al-Daghistani HI, Abdel-Dayem M (2002). Hyperprolactinemia and hypergonadotropins in infertile males with severe oligospermia and azoospermia. *Internet J. Endocrinol.*, pp. 1540-2606.
- Alternative Medicine Review (2007). Description of *Momordica charantia*, a member medicine. *Monogr. Clin. Rep.*, 12 (4): 360-363.
- American Physiological Society (2002). Guiding principles for research involving animals and human beings. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 283: 281-283.

- Animal tissue techniques (2009). Gretchen L. Humason Los Alamos Scientific Laboratories. On open library.org. Chap., 1(3): 3-39.
- Bassey RB, Yama OE, Osinubi AA, Noronha CC, Okanlawon AO (2011). Effects of Tahitian Noni dietary supplement on caffeine-induced testicular histo-pathological alterations in adult Sprague-Dawley rats. *Middle East Fertil. Soc. J.*, 16: 61-66.
- Bushra R, Aslam N, Khan AY (2011). Food-Drug Interactions. *Oman. Med. J.*, 26(2): 77-83.
- Dhanasekar S, Sorimuthu S (2005). Antioxidant properties of *Momordica Charantia* (bitter gourd) seeds on Streptozotocin induced diabetic rats. *Asia Pac. J. Clin. Nutr.*, 14(2): 153-158
- Heinicke R (1985). The Pharmacologically Active Ingredients of Noni. *Pac. Trop. Bot. Garden Bull.*, 15: 10-14.
- Jegou B, Pineau C, Toppari J (2002). Spermatogenesis *in-vitro* in mammals. *Assisted Reproductive Technology: Accomplishments and New Horizons*. Cambridge: Cambridge University Press, 1: 3-60.
- Tan D, Manchester LC, Reiter RJ, Qi W, Karbownik M, Calvo JR (2000). Significance of Melatonin in Antioxidative Defense System: Reactions and Products. *Neurosignals*, 9 (3-4): 137-159.
- Technical data on *Momordica charantia* (2002). 103: 3-7.
- Teen prescription and OTC drug abuse (2008). The Dangers and Effects of Herbal Drugs. <http://www.teenoverthecounterdrugabuse.com/dangers-effects-herbal-drugs.html>.
- Vandana P (2005). Herbal Medicines: Its Toxic Effects & Drug Interactions. The Indian Anaesthetists' Forum. <http://www.theiaforum.org> Online ISSN 0973-0311.
- Wang M, West B, Jensen C, Nowicki D, Palu A, Anderson G (2002). *M. citrifolia* (Noni): A literature review and recent advances in Noni research. *Acta Pharmacol. Sin.*, 23: 1127-41.
- Yama OE, Duru FI, Oremosu AA, Osinubi AA, Noronha CC, Okanlawon AO (2011a). Sperm quotient in Sprague-Dawley rats fed graded doses of seed extract of *Momordica charantia*. *Middle East Fertil. Soc. J.*, 16 (2): 154-158.
- Yama OE, Duru FIO, Oremosu AA, Noronha CC (2011b). Testicular oxidative stress in Sprague-Dawley rats treated with *Momordica charantia*: The effect of antioxidant supplementation. *Bangladesh J. Med. Sci.*, 10: 2.
- Yama OE, Osinubi AA, Noronha CC, Okanlawon AO (2010). Effect of methanolic seed extract of *Momordica charantia* on body weight and serum cholesterol level of male Sprague-Dawley rats. *Niger. Q. J. Hosp. Med.*, 20(4): 209-213.