

*Full Length Research Paper*

# **Preliminary study on the effect of sugar cane (*Saccharum officinarum*) molasses on steroidogenesis in testicular cell cultures**

**Farzana Rahiman and Edmund John Pool\***

Department of Medical Bioscience, University of the Western Cape, South Africa.

Accepted 10 November, 2009

**Blackstrap molasses has been used for centuries in the preparation of food products and have also been used to treat numerous ailments such as high blood pressure, arthritis, ulcers, dermatitis, eczema, constipation, colitis, varicose veins and many other health problems. Reports suggest that blackstrap molasses may have endocrine disruptive effects in cattle. The present study investigated the effect of sugar cane (*Saccharum officinarum*) molasses on steroidogenesis in testis cell culture. Testis cell cultures were incubated with various concentrations of molasses. Luteinizing hormone was used to stimulate the production of testosterone in the testes cell culture. Supernatants of the testes cell cultures were then assayed for testosterone, oestradiol and cytotoxicity using commercially available kits. Results showed that low concentrations (12 - 50 ug/ml) of molasses increase testosterone secretion relative to control cultures ( $P < 0.05$ ). None of the molasses concentrations tested were cytotoxic. This study has shown that molasses stimulate testosterone production. Therefore, molasses may potentially be used as a diet supplement to increase testosterone levels.**

**Key words:** Molasses, testosterone, male reproductive system, immunostimulation.

## **INTRODUCTION**

The endocrine system is an important component of the body that functions in the growth, development and reproductive activities of both humans and wildlife (Gunnarson, 2008). There is concern regarding the impact of various substances, such as environmental contaminants and chemicals, on the endocrine system and its subsequent effect on the health of humans and animals (Gutendorf and Westendorf, 2001).

An endocrine disruptor (ED) may be defined as a compound that alters the function of the endocrine system and may act by mimicking natural hormones, blocking hormone receptors as well as initiating change in the metabolism of endogenous hormones (DiDiego, 2005). Occupational exposure and diet are sources whereby endocrine disruptors may enter the body of a human or animal, followed by their attachment to specific

endogenous receptors. This initiates an alteration in the hormonal balance of the body and may induce diseases such as cancer, cryptorchidism, and suppression of male reproductive fitness, low sperm counts and skin diseases (Kumar et al., 2008).

Reproductive problems such as decreased fertility, malformations and impaired growth have increasingly become a major issue among the scientific and general public. Therefore, it is necessary to screen for possible endocrine disrupting compounds, especially those that we are exposed to on a daily basis, in order to maintain reproductive health.

Molasses is a dense, viscous substance derived as a by-product of sugar refinement. This residual product has a long history of use in animal feeds with usage ranging from eradication of dust and feed wastage to becoming an important supply of dietary energy (Curtin, 1983). Molasses has also become a very popular ingredient of the human diet and is being used to treat numerous diseases. This includes diseases such as arthritis, ulcers, dermatitis, hair damage, eczema, high blood pressure,

\*Corresponding author. E-mail: [epool@uwc.ac.za](mailto:epool@uwc.ac.za). Tel: +2721 959 3535. Fax: +2721 959 2433.

constipation, colitis, varicose veins, nerve damage, anemia and bladder problems (Kirschmann, 2007).

A study conducted by Rowe et al. (1977) shows that cattle with free access to molasses (3% urea) and limited amounts of roughage developed a syndrome termed molasses toxicity. It has also been demonstrated that molasses toxicity induces effects such as decreased body temperature, fatigue, excessive salivation, rapid breathing and a drunken appearance seen in animals (Pate, 1983). Reports also suggest that blackstrap molasses may have endocrine disruptive effects in cattle (Cellar, 2006).

Research suggests that exposure to endocrine disruptors such as environmental contaminants may be linked to the occurrence of various reproductive diseases (Sanderson and van den Berg, 2003). The male sex hormone, testosterone and female sex hormones progesterone and oestradiol play a vital function in ensuring reproductive health (Stocco, 1997). Gonadal steroid hormones are derived from the common precursor cholesterol, a process which requires movement between the mitochondria and smooth endoplasmic reticulum as well as various enzymatic reactions. This process is termed steroidogenesis (Whitehead and Rice, 2006; Sanderson, 2006). In the male, testosterone functions as an active androgen that is essential to the growth and development of the testes, epididymides, vas deferens, seminal vesicles and other important parts of the male reproductive tract (United States Environmental Protection Agency (U.S. EPA, 2005). Some endocrine disrupting chemicals affect the production of steroid hormones and/or the enzymes responsible for steroid hormone synthesis. As a result, any change induced by endocrine disruptors on sex hormones, such as testosterone or steroidogenic enzymes may have a significant effect on sexual differentiation and maturity (Whitehead and Rice, 2006).

It is evident from previously mentioned reports that molasses may have potential adverse effects on health. It is therefore imperative that research is conducted to determine the biological activity of molasses. This study investigated the potential effect of sugar cane (*Saccharum officinarum*) molasses on steroidogenesis in testis cultures. Testosterone and oestradiol synthesis were used as biomarkers to determine the effect of sugar cane molasses on the male reproductive system.

## MATERIALS AND METHODS

### Animals

Healthy Balb/C, male mice were purchased from the University of Cape Town's Animal Unit (Cape Town, South Africa) after obtaining animal ethical clearance from the University of the Western Cape. Experiments were conducted in accordance with the guidelines of the institutional animal ethics committee. The mice were kept in a well-ventilated animal house (temperature  $20 \pm 2^\circ\text{C}$  and 12 h light/12 h dark cycles) in which they had access to normal drinking water and fed standard mouse feed (Medical Research Council, Cape

Town, South Africa).

### Cell preparation

Testes of three month old mice, sacrificed by cervical dislocation, were removed under aseptic conditions. The testes were minced with scissors and transferred to a tube containing 10 ml serum free medium. Serum free medium consisted of 0.2% bovine serum albumin (Sigma, USA), 1% glutamax, 1% combination of streptomycin, penicillin and fungizone used to avoid contamination and RPMI-1640 (Modified) medium (Sigma, USA). The cell debris was allowed to settle, after which the supernatant (containing cells) was transferred to a new tube. The cells were then centrifuged (Super mini centrifuge, MiniStar Plus, Hangzhou Allsheng Instruments, China) at  $1000 \times g$  for 10 min. The supernatant was discarded and the cell pellet was suspended in serum-free medium making up a final volume of 20 ml and incubated at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$  for 1 h. The cells were then centrifuged at  $1000 \times g$  for 10 min. The supernatant was discarded and the cell pellet was suspended in 20 ml serum-free medium and incubated at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$  for 30 min. The cells were then centrifuged at  $1000 \times g$  for 10 min. The supernatant was discarded and the cell pellet was suspended again in 20 ml serum-free medium and incubated at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$  for 20 min to determine background testosterone and oestradiol production.

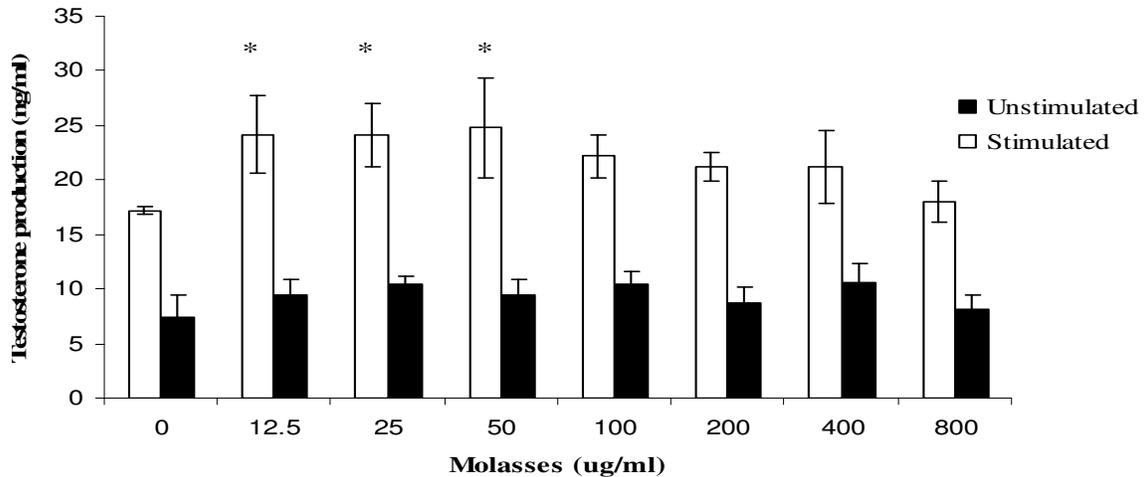
Cell preparations were activated or not activated with 10  $\mu\text{u/ml}$  human luteinizing hormone (LH) (Sigma, USA) and counted using a Neubauer hemacytometer ( $10^7$  cells per ml) (West Germany). Dilution ranges of molasses in distilled water or distilled water controls at a volume of 3  $\mu\text{l/well}$  were added to a 96 well culture plate (four replicates per dilution of molasses). The cell suspensions (100  $\mu\text{l/well}$ ) were then added to the molasses samples and the plate was incubated at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$  for 4 h (U.S. EPA, 2005). Following the incubation period, supernatants were collected and assayed for testosterone. These assays were conducted using commercially purchased testosterone Enzyme-linked immunosorbent assay (ELISA) kits (DRG Instruments GmbH, Marburg, Germany) and were performed in accordance with the manufacturer's instructions. The kit contained all components, including positive controls required to perform the assay. The experiment was done in quadruplicate to avoid statistical errors.

### Cytotoxicity of molasses

Cells and cell culture supernatants were used for cytotoxicity analysis after incubation with molasses as described above. Lactate dehydrogenase (LDH) activity in cell culture supernatants was used to determine cytotoxicity of samples. LDH was measured using a Cytotoxicity Detection kit (Biovision, USA). The kit contains all components required for the assay. Cells were lysed with cell lysis solution. The cell lysate was used to determine total cellular LDH. Cell culture supernatants and cell lysate were transferred to a 96 well plate (Nunc-Immuno plate, Serving Life Science, Denmark). 100  $\mu\text{l}$  of cytotoxicity kit reaction mixture was added to each well and incubated for 15 min. The absorbance of reaction mixtures were then measured at 492 nm using a plate spectrophotometer (Original Multiskan EX, Type 355, Thermo Electron Corporation, Shanghai, China). The amount of LDH in the culture supernatants were expressed as a percentage of the total cellular LDH.

### Statistical analysis

Data was statistically analysed via one-way ANOVA ( $P < 0.001$ ) and regression analysis using SigmaStat software (Systat Software Inc., USA).



**Figure 1.** The effect of molasses on testosterone production by testes cell cultures (\* indicates  $P < 0.05$  relative to the control). Each point is the mean and standard deviation of four replicates.

## RESULTS

Molasses extracts were tested for cytotoxicity using LDH release as a biomarker. Treatment with a toxic compound initiates the release of the LDH enzyme into culture medium due to cell membrane damage (Fotakis and Timbrell, 2005). None of the molasses extracts resulted in a significant increase in LDH release compared to controls ( $P > 0.05$ ) indicating that molasses is not cytotoxic (data not shown). Molasses addition (12.5 - 50  $\mu\text{g/ml}$ ) to LH stimulated testes cultures increased testosterone production ( $P < 0.05$ ) (Figure 1). This experiment was repeated with testis from three mice and all of these experiments gave similar results. Molasses has no effect on testosterone secretion by unstimulated cultures (Figure 1).

## DISCUSSION

Testosterone is a sex steroid that is required for the growth of both the internal and external male reproductive system (Fisher, 2004). Testosterone functions in maintaining libido, sperm production, muscle and bone mass as well as male hair patterns (Vassan, 2006). Therefore, it is vital that normal levels of testosterone are established in order to promote male reproductive health. This study shows that low concentrations of molasses extracts elevate testosterone production by LH stimulated testicular cells *in vitro* and as a result may therefore be used in the regulation of the above mentioned male biological processes. There appears to be little or no scientific evidence that directly associates molasses with increased testosterone production. On the contrary, reports suggest that molasses is an endocrine disruptor (Cellar, 2006). This study has shown that molasses caused a significant increase in LH stimulated testosterone

production *in vitro*.

Further research is needed to elucidate if molasses stimulates testosterone production *in vivo*. This could prove molasses to be beneficial as a supplement in diets of men with low testosterone levels. Numerous studies with men ranging from middle to older age have shown an association of decreased testosterone levels with various diseases such as poor memory and cognitive potential, metabolic syndrome, osteoporosis, sarcopenia and Type 2 diabetes (Yeap, 2008). Perhaps the supplementation of molasses in diets of older men may prove to be favourable for future use.

## Conclusion

This study has shown that molasses has an impact on the male reproductive system by acting as a stimulant of testosterone production, which may prove beneficial to reproductive health. Research on sugar cane (*S. officinarum*) molasses is limited and we therefore suggest further investigations on the biological actions of molasses be conducted.

## ACKNOWLEDGEMENTS

We are grateful for the financial support received by the National Research Foundation of South Africa and the DAAD (Deutscher Akademischer Austausch Dienst) foundation in Germany.

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