African Journal of Pharmacology and Therapeutics Vol. 1 No. 2 Pages 67-70, 2012
Open Access to full text available at http://www.uonbi.ac.ke/journals/kesobap/

Research Article

Dose-dependent myocardial toxicity of *Mangifera indica* during diarrhoea treatment

Faraj O. Alkizim[a,*], Duncan M. Matheka [a], and Anne W. Muriithi [a]

[a] *Department of Medical Physiology, School of Medicine, University of Nairobi*

* **Corresponding author:** Department of Medical Physiology, School of Medicine, University of Nairobi, P.O. Box 30197-00100 Nairobi, Kenya; **Tel**: +254-72-1336955 ; **Fax**: +254-20-4451770; **Email**: faralkizim@hotmail.com

Background: The use of alternative medicine is on the rise worldwide. *Mangifera indica* kernel has been used as a diarrhoea remedy. Studies have demonstrated its mechanism of action to be via the sympathomimetic pathway. Possible adverse effects on the cardiovascular system were however unknown.

Objective: The current study aimed to investigate the *in-vitro* effects of *Mangifera indica* kernel extract (MIE) on myocardial activity.

Methodology: The dose-related effects of MIE were tested on isolated rabbit hearts, and the mechanism of action verified. This was then compared against its efficacy on the jejunum, and the likelihood of myocardial toxicity investigated.

Results: MIE significantly enhanced myocardial contractility with no effect on the frequency. However, the doses required for this effect were much higher than those that would be required to treat diarrhoea.

Discussion: The use of MIE as a diarrhoea remedy is probably safe on the cardiovascular system provided the dose is maintained within an anti-diarrhoeal therapeutic window.

Key words: Diarrhoea, adverse effects, mango kernel, myocardial activity.

Received: June, 2012
Published: July, 2012

1. Introduction

The use of herbal medicine is on the rise worldwide (Trindle et al, 2005). Many cultures have beliefs and prejudices towards the treatment of various morbidities that often affect their attitudes towards conventional medical practices. It would therefore be expedient to supplement, rather than to supplant such beliefs and cultural customs. This is the basis of ethnopharmacology, a science that aims to validate (or invalidate) the use of such plants for their alleged beneficial properties. The easy availability, low cost and belief of fewer associated side effects are the major attractions to herbal medicine among such communities.

One such traditional practice is the use of mango (*Mangifera indica*) seed in the treatment of diarrhoea. This condition has remained a major cause of infant mortality worldwide, despite the wide range of treatment modalities available (Alkizim et al, 2011). Diarrhoea accounts for 16% (1.5 million) of deaths in children under 5 years (UNICEF and WHO, 2009). Such alarmingly high rates of morbidity and mortality have made it imperative to search for novel modalities of diarrhoea management, both pharmaceutical and alternative.

Mangifera indica grows in tropical and subtropical regions and its parts are commonly used in folk medicine for a wide variety of remedies. The chemical composition of this plant has been studied extensively over the past years and has been shown to have numerous therapeutic uses (Shah et al, 2010). Different parts of the plant, including the seed kernel, have been shown to be therapeutically beneficial. Studies have

 ISSN 2303-9841

demonstrated its efficacy in the treatment of diarrhoea and elucidated its sympathomimetic mechanism of action (Alkizim et al, 2012). This however, raised the question of its safety, since sympathomimetic agents have the potential of causing adverse chronotropic and inotropic reactions on the heart.

It is worth noting that herbal medicines can cause severe toxicity and even mortality as a result of their unintended effects on other systems of the body i.e. adverse drug reaction (ADR). The World Health Organization (WHO) reported that ADRs are among the leading causes of mortality worldwide (WHO, 2008).

Having identified the mechanism of action of the mango kernel, pharmacovigilance is necessary in order to assess the possible sympathomimetic ADR on the heart. The present study therefore aimed at investigating the extract's effect on the myocardial activity. It is worth keeping in mind that sympathomimetic antidiarrhoeal agents should ideally be specific to the gastrointestinal system, and have no effect on the heart (Lyrenas et al, 1985).

2. Methods

2.1 Study animals

Fifteen healthy male New Zealand white rabbits aged 7 - 8 months and weighing 2000 - 2500 g were used. They were kept on a twelve hour light/dark cycle at normal laboratory conditions (temperature and humidity). Commercial rabbit pellets (Unga feeds Ltd) and water were provided *ad libitum*. Before experimentation, each rabbit was fasted for 18-24 hour; water however was continued *ad libitum.*

2.2 Preparation of *Mangifera indica* aqueous extracts (MIE)

Mangoes were obtained from a local grocery and identified by species with the help of the Herbarium of the University of Nairobi. Preparation of the extracts began one day prior to each experiment. The seeds were separated from the flesh, and the endocarp slit open to expose the kernel. The kernel, after being chopped into small pieces, was dried at 50 °C for 1 hour, and thereafter ground to a fine powder. When required, the powder was accurately measured and dissolved in distilled water to prepare the various concentrations of MIE. The mixture would then be allowed to macerate overnight at 4 °C. The following morning, the mixture would be centrifuged and decanted prior to use.

2.3 Preparation of physiological solutions

Ringer's solution (mg/ml: NaCl 9.0, $NaHCO_3$ 0.2, KCl 0.42, $CaCl_2$ 0.24, D-Glucose 1.0) and Tyrode's solution (mg/ml: NaCl 8.0, $NaHCO_3$ 1.0, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, NaH_2PO_4 0.05, D-Glucose 1.0) were prepared for *in vitro* physiological perfusion of the heart and jejunal tissues respectively.

2.4 Isolation of the tissues and drug administration

Each animal was rendered unconscious via cervical dislocation. The heart was isolated and cannulated, through the aorta, to a supply of Ringer's solution, maintained at 37 °C and equilibrated with a mixture of 95% O_2 and 5% CO_2. This reverse flow through the aorta caused closure of the semilunar valves, forcing the perfusate to flow through the coronary vessels and perfuse the heart muscle. The heart apex was then hooked and connected to an isotonic transducer which was, in turn, connected to a ''Graphtec'' recording apparatus. Time was then allowed for acclimatization, and after stable contractions had been recorded (identically reproducible for a period of at least 60 s), the contractile responses were recorded in the presence of cumulative concentrations of MIE (0.00 – 24.00 mg/ml). The contact time for each concentration was 10 seconds. This was determined by preliminary experiments which showed that *Mangifera indica* reached its maximal excitatory effect within this time period. The effect of MIE was then evaluated after the administration of propranolol (2 µg/ml), a known β adrenoceptor blocker.

In the meantime, the jejunum was isolated and placed in Tyrode's solution. Three centimetre pieces of the isolated tissue were then longitudinally set up in an organ bath containing 80 ml Tyrode's solution, maintained at 37 °C and equilibrated with a mixture of 95% O_2 and 5% CO_2. The tissues were connected to an isotonic transducer which was in turn connected to a ''Graphtec'' recording apparatus. Time was allowed for acclimatization, and after stable contractions had been recorded (identically reproducible for a period of at least 120 sec), the contractile responses were recorded in the presence of cumulative concentrations of MIE (0.00 – 4.00 mg/ml). The contact time for each concentration was 120 sec. This was determined by preliminary experiments which showed that *Mangifera indica* reached its maximal inhibitory effect within this time period. Adrenaline (0.2 µg), and distilled water were used as positive and negative controls.

2.5 Data analysis

Data were expressed as mean ± standard error of mean (SEM). The Kruskal Wallis test was used to test statistical differences across groups, while the Man Witney test was used to compare two groups. Significance levels were set at $p<0.05$.

2.6 Ethical considerations

The study protocol was approved by the Postgraduate Research Committee, Department of Medical Physiology, University of Nairobi. Animals were handled in accordance to the guidelines of the US National Research Council for the care and use of laboratory animals (National Academy Press, 1996).

 ISSN 2303-9841

3. Results

A dose related increase in myocardial contractility, with a mean of 18.70 ± 4.71%, occurred in response to MIE. This tested highly significant (p < 0.01). Post hoc analysis showed the highest significance to be between 4.00 - 8.00 mg/ml. Doses below 4.00 mg/ml showed no significant change, and doses above 12.00 mg/ml caused dose-related reduction in contractility. Furthermore, there was no significant change in frequency at all doses (**Figure 1**).

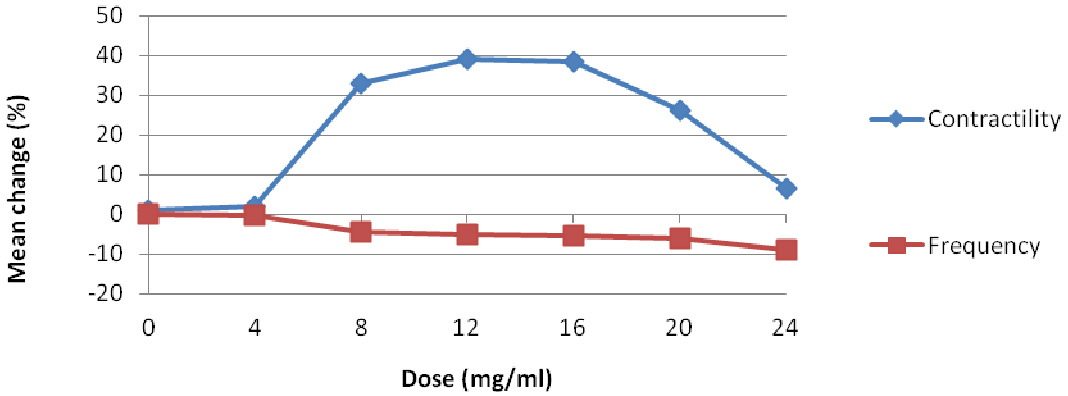

Figure 1: Dose-related change in myocardial contractility in response to *Mangifera indica* with a mean of 18.70% ± 4.71 (p<0.01). Decrease in frequency is however not statistically significant

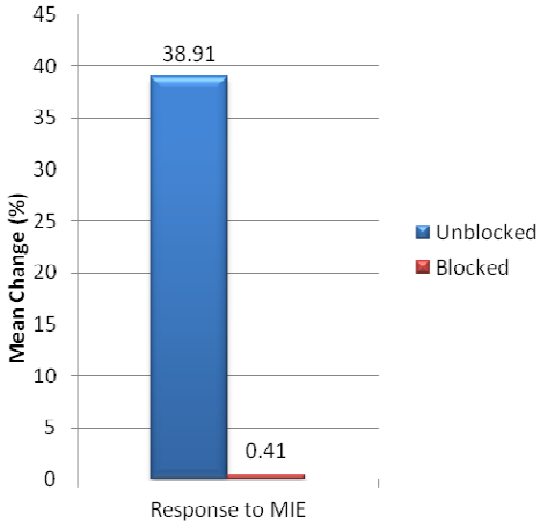

Figure 2: Comparison of the percentage increase in myocardial contractility in the presence of unblocked and blocked adrenoceptors. The difference was highly significant (p<0.01)

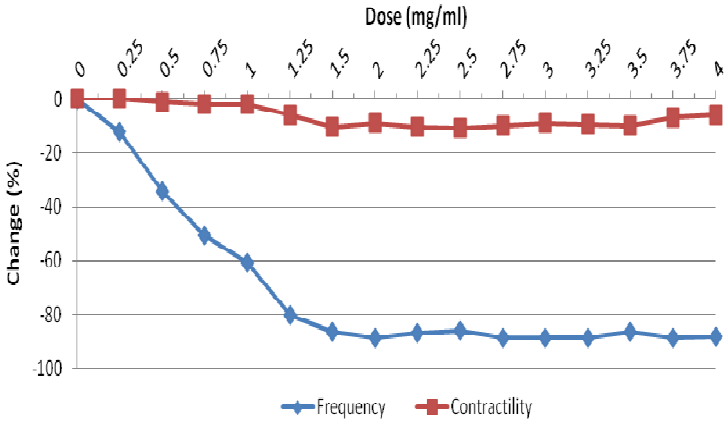

Figure 3: Dose-related effects of *Mangifera indica* on jejunum contractility and frequency

Propranolol significantly abolished the effect of *Mangifera indica* (p < 0.01). In the presence of the antagonist, the mean increase in contractility in response to MIE, was 0.41 ± 0.32%, compared to a mean increase of 38.91 ± 3.74% in the absence of the antagonist (**Figure 2**).

A highly significant (p < 0.01) dose-related decrease in jejunal contractility was observed in response to MIE, from doses 0.00 mg/ml to 4.00 mg/ml. The highest effective dose was noted to be 2.00 mg/ml. Higher doses had similar efficacy, but with a longer duration of action. This dose had no significant effect when administered to the heart (**Figure 3**).

4. Discussion

Medicinal plants are important sources of potentially useful phytochemicals for the development of novel pharmacotherapeutic agents (Wauthoz, 2007). *Mangifera indica* is one such plant, which has been used in the management of diarrhoea in folk medicine. Studies have demonstrated its efficacy and elucidated its sympathomimetic mechanism of action. However, until the current study, it's possible side effects on the heart during treatment of diarrhoea had not been investigated.

On cardiac administration of MIE, a significant dose-related increase in myocardial contractility was observed between the doses 4 mg/ml and 12 mg/ml. Higher doses caused the inverse response which were initially reversible, but non-reversible at even higher doses (above 20 mg/ml). This was indicative of toxicity to the myocardium at high doses of MIE.

Myocardial activity has two major components: the contractility (force) and the rate (frequency). These are regulated separately, where the rate is regulated by β_1 adrenoceptors alone while contractility is regulated by both β_1 and β_2 adrenoceptors (Barrett et al, 2010). It is worth noting that the extract only increased the contractility of the myocardium. The frequency change

was statistically insignificant, and the slight decrease was probably due to dilution of the perfusate, during administration of the extract. This positive inotropic and negligible chronotropic effects are an indication of specificity of MIE to β_2 adrenoceptors.

To confirm that MIE was inotropic purely via a sympathomimetic mechanism, a known concentration of propranolol was used to block the β adrenoceptors in the myocardium. The inotropic effect was significantly abolished in the presence of the antagonist ($p < 0.01$), thereby confirming the mechanism of action. Furthermore, in the presence of the antagonist, the slight increase in contractility tested statistically insignificant.

The highest effective dose administered to the jejunum (2.0 mg/ml) caused no significant response to the myocardium, where as the lowest effective dose on the myocardium (4.0 mg/ml) had a highly significant effect on the jejunum. It is therefore clear that a significant response to the extract by the heart occurred at doses much higher than the dose required to inhibit jejunal motility. This demonstrates that when used at the right dose, *Mangifera indica* seed kernel would be a suitable treatment for diarrhoea, without any side effects to the heart. Furthermore, although the bioavailability is yet to be demonstrated, oral administration of a particular dose of MIE for its local effect on the gut, during the treatment of diarrhoea, would probably lead to lower plasma concentration in systemic circulation, hence further assuring the heart's safety.

The use of *Mangifera indica* kernel as a diarrhoeal remedy is therefore probably safe on the cardiovascular system, provided that the dose is maintained within an anti-diarrhoeal therapeutic window. Further studies are however necessary, in order to investigate its safety on other body systems.

Conflict of Interest declaration

The authors declare no conflict of interest

Acknowledgements

The authors acknowledge the department of Medical Physiology, University of Nairobi, where this study was conducted. The authors also thank Mr. Karume and Mr. Kinyungu, for their technical assistance.

References

Alkizim F, Matheka D, Muriithi A (2011). Childhood Diarrhoea: Failing Conventional Measures, what Next? *Pan Afr. Med. J.* **8**: 47.

Alkizim F, Matheka D, Abdulrahman F, Muriithi A (2012). Inhibitory effect of *Mangifera indica* on gastrointestinal motility. *Med. Chem. Drug Discov.* **2**: 9-16.

Barrett K, Barman S, Boitano S, Brooks H (2010). Ganong's Review of Medical Physiology, 23rd ed. The McGraw-Hill Companies, Columbus.

Guide for the Care and Use of Laboratory Animals (1996). Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. National Academy Press, Washington DC.

Lyrenas E, Abrahamsson H, and Dotevall D (1985). Rectosigmoid Motility Response to Beta-Adrenoceptor Stimulation in Patients with the Irritable Bowel Syndrome. *Scand. J. Gastroenterol.* **20**: 1163-8.

Shah K, Patel M, Patel R, Parmar P (2010). Mangifera Indica (Mango). *Pharmacogn Rev.* **4**: 42-8.

Tindle H, Davis R, Phillips R, Eisenberg D (2005). Trends in use of complementary and alternative medicine by US adults: 1997–2002. *Altern. Ther. Health Med.* **11**:42-9.

UNICEF and WHO (2009). Diarrhoea: Why children are still dying and what can be done. WHO press, Geneva, pp 4-7.

Wauthoz N, Aliou B, Elhadj S, Marc V, Pierre D (2007). Ethnopharmacology of Mangifera indica L. Bark and Pharmacological Studies of its Main C-Glucosylxanthone, Mangiferin. *Intl. J. Biomed. Pharma. Sci.* **1**: 112-9.

World Health Organization. Medicines: safety of medicines – adverse drug reactions. http://www.who.int/mediacentre/factsheets/fs293/en/index.html. (Accessed 31 May 2012).

 ISSN 2303-9841