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Inhibitory effect of *Mangifera indica* on gastrointestinal motility

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Abstract

Diarrhoea, the second leading cause of child mortality, accounts for 1.5 million deaths, impeding the realization of the fourth Millennium Development Goal. A review of existing treatment modalities and formulation of newer ones is therefore necessary. Mango fruit (*Mangifera indica*) kernel has been used as anti-diarrhoea remedy. Its mechanism is however uncertain. The current study therefore aimed to investigate the *in-vitro* effect of *Mangifera indica* kernel extract (MIE) on intestinal motility, and elucidate its mechanism. The dose related effects of MIE was tested on sections of jejunum freshly isolated from rabbits. Various receptors were then selectively blocked to investigate its mechanism. Finally the effect of MIE was compared with that of loperamide. MIE reversibly inhibited motility by -46.38 ± 5.83 % (p<0.001), via a sympathomimetic mechanism. Its efficacy was comparable to loperamide, making it a potential antidiarrheal agent.

Keywords: Diarrhoea; mango kernel; gut motility; *Mangifera indica*

Introduction

Diarrhoea is the passage of loose stool, by an individual, at least three times a day, or more frequently than normal. It is most commonly caused by intestinal infection, mainly viral, transmitted faecal-orally (Koletzko *et al*., 2009). For decades, diarrhoea has been described as one of the leading causes of mortality not only in the developing world, but also in the developed too. The implications are however more evident in the former (Farthing, 2000). In 1979, WHO reports (WHO, 1979) expressed concern on the matter and more than 2 decades later the same agency reported diarrhoea as the second leading cause of infant mortality worldwide (Figure 1), causing up to 1.5 million deaths annually (UNICEF and WHO, 2009). This accounts 16% of deaths in this age group, a toll greater than that of AIDS, malaria and measles combined (UNICEF and WHO, 2009).

Even these figures may well be an underestimate as they may not include extreme rural cases that may not have been fortunate to make it to a medical facility. The immediate
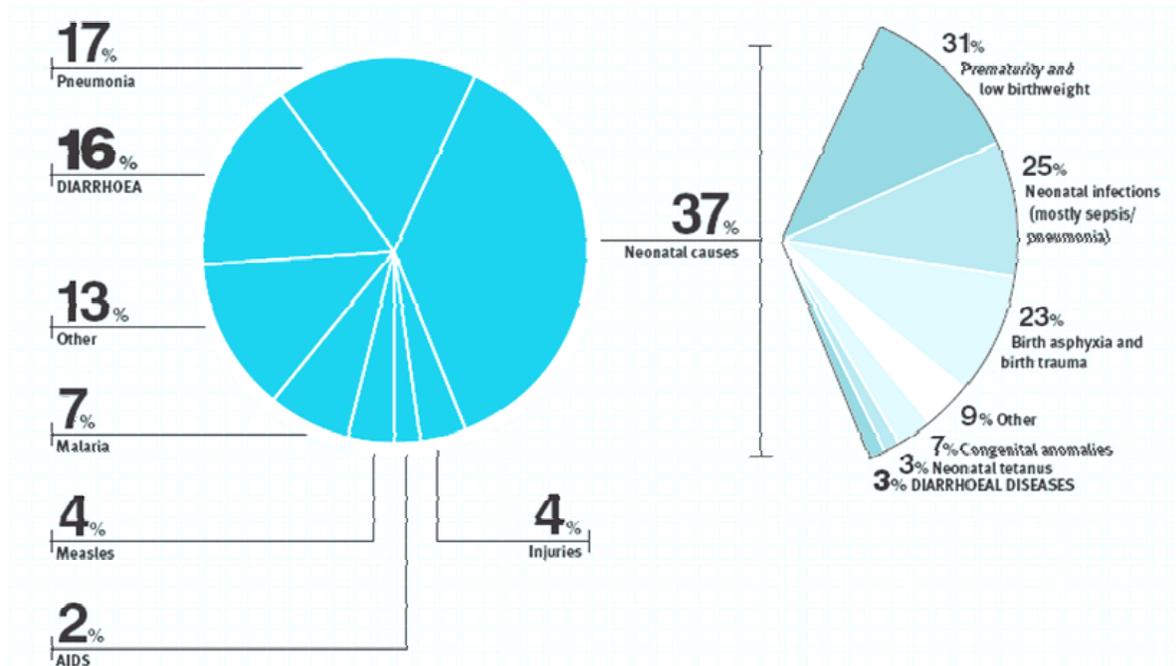
Figure 1. the common causes of infant mortality. Source: UNICEF and WHO. (2009). Diarrhoea: Why children are still dying and what can be done. WHO press, Geneva .

source of concern is dehydration and the electrolyte imbalance. The type of dehydration: hypertonic, isotonic or hypotonic is independent of the pathogen (Koletzko and Stephanie, 2009). With repeated episodes, protein energy malnutrition (PEM) results, followed by complications such as shock and renal failure, and subsequently death, if the diarrhoea is not restricted. PEM on the other hand is not only a consequence, but a predisposing factor of diarrhoea (Cutting, 1979). This interrelation is known as the vicious cycle of diarrhoea (Guerrant *et al.*, 2002), and it could consequently result in impaired growth and development (Farthing, 2000).

Long term effects of childhood diarrhoea include: decreased fitness index and cognitve function, delayed school commencement, and poor school performance (Guerrant *et al.*, 2002). This makes the disease far more costly, economically and on its toll on community health, and is therefore far more important to control than previously thought.

It is interesting to note that despite the wide range of treatment and prevention modalities available, diarrhoea still remains a major contributor to infant mortality worldwide (Alkizim *et al.*, 2011). This poses a major obstacle in the achievement of Millennium Development Goal number 4. Recent estimates on its realization show minimal progress, and the MDG target is, therefore, likely to be unattained (Murray *et al.*, 2007). Despite the fact that the annual death toll has fallen from 4.5 million in 1979 to 1.6 million currently, a lot still needs to be done to achieve the goal.

The use of herbal products has continued to increase despite the rapid development of pharmaceutical products (Dahunakar *et al.*, 2000). This is attributed to various reasons including cultural beliefs, poor distribution and availability of modern medication, and poverty. Ethnopharmacology (the science of ethnic drugs), has therefore grown with the aim to invest-

igate the use and effects of these ethnic drugs (Wauthoz, 2007). It has contributed to drug discovery since the 19th century (Heinrich and Gibbons, 2001).

Mango fruit (*Mangifera indica*)*,* often called the "King of fruits", originated in India, Myanmar, and the Andaman Islands (Wauthoz, 2007). It is now cultivated throughout the tropical and subtropical world. 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). The use of its kernel has been recommended for the treatment of diarrhoea, in folk medicine. An *in vivo* study has been attempted by Sairam *et al*. (2003). The mode of action was however not elucidated and has remained unclear prior to the current study.

Considering the failing conventional diarrhoea treatment modalities (Alkizim *et al*., 2011), the widespread mango fruit cultivation, the enormous availability of the seeds at factory sites which would otherwise cause environmental pollution (Dhingra and Kapoor, 1985), the present study is justified as an attempt to establish credibility, awareness, and scientific data to support the therapeutic use of *Mangifera indica* as an alternative to conventionally used pharmaceutical drugs for the treatment of diarrhoea.

Materials and methods

Animals

Fifteen male New Zealand white, pure breed, rabbits (7-8 months, 2000-2500g) were used. Exclusion criteria included disease, femininity, and non-pure breed. They were kept on a twelve hour light/dark cycle and fed on commercial rabbit pellets (Unga feeds), and water *ad libitum.* Normal laboratory conditions (temperature and humidity) were maintained. Before each rabbit was used, food was withdrawn 18-24 h before the experiment, water was however continued *ad libitum.*The animals were handled in accordance to the "Principle of Laboratory Animal Care" (NIH publication No. 85-23) adhering to the set guidelines and procedures (NIH publication revised 1985)

Preparation of Mangifera indica aqueous extracts (MIE)

Mangoes were purchased from a local grocery and identified 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 flesh, and the endocarp slit open to expose the kernel. The kernel, chopped into small pieces, was dried at $50^{o}C$ for 1 hour, and thereafter ground to fine powder. Whenever needed, powder was accurately measured and dissolved in distilled water to prepare the various concentrations of MIE (0.00, 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 mg/ml). These were then allowed to macerate overnight, at $4^{o}C$, and centrifuged the following morning so as to decant the supernatants for use.

Isolation of jejunum and drug administration

The rabbits were, one at a time, rendered unconscious via cervical dislocation. The jejunum was then isolated and placed in Tyrodes solution (mg/ml: NaCl 8, $NaHCO_3$ 1, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, NaH_2PO_4 0.05, D Glucose 1). Three centimetres pieces of the isolated organ were longitudinally set up in an organ bath containing 80 ml Tyrodes 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 (0.00–1.50 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.

The effect of MIE was also evaluated after the administration of Propranolol (2 μg) to block β adrenoceptors, Phenoxybenzamine (2 μg) to block α adrenoceptors, and Naloxone (10^{-6} M) to block μ opioid receptors. Finally the effect of loperamide (0.5 mg/ml), used as the reference drug, was recorded and compared against that of MIE. Contact time for loperamide was 360 seconds, established by preliminary studies.

Analysis

Results are expressed in mean ± Standard error of mean (SEM). Kruskal Wallis was used test statistical differences across groups, while Man Witney U test was used to compare two groups. P<0.05 was considered significant.

Results

A dose related decrease in jejunal contractility, with a mean of -46.38% ± 5.83, occurred in response to MIE. This tested very highly significant (P < 0.001). Post hoc analysis showed the highest significance to be between 0.25 and 0.50 mg/ml (P<0.01), and least significance between 1.25 and 1.5 mg/ml. There was however no significant decrease in frequency (Figure 2)

Propranolol and phenoxybenzamine significantly abolished the inhibitory effect of *Mangifera indica* (P<0.05). In the presence of the antagonists, the mean decrease in contractility, in response to MIE, was -19.56% ±7.21 compared against a mean decrease of 60.48% ± 1.41 in the absence of antagonists. Naloxone however failed to block the extracts effect. (Figure 3)

In comparison to the reference drug, 0.5 mg/ml *Mangifera indica* caused a 34.28 ± 4.63 % decrease in contractility, while 0.05 mg/ml loperamide caused an 85.78 ± 1.29 % decrease. Furthermore loperamide had 46.67 seconds duration to onset, while *Mangifera indica's* effect was immediate. The effect of loperamide persisted till washout while that of *Mangifera indica* was transient, lasting a few seconds.

Discussion

Ethnopharmacology, a relatively new science, has over the years been focussing on evaluating indigenously used drugs. Medicinal plants are considered as an important source of potentially useful phytochemicals for the development of novel pharmacotherapeutic age-
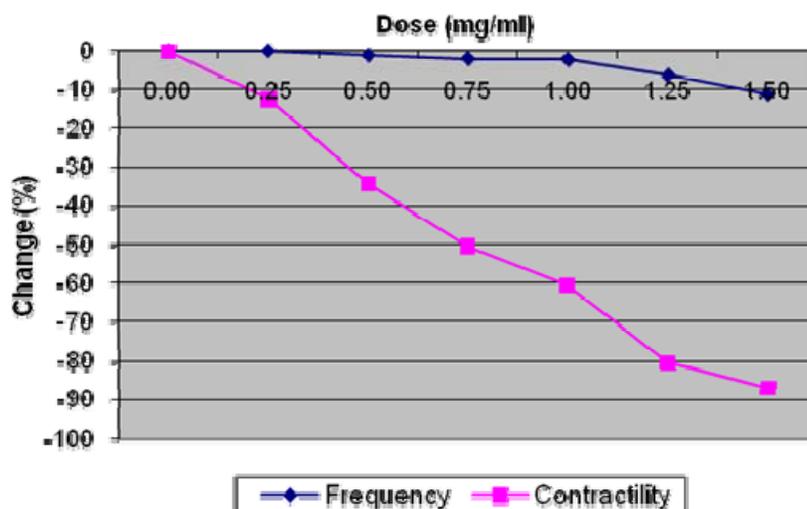
Figure 2. Dose-related decrease in jejunum contractility in response to *Mangifera indica* with a mean of -46.38 = 5.83 (p<0.01). Decrease in frequency is however not statistically significantly.

nts, and the main aim is to validate (or invalidate) the plants through the isolation of their active substances (Wauthoz, 2007).

The gut is a neurological organ (Holzer *et al*., 2001) that functions not only to assimilate food, but also to sort the ingested in terms of nutritive, toxic, and pathological properties (Holzer, 2004). To perform its functions, the gut requires electric activity generated by the Interstitial Cells of Cajal (ICC), within the Enteric Nervous System (ENS).
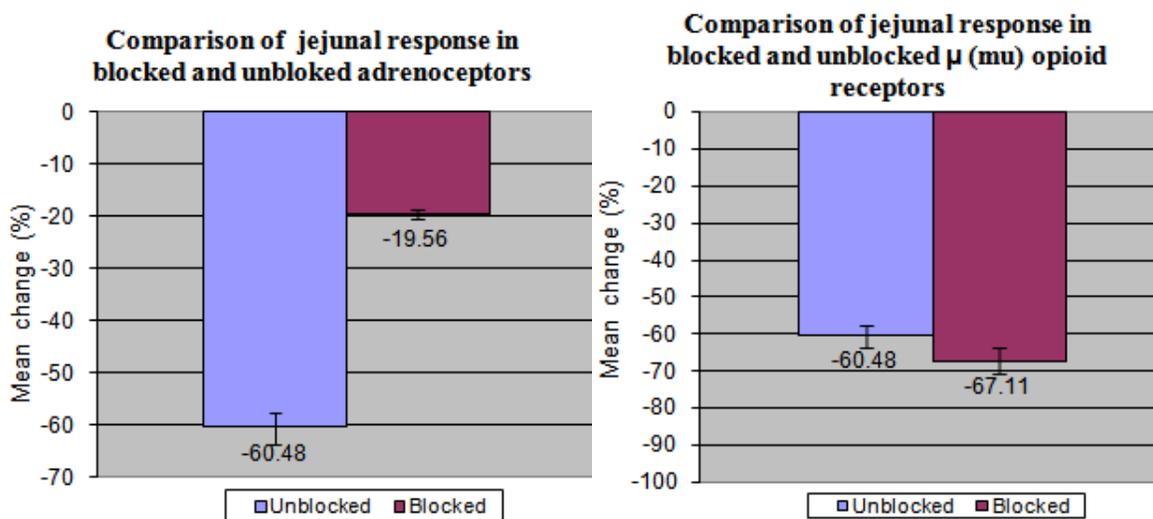

Figure 3. Receptor blockage to elucidate the mechanism of action of *Mangifera indica*. (Left): Percentage decrease in jejunal contractility with unblocked adenoceptors had a mean of -60.48±1.41%. where as that with blocked adenoceptors had a mean of -19.56±7.21%. The difference was significant (p<0.05). (Right): Percentage decrease in jejunal contractility with unblocked mu receptors had a mean of -60.48±1.41%. where as that with blocked adenoceptors had a mean of -67.11±7.21%. The difference was not statistically significant (p<0.05).

Mangifera indica kernel extract was seen to inhibit jejunal contractility, in the current study, with higher doses causing more pronounced effects. This was probably due to increasing availability of agonistic molecules to bind to receptors, hence inhibiting the ENS, which inturn inhibit *muscularis externa* activity.

The responses were noted to be immediate but short lived. An immediate response is probably an added advantage to its efficacy, whereas the short duration, showed that the binding to receptors was reversible. This too is likely to be an advantage, as an irreversible effect would undesirably cause paralytic ileus. The duration of effect could however be prolonged pharmaceutically, for example using slow releasing preparations, once the active drug has been isolated.

The sympathetic nervous system is one of the regulatory mechanisms of the gut. This works to inhibit gut function, and does so by two functional pathways, a direct and an indirect. Whereas the direct pathway is specific for sphincter contraction and vasoconstriction in the gut, the indirect pathway causes inhibition of gut motility on non-sphincteric regions, where it works by inhibition of excitatory enteric pathways (Wood *et al.*, 1999). This is effected via the presynaptic α_2 adrenoceptors (Holzer, 2004). The direct pathway is explored in this study, since a piece of non-sphincteric jejunum was used each time.

An attempt to establish the mode of action was made using known concentrations of phenoxybenzamine and propranolol together to block the α (alpha) and β (beta) adrenoceptors of the jejunum. The inhibition of motility by *Mangifera indica* was significantly less in the presence of the antagonists. This indicated that the extract exerted its effect through adrenoceptors. This activated the SNS to inhibit jejunal motility (Kellow *et al.*, 1999). The slight inhibition that persisted in the presence of the antagonists was probably due to incomplete blocking of the receptors, leaving some available for the agonist. Identification of the adrenoceptor subgroups was, however, beyond the scope of this study.

An attempt to block the μ_3 opioid receptors, was also made using naloxone. The gut response to *Mangifera indica* with and without the antagonist was statistically similar, ruling out the likelihood of opioid receptors involvement.

The effects of 0.5mg/ml of Loperamide, and 1.0 mg/ml of *Mangifera indica* were comparable on the jejunum. Loperamide was however seen to have a slow onset of response, yet a long duration. The extract was approximately half as effective as the standard drug. This was probably because Loperamide is a pure drug concentrate whereas the MIE was in form of a crude extract, probably containing very small amounts of the active ingredient. Nevertheless, this was an indication that a pure extract of the active phytochemical has the potential of being, if not more effective, as effective as Loperamide.

Antidiarrhoea drugs like Loperamide, despite their efficacy in inhibiting gut motility, are not recommended for use in infants due to their potential side effects (Caleb *et al.*, 2010), as well as the fact that they prevent expulsion of the pathogens, by inhibiting gut motility, thus allowing them to thrive.

It is evident, from this study, that Mangifera indica seed kernel decreases rhythmic motility of the jejunum by a sympathomimetic mechanism. This will reduce stool output, hence shortening the disease duration, and preventing complications. Furthermore, decreased motility allows for increased absorption of electrolytes and water, hence ultimately decreaseng faecal bulk, dehydration and electrolyte imbalance (Sairam *et al*., 2003). Anti-diarrhoeal activity of the extracts may also be due to the presence of tannins and tannic acids (Sairam *et al*., 2003), which probably precipitates pathogenic proteins (e.g. toxins) forming protein tannates, hence detaching them from the mucosa and reducing their effect. Furthermore, the kernel of *Mangifera indica* has been reported to have anti-inflammatory activity (Garrido *et al*., 2001), which facilitates diarrhoea management by inhibition of prostaglandin production (Sairam *et al*., 2003). The SNS has been shown to enhance gut immunity, and immune responses, by influencing the migration and accumulation of naïve and memory lymphocytes in mucosal lymphoid tissue (Ariki and Husband 1998), hence enhancing acquired immune responses. Having demonstrated the sympathomimetic effect, MIE may therefore enhance gut immunity, facilitating diarrhoea management, via the above immune mechanism.

Keeping in mind the prevalence of diarrhoea in regions where mangos are readily available, this study supports the potential use of *Mangifera indica* as a safe and efficacious antidiarrheal agent as claimed by traditional medicine.

Aacknowledgment

Conflict of interest

The authors declare no conflict of interest.

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