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Review

Nanotechnology: A non-invasive diagnosis and therapeutic tool for brain disorders

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Presently, nanotechnology appears as a powerful and innovative tool in the medical field. It has various advantages over the conventional drug therapy such as therapeutic specificity, has less side effects, reduces dose of drug, has more precise treatment and can access the inaccessible areas of the body like brain, tumor cells, etc. The human brain is the most complicated part of the body which is highly protected with the blood-brain barrier (BBB) and the other protective measures of the body. The available treatments for brain disorder are highly invasive (parenteral or surgical procedures) in nature or cause high peripheral toxicity (oral administration). Thus, a prominent strategy is needed which can easily approach the brain and offers a more specific treatment. Nanomedicines are effective tools used for the diagnosis and treatment of brain disorders.

Key words: Brain, neurodegenerative disorders, nanotechnology, gold nanoparticle, quantum dots.

INTRODUCTION

The brain is the most delicate, complex and vital organ of the human body. It regulates all the responses and stores information (Sonali et al., 2018). Our brain makes us feel,

sense and be aware of every little sense and stimulus outside as well as inside the body. The body has its protective measures to shield the brain from any

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damage. Among these, the very first protective structure is the skull which covers the whole brain and protects it from external injuries. The other primary protective layers include the meninges, blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (Tsou et al., 2017). Throughout life, our brain comes in contact with various types of stimuli which sometimes harm the integrity and function of brain. The brain disorders may vary from accidental injury, shock, trauma, stroke from poisoning, infection, physiological dysfunctioning, genetic disorders, aging, etc. which damage any particular part or group of neurons (Orive et al., 2009). Concerning the treatment of brain disorder, medical science still has not any answer to brain disorders. There are some factors associated with brain physiology which restrict the therapy. A significant factor is the presence of physiological barriers like BBB which limits the entry of almost all the external molecules (including drug and nutrients) to the brain. Such protective feature makes the brain inaccessible to the drug and diagnostic aids (Blanco et al., 2015). Hence, the treatment needs a large dose of the drug to attain the therapeutic level that causes a systemic side effect. Along with this, the other treatment strategy includes surgical procedures or intracerebroventricular injections, a highly invasive and painful approach (Silva Adaya et al., 2017).

Considering such challenges, researchers are continuously trying to find out a promising and convenient tool to deliver the drug to the brain without interfering with the efficacy of the drug. One such approach is nanotechnology, the engineered nano-sized tools that regulate and manipulate the physicochemical prospects of any material or device at the molecular level (Huang et al., 2017). Research proves that nanotechnology is a useful tool that can replace or improve the presently available drug therapies and modify the diagnostic aids by using molecular imaging and modeling (Fan and Yeh, 2014; Silva et al., 2017; Sonali et al., 2018). The nanotherapeutics utilize various nanocarrier systems like liposome, nanoparticle, nanogels, dendrimer, nanotube, nanofiber, etc. and different targeting strategies to enhance the specificity of the treatment (Saraiva et al., 2016; Agrawal et al., 2017; Tsou et al., 2017; Poupot and Bergozza, 2018). The significant advantage of nanotechnology firstly involves its ability to deliver the drug across the BBB (Blanco et al., 2015), owing to the smaller size, secondly, the controlled and sustained release behavior (Saraf et al., 2015), thirdly, the targeting ability and lastly the ability to protect the drug integrity (Silva Adaya et al., 2017). Along with this, nanocarriers are highly stable, able to carry both the lipophilic and hydrophilic molecules and biodegradable in nature (Alexander et al., 2016; Kumar et al., 2017). This behavior makes nanocarrier a suitable tool for accessing the brain (Hassanzadeh et al., 2017). This novel technology is not only limited to the oral and parenteral

administration, but it can also be given through topical, transdermal and intranasal route to the brain. By this way, it offers a non-invasive treatment over the painful parenteral and highly invasive surgical measures.

ADVANCED NANO-TOOLS

For the effective treatment and diagnosis of brain disorders, some advanced form of nanomaterials is presently under investigation like gold nanoparticle, carbon nanotube, a magnetic nanoparticle, silicon nanowires, etc.

Silicon nanowires

Silicon nanowires are a semiconductor nanowire made of silicon precursors. These are quasi-1D structures with a size of approximately 100 nm. The small size of the nanowires offers a larger surface area. Its unique mechanical, optical and electronic properties, biocompatibility and low toxicity profile makes it a promising therapeutic and diagnostic tool. Moreover, owing to the high surface-volume ratio, the silicon nanowires are used in biotechnology, for development of nanosensor, high-performance silicon nanoprobe, nanowires, etc. for biological investigation and recognition and treatment of various brain disorders (Peng et al., 2014). Also, the fluorescence silicon nanowires can be used for identification and imaging of tumor or cancer cells, detection of plaque formation, and recognition of DNA and proteins. Parmeshwaran et al. (2018) have developed a photosensitive silicone nanowire to modulate the neuronal activity and regulate cellular excitability in a non-genetic and non-invasive manner. The nanowires are administered in a drug like fashion and get activated inside the body by absorbing light (Lanzani, 2018). The study shows the nanotool as an effective and alternative approach over highly invasive surgical procedures for the treatment of brain disorders (Parmeswaran et al., 2018).

Gold nanoparticle

Another important strategy is the gold nanoparticle which is now popular in neuroscience. These are nanosized colloidal particles made of gold, having a size of less than 100 nm and offer multiple surface functionalization (Kerdi et al., 2010). Due to the high surface functionality, it can easily interact with different antibiotics, proteins and oligonucleotides, thus, can serve as a promising diagnostic tool. It can also bind with targeting ligands and can carry various drug substances, hence, used as a potential drug carrier (Brown et al., 2010). These are the

potential oxidizing catalyst of liquid and gases. Thus, a suitable stabilizer or chemical method is needed to stabilize nanoparticles (Kerdi et al., 2010). It offers various advantages like drug targeting, cell signaling, nerve modulation, and signaling, etc. The application of gold nanoparticle in the treatment of neurological disorders includes neuro suppression (Yoo et al., 2014), nerve depolarization (Eom et al., 2014; Yong et al., 2014), neuromodulation by interfering with intracellular calcium signaling (Paviolo et al., 2014; Nakatsuji et al., 2015), neurite outgrowth enhancement (Paviolo et al., 2013; Papastefanaki et al., 2015). Along with this, gold nanoparticles are also used for gene therapy (Paviolo and Stoddart, 2017)

Carbon nanotubes

Carbon nanotubes were firstly prepared by Sumio Iijima in 1991, from the arc discharge of graphite electrode in an experiment (Iijima, 1991). The carbon nanotubes are entirely made of carbon atoms, arranged as a benzene ring; these benzene ring-like structures are made as graphene sheet which further turns into a cylindrical shape. By the arrangement, the carbon nanotubes are of two types (i) single-walled carbon nanotube and (ii) multi-walled carbon nanotube. The single-walled carbon nanotube consists of a single graphene sheet and having a cylinder diameter of less than 2.5 nm; while the multi-walled carbon nanotube is made of 2 or more graphene sheets with a cylindrical diameter up to 100 nm (Silva Adaya et al., 2017). The unique structure of the carbon nanotube is attributed to the high tensile strength, semiconductor property, high thermal conductivity, sustainability to the high current density, high resilience, etc. Thus it can be used as scanning probes, nanosensors, gas sensors and various nano-electric devices for bioanalysis (Esawi and Farag, 2007). The carbon nanotubes possess excellent plasma membrane permeability (Pantarotto et al., 2004), high drug loading, intrinsic spectroscopic behavior, hence, found suitable in disease diagnosis, imaging, and application in biotechnology (Lin et al., 2004). Recently it is also proposed as a component of protein biosensor, DNA (Li et al., 2005; Allen et al., 2007) and as ion channel blocker (Manish et al., 2005).

Super paramagnetic iron oxide nanoparticles (SPIONs)

Owing to the unique magnetic behavior, the SPIONs can serve as a promising diagnostic and therapeutic tool for CNS disorders. Their magnetic properties and surface modification assist in the identification of tumor cells, lesions and other pathological factors of CNS disorders.

At the same time, they can be used as a potential drug carrier system which specifically delivers the drug to its target site (Krupa et al., 2014). The main advantage and sometimes limitation of the magnetic nanoparticle is the necessity of an external magnetic field. The SPIONs get activated by the application of an external magnetic field and are inactive in its absence. Such property offers a great control over drug release, targeting and imaging responses of SPION. The average size of the magnetic nanoparticle is approximately 100 nm (SPION), and the particles with size less than 50 nm are known as ultra-super paramagnetic iron oxide nanoparticle (USPION) (Corot et al., 2006; Lodhia et al., 2010). In the past decade, the USPION became more popular as diagnostic agent than the SPION due to its unique ability to be visualized in both T1-MRI sequence and T2-MRI sequence as hyperintense (bright) and hypointense (dark) signal, respectively (Bridot et al., 2007; Na et al., 2007; Pan et al., 2008). Due to its smaller size, USPION can be absorbed by tumor cells via phagocytosis. However, the surface modification with an antibody or specific ligands could increase the targeting efficiency (Hadjipanayis et al., 2010). In addition, the USPION is a more patient-friendly carrier system with very less or no renal side effects (Neuwelt et al., 2007; Neuwelt et al., 2009).

Quantum dots

Quantum dots are one of the most studied nanotools nowadays. These are nano-sized crystalline colloidal semiconductor materials commonly consist of metallic crystals like selenium, cadmium, etc. At the core, they are enclosed by inert metal shell like zinc sulfide. These are the inorganic nanomaterials with high brightness, greater photostability and easy tunability to the narrow emission spectra. Such properties make them a promising diagnostic and therapeutic tool (Li et al., 2017). They are broadly used in biolabelling, biosensors, lasers, light emitting diodes and in the medical field for diagnosis as well as in therapy. In general quantum dots are fluorescent nanocrystals made of semiconductor materials which continuously emit energy. Because of the light emission property, quantum dots are found very useful in biological diagnosis and imaging (Silva Adaya et al., 2017).

Nanotechnology generally deals with the formulation of nano-sized particles or carrier system used for delivery of drug to treat various diseases. The NIH (National Institute of Health) of USA approved nanomedicines for diagnostic and therapeutic application (Markman et al., 2013). The nanocarriers have great advantages over conventional therapies and diagnosis techniques. They are easy to prepare by adopting standard techniques, can provide target specific action or recognition by surface

functionalization with specific ligands. Moreover, the radiolabelled or fluorescent probes or other nano-sized devices offer a significant degree of monitoring and produce more precise results (Sonali et al., 2018). Also, they are biocompatible and can be used as a component of various biotechnological devices, DNA, protein and can offer ease of permeation across the biological membranes like BBB. The radiolabelled nanoparticles, nanoprobe, magnetic nanoparticle, gold nanoparticle, photoresponsive nanomaterials, surface modified (with a biomarker, ligand or specific antibody) nanoparticle represent an efficient and non-invasive method of diagnosis (Viola et al., 2015). However, their application is limited due to their toxicity profile and unpredictable *in vivo* response of these devices.

INTRANASAL APPROACH AS AN ALTERNATIVE ROUTE FOR BRAIN DRUG DELIVERY

One more interesting strategy is the nose-to-brain delivery of nanoparticle. The intranasal route directly delivers the drug to the brain through the olfactory route and bypasses the BBB. By this way, it enhances the drug efficacy, brain targeting and reduces the dose of the drug and systemic side effect. The amalgamation of nanotechnology with the intranasal route improves the retention time of drug in the nasal cavity, protects the drug from enzymatic degradation and target the drug to a particular part of the brain. Hence, the delivery of nanomedicines via nasal route offers an attractive, non-invasive and cost-effective therapy for brain disorders (Sonvico et al., 2018).

CONCLUSION

In this work, we have discussed a few examples of nanotools used as a diagnostic and therapeutic tool for brain disorders. There are a wide number of different nanotechnological approaches available for application or under research which overcomes the limitation of the conventional medical system. It offers a very convenient, more precise and quality services in the healthcare system. Although, some pitfalls are also there like the toxicity profile of nanomedicines which restricts the clinical application of such a prominent tool. Some of the nanomedicines were approved by the FDA for commercial application as anti-cancer drugs, analgesic agents and many more. Although, most of the works are in the pipeline. On the basis of the extensively successful research attempts, we the evidence of nanotechnology will be the next era of medical science.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Myorelaxant effect of aqueous root bark extract of *Carissa edulis* (Forssk.) Vahl. is mediated through activation of nitric oxide synthase

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Many patients in Africa often utilize herbal medicine for the management of hyperactive gut disorders such as diarrhea and abdominal colic. There is therefore a need to scientifically evaluate efficacy of this herbal remedies. This study investigated the myorelaxant effect of *Carissa edulis* (Forssk.) Vahl and its possible mechanism of action using isolated rabbit jejunum preparations. Pieces of jejunum were isolated from adult New Zealand white rabbits. They were then mounted in an organ bath containing Tyrode's solution. The rate and force of jejunal contraction were recorded and analyzed using a Powerlab that was coupled to Chart5 software for windows. The effects of the extract (0.1-10.0 mg/ml) on spontaneous contraction were investigated. Its effect (3.0 mg/ml) was also studied in the presence of L^G-NAME (nitric oxide synthase inhibitor), methylene blue (soluble guanylyl cyclase inhibitor) and propranolol (non-selective β adrenergic receptors antagonist). The extract dose-dependent significantly decreased the force but not the rate of spontaneous rabbit jejunal contraction. Among the blockers used, only L^G-NAME significantly blocked the effect of the extract. Aqueous root bark extracts of *C. edulis* possess significant myorelaxant effect on isolated rabbit jejunum. This appears to be mediated through stimulation of nitric oxide release by nitric oxide synthase.

Key words: *Carissa edulis*, diarrhea, jejunum, motility, myorelaxant.

INTRODUCTION

Spasms of the gastrointestinal tract underlie hyperactive gut disorders such as diarrhea and inflammatory bowel

disease (Field, 2003). These conditions are highly prevalent; for example, 1.731 billion episodes of diarrhea

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occur annually and result in 700,000 deaths of children - especially those below 2 years of age (Walker et al., 2013); while inflammatory bowel disease is estimated to have a prevalence of over 1 million in the USA and 2.5 million in Europe (Kaplan, 2015). These conditions are currently managed by anticholinergics, calcium channel blockers and musculotropic agents (Annaházi et al., 2014). The remedies are associated with side effects, limited availability (Annaházi et al., 2014) and higher health care cost (Kaplan, 2015). It is for these reasons that there is an increased interest in finding cheaper, safer and locally available compounds with potential use for these conditions.

Majority of the world population rely on herbs for their medicinal needs (Ondicho et al., 2016), showing the need to scientifically evaluate the efficacy of herbal medications. *Carissa edulis* (Forssk.) Vahl is a member of the Apocynaceae family. Its root decoction is used to manage hyperactive gut disorders such as abdominal colic and diarrhea in the Rift Valley and Nyanza parts of Kenya which are mainly inhabited by Keiyo and Luo community respectively (Geissler et al., 2002; Kigen et al., 2014). Despite its extensive use for these conditions, no study has been done to investigate its effects on gastrointestinal motility. Therefore, this study investigated the effect of aqueous root bark extract of *C. edulis* (Forssk.) Vahl on gastrointestinal motility and its possible mechanisms of action of using isolated rabbit jejunum preparations.

MATERIALS AND METHODS

Plant material and extract preparation

C. edulis (Forssk.) Vahl roots were collected from Homa Bay, Kenya in July 2017, and were identified at the Department of Botany, University of Nairobi and a voucher specimen deposited (Voucher No. LLO 2017/01). The roots were cleaned with tap water, and their barks were peeled off and cut into smaller pieces. The material was then air dried at room temperature for 7 days after which it was ground into coarse powder using an electric mill.

The powdered material (100 g) was boiled in distilled water (1000 ml) for 10 min; the mixture left standing for 4 h; and the resultant mixture filtered using Whatmann No.1 filter paper- then the filtrate transferred into a round-bottomed rotary vapor flask where water was removed at a temperature between 35 and 40°C at a pressure of -760 mmHg.

Study animals

New Zealand White rabbits of both sexes (1.5-2 kg) were used in this study. They were housed singly in metallic cages measuring 46 x 48 x 36 cm at standard temperature (22–25°C) and humidity with a normal light/dark cycle. The animals were fed on standard rabbit pellets, vegetables and water *ad libitum*, and they were acclimatized for two weeks. This study was approved by the Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary

Medicine, University of Nairobi (FVM BAUEC/2018/141).

Isolation and preparation of rabbit jejunum

The animals were fasted for 24 hours before experiments but they had access to water *ad libitum*. They were sacrificed by cervical dislocation, their abdomen were cut open and the jejunum portion (6-8 cm) from the ligament of Treitz was isolated out (Grasa et al., 2005).

Two-centimeter segments of rabbit jejunum were mounted individually in an 80 ml organ bath containing Tyrode's solution. The solution was aerated with 95% O₂ and 5% CO₂ and was maintained at 37°C. The composition of the Tyrode's solution in mM was: NaCl 136.9, KCl 2.68, MgCl₂ 1.05, NaHCO₃ 11.90, Na₂HPO₄ 0.42, CaCl₂ 1.8, glucose 5.55 and a pH 7.4 (Cortés et al., 2006).

The upper end of the segment was hooked to an isometric force transducer (ML500/A, AD Instrument) coupled to a Power Lab data acquisition system (Power Lab 8/30). The force (g) and rate (number per minute) of contraction were recorded and analyzed using Chart 5 software for windows.

The tissues were allowed to equilibrate for 30 min before addition of any drug; the bath fluid drained at 20 min interval; and only jejunal segments that contracted spontaneously were used in this study.

Pharmacological evaluation

To investigate the myorelaxant effect of the extract, its effect on spontaneous contraction was studied. After stabilization period, spontaneous contractions of the jejunum were recorded for 2 min. Extract at concentrations of 0.1, 0.3, 1.0, 3.0, and 10.0 mg/ml was added cumulatively into the organ bath at an interval of 3 minutes before washing out. The inhibition of contractions by the extract was expressed as a percentage of the spontaneous contractions of the rabbit jejunum.

To investigate the extract's possible mechanism of action, 3.0 mg/ml of *C. edulis* extract was studied in the presence of L^G-NAME (nitric oxide synthase inhibitor), methylene blue (soluble guanylyl cyclase inhibitor), and propranolol (a non-selective β adrenergic blocker) respectively. Normal jejunal activity was first recorded for 2 min prior to the addition of a given blocker. The extract was added 15 min after the addition of either 10 μM methylene blue or 100 μM L^G-NAME (Chda et al., 2016) or 2 min after the addition of 1 μM propranolol (Kinuthia et al., 2016) followed by a 3 min record of jejunal activity before washing out.

Data and statistical analysis

The results were expressed as Mean ± SEM; n represents the number of experiments. Data were analyzed using SPSS version 20, while graphs were generated using GraphPad Prism version 7. One-way ANOVA, followed by the Bonferroni post hoc test were used to compare between groups. The difference was considered significant only if p < 0.05.

RESULTS

Extract yield

100 g of powdered root barks produced 8.21 g of aqueous extract. This represents 8.21% extract yield.

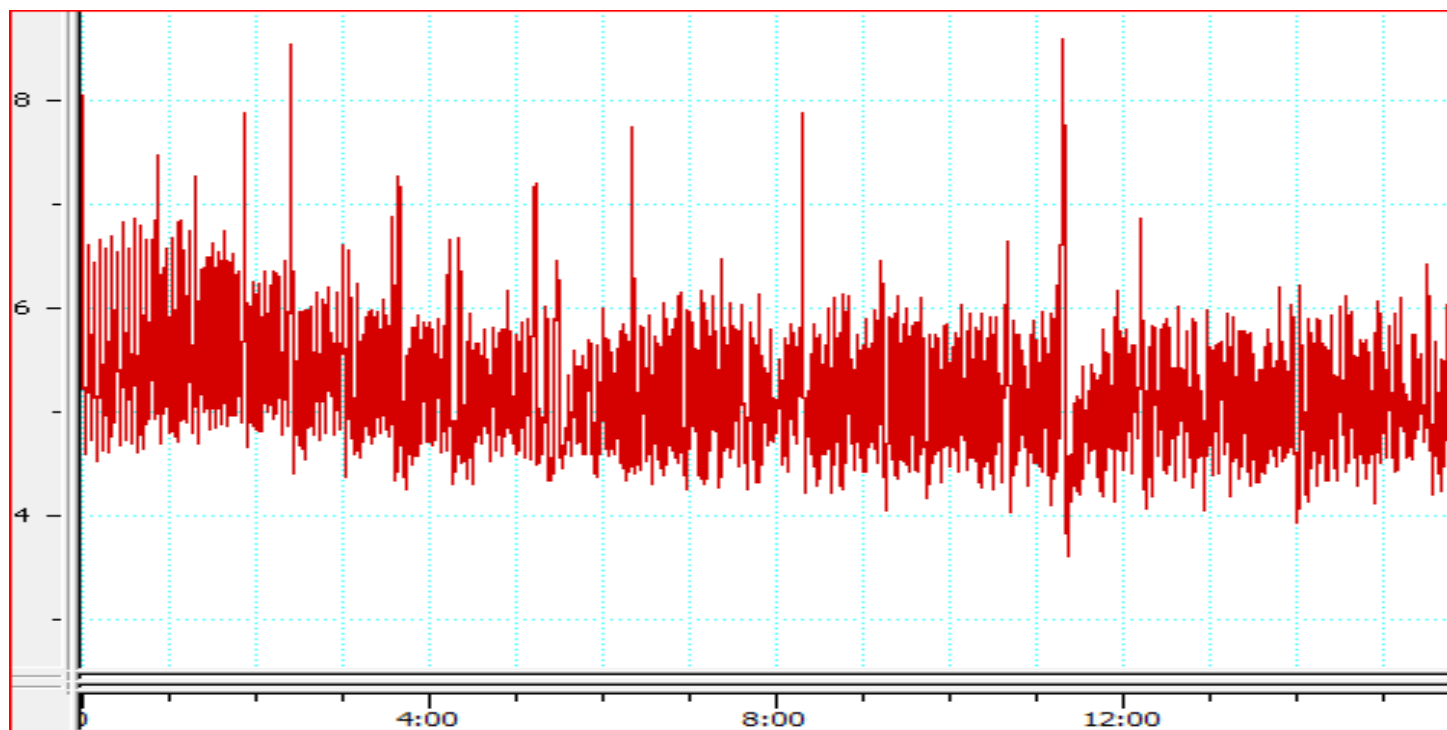


Figure 1. Spontaneous rabbit jejunal contractions.

Effect of extract on rate and force of spontaneous rabbit jejunal contraction

The isolated rabbit jejunum showed rhythmic spontaneous contractions that did not change with time as presented in Figure 1. Addition of the extract caused a dose-dependent decrease in the force of spontaneous jejunal contractions, [100.00 ± 0.00 (control) vs. 92.94 ± 1.46% (0.1 mg/ml) vs. 71.16 ± 4.13% (0.3 mg/ml) vs. 49.21 ± 2.38% (1.0 mg/ml) vs. 24.23 ± 2.42 (3.0 mg/ml) vs. 7.74 ± 0.50 (10.0 mg/ml)]. Doses of 0.3, 1.0, 3.0 and

10.0 mg/ml of the extract caused a significant decrease in the force of spontaneous jejunal contraction as presented in Figures 2 and 3. The effect of the extract on the force of spontaneous contraction was partially reversible on washing out as shown in Figure 2. The extract had no significant effect on the rate of spontaneous jejunal contractions as determined by one way ANOVA analysis, [100.00 ± 0.00% (control) vs. 99.19 ± 1.61% (0.1 mg/ml) vs. 104.56 ± 2.24% (0.3 mg/ml) vs. 99.33 ± 2.39% (1.0 mg/ml) vs. 100.11 ± 3.03% (3.0 mg/ml) vs. 104.76 ± 4.06%

(10.0 mg/ml); $p > 0.05$] as shown in Figures 2 and 3.

Effect of blockers on myorelaxant effect of the extract

L^G-Nitro arginine methyl ester (L^G-NAME)

The myorelaxant effect of the extract was significantly reduced by L^G-NAME, [24.23 ± 2.42% (3.0 mg/ml ARCE) vs. 76.06 ± 8.67% (L^G-NAME + 3.0 mg/ml ARCE), $p < 0.001$]. The extract showed

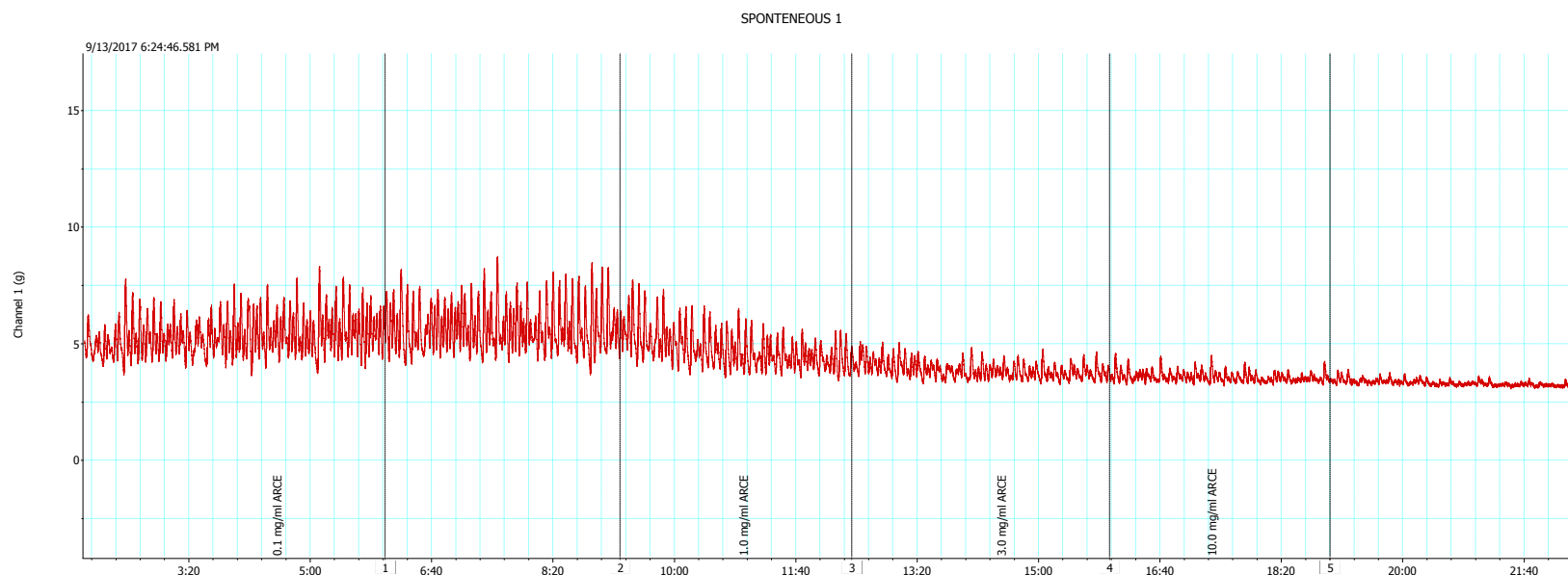


Figure 2. Powerlab tracing showing the effect of extract (0.1-10 mg/ml) added cumulatively on force and rate of spontaneous rabbit jejunal contraction.

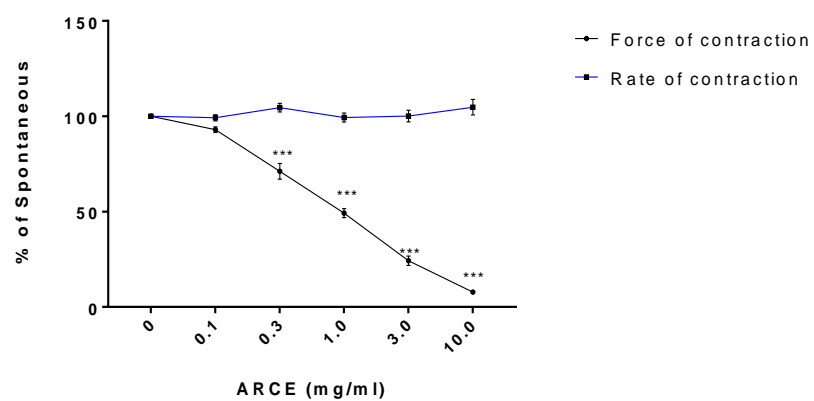


Figure 3. Graph showing the effect of extract (ARCE) on rate and force of spontaneous rabbit jejunal contraction. They are shown as mean ± SEM, (n= 5), *p<0.05, **p<0.01, ***p<0.001 vs. control.

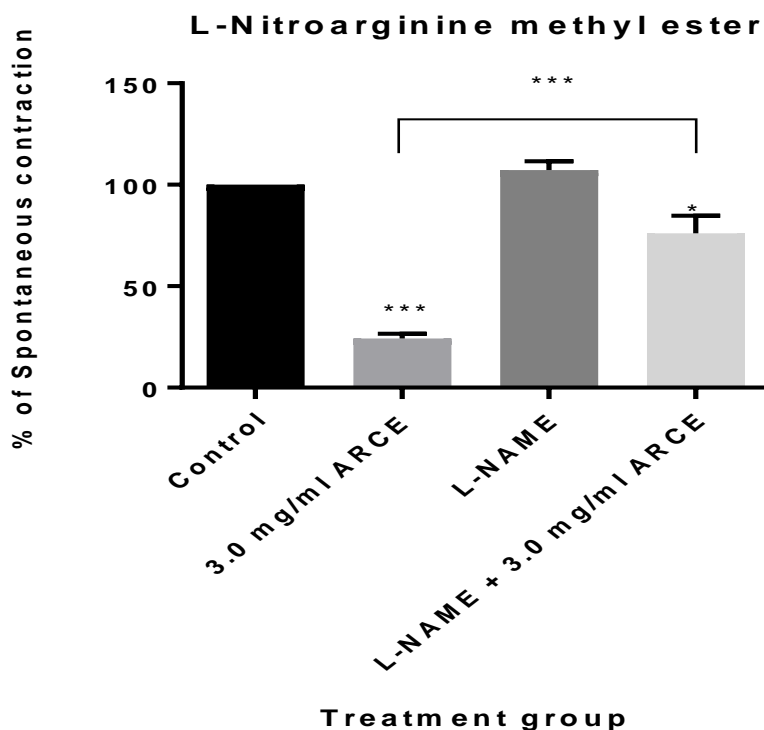


Figure 4. Bar graph showing the effect of L^G-NAME on myorelaxant effect of the extract. Results are presented as mean ± SEM (n= 5), *p<0.05, **p<0.01, ***p<0.001 vs. control.

a significant myorelaxant effect both in the absence and presence of L^G-NAME, [100% (Basal tone) vs. 24.23 ± 2.42% (3.0 mg/ml ARCE); p < 0.001] and [100% (Basal tone) vs. 76.06 ± 8.67 % (L^G-NAME + 3.0 mg/ml ARCE; p < 0.05). L^G-NAME caused an increase which was not significant on basal jejunal tone, [100% (Basal tone) vs. 107.20 ± 4.367% (L^G-NAME); p > 0.05]. These results are summarized in Figures 4 and 5.

Methylene blue

Methylene blue partially reduced the myorelaxant effect of the extract; however, this was not significant, [24.23 ± 2.42% (3.0 mg/ml ARCE) vs. 33.53 ± 2.66% (methylene blue + 3.0 mg/ml ARCE); p > 0.05]. Methylene caused an increase which was not significant on basal jejunal contraction, [100% (Basal tone) vs. 119.57 ± 12.49% (methylene Blue); p > 0.05]. The extract caused a significant myorelaxant effect both in the absence and presence of methylene blue, [100% (Basal tone) vs. 24.23 ± 2.42% (3.0 mg/ml ARCE); p < 0.001] and [100% (Basal tone) vs. 33.53 ± 2.66% (Methylene Blue + 3.0 mg/ml ARCE); p < 0.001]. These results are summarized in Figures 6 and 7.

Propranolol

The myorelaxant effect of the extract was slightly higher in the presence of propranolol than in its absence although they were not significantly different, [24.23 ± 2.42% (3.0 mg/ml ARCE) vs. 18.49 ± 1.63% (Propranolol + 3.0 mg/ml ARCE); p > 0.05]. The extract had a significant myorelaxant effect both in the absence and presence of propranolol, [100% (Basal tone) vs. 24.23 ± 2.42% (3.0 mg/ml ARCE); p < 0.001] and [100% (Basal tone) vs. 18.49 ± 1.63% (Propranolol + 3.0 mg/ml ARCE); p < 0.001]. Propranolol caused a significant decrease in basal tone of jejunal contraction, [100% (Basal tone) vs. 74.85 ± 10.26% (Propranolol); p < 0.05]. These results are summarized in Figures 8 and 9.

DISCUSSION

The rate and force of the isolated rabbit jejunum spontaneous contractions remained constant with time in this study. This is due to periodic membrane depolarization and repolarization by the slow waves generated by the interstitial cells of Cajal (Kito et al., 2015).

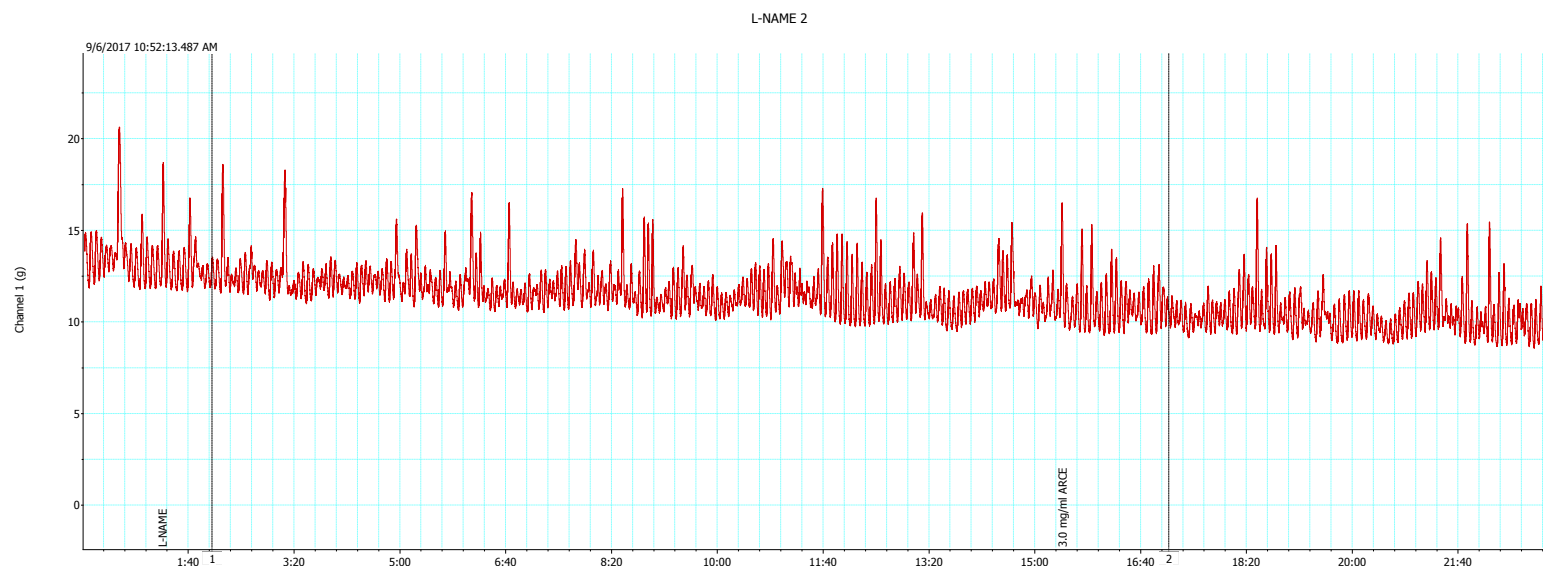


Figure 5. Powerlab tracing showing the effect of LG-NAME on myorelaxant effect of 3.0 mg/ml of the extract.

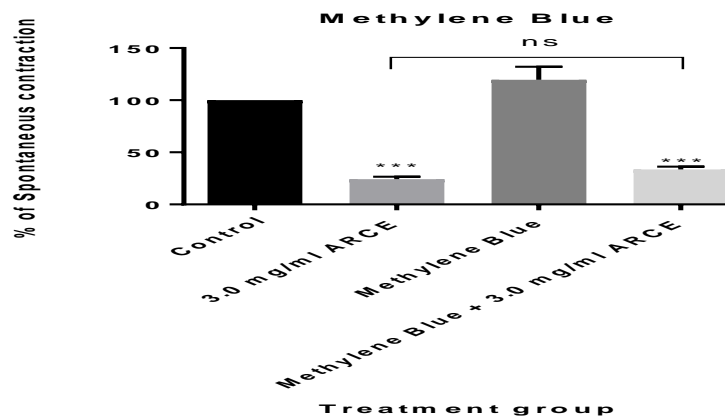


Figure 6. Bar graph showing effect of methylene blue on myorelaxant effect of ARCE. They are shown as mean \pm SEM, (n= 5), ns –not significant, *p<0.05, **p<0.01, ***p<0.001 vs. control.

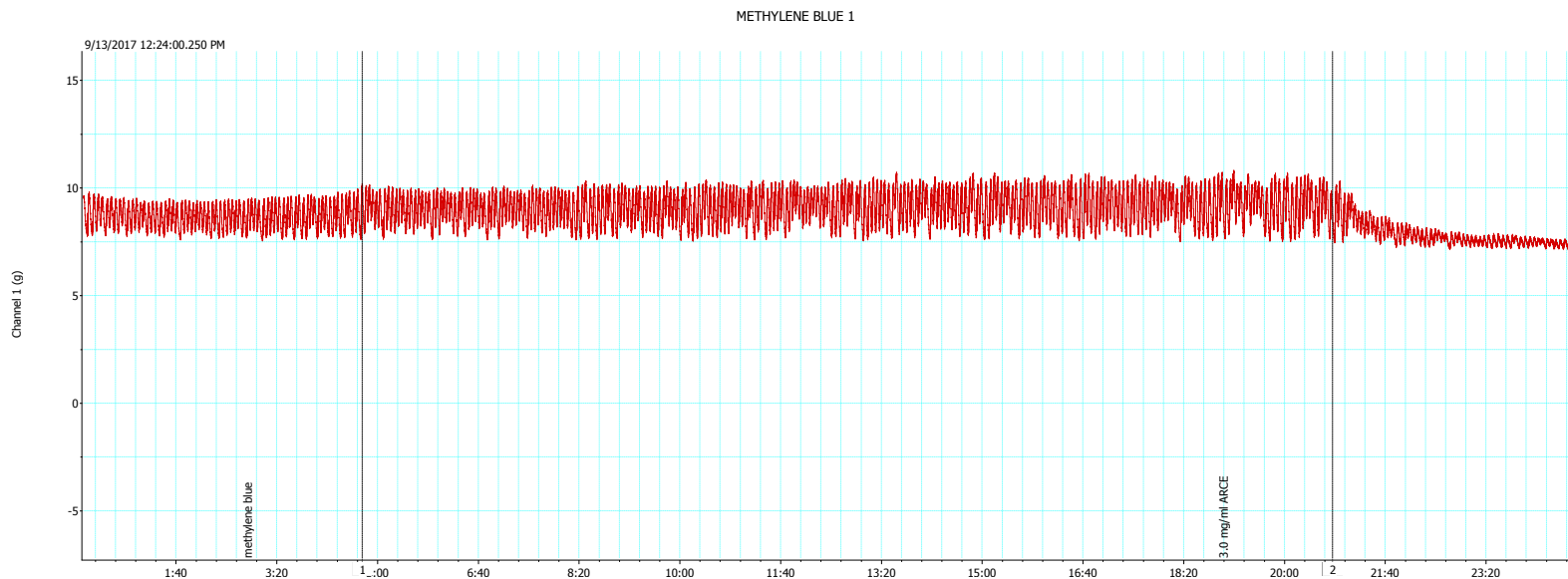


Figure 7. Powerlab tracing showing the effect of methylene blue on myorelaxant effect of the extract.

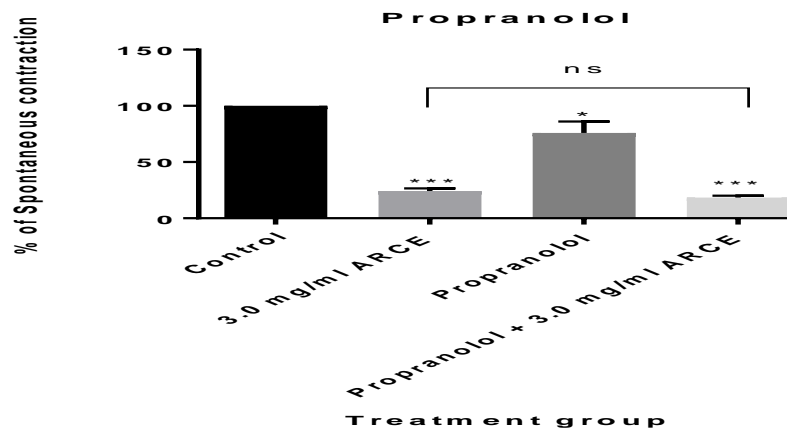


Figure 8. Bar graph showing the effect of propranolol on myorelaxant effect of ARCE. They are shown as mean \pm SEM, (n= 5), ns-not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control.

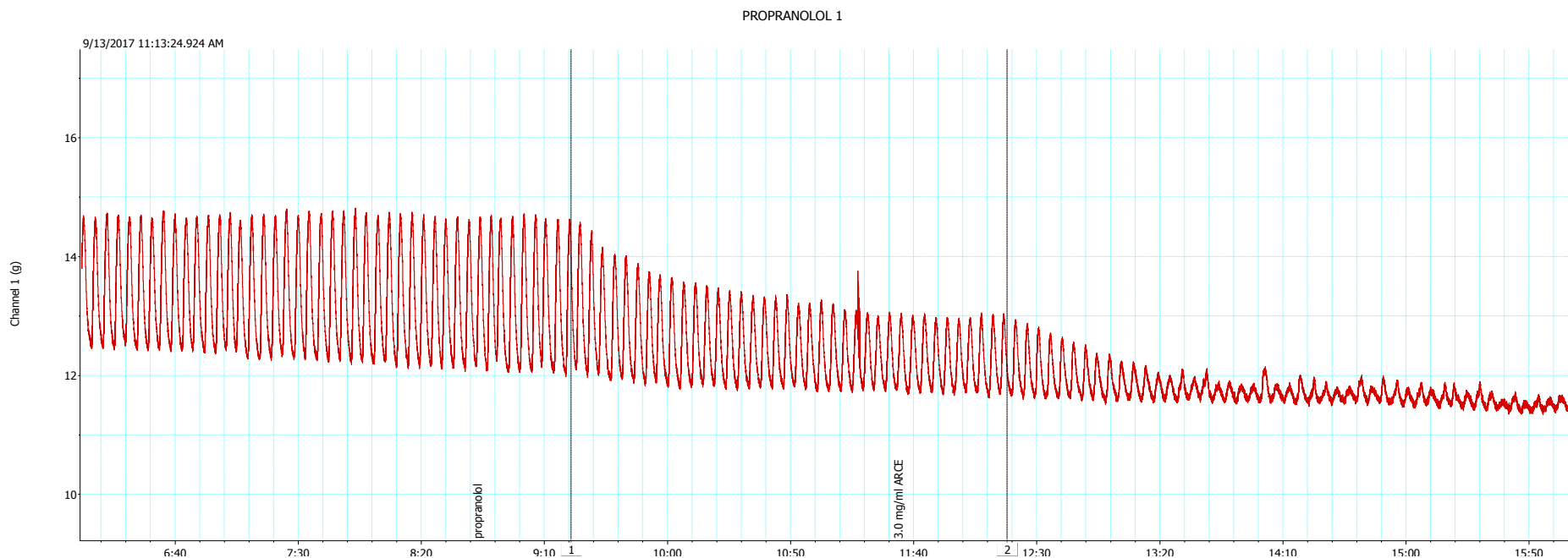


Figure 9. Powerlab tracing showing the effect of propranolol on myorelaxant effect of 0.3 mg/ml of the extract.

The extract had no significant effect on the rate of spontaneous rabbit jejunal contractions. These findings are similar to those of *Bidens Biternata* (Lour.) Merr and Sheriff (Kinuthia et al., 2016). This finding suggests that *C. edulis* (Forssk.) Vahl extract did not modify the frequency of pacemaker cells (Devi et al., 2011).

C. edulis (Forssk.) Vahl in a dose-dependent manner significantly inhibited the force of spontaneous rabbit jejunal contraction. These findings are similar to those in published literature. In deed several plants such as *Matricaria recutita* (Yazdi et al., 2017), *Salsola imbricata* (Aslam and Janbaz, 2017) and *Viscum album* (Khan et al.,

2016) also showed a dose-dependent inhibition of the force of isolated rabbit jejunum contractions. This suggests that the inhibitory effect of the extract on the force of spontaneous rabbit jejunal contraction may have been due to its interference with calcium release or influx or by the stimulation of release of inhibitory transmitter substances.

The effect of the extract on the force and rate of spontaneous rabbit jejunal contractions were partially reversible on washing out. This is advantageous since an irreversible effect is likely to cause paralytic ileus (Alkizim et al., 2012). Nitric oxide is the major non adrenergic non cholinergic Inhibitory transmitter in the gastrointestinal tract

(Sanders et al., 2012). It is synthesized by nitric oxide synthase and it activates soluble guanylyl cyclase (sGC) which activates cGMP, and subsequently activating PKG (Sanders and Ward, 2019). LG-Nitro arginine methyl ester (a non-selective nitric oxide synthase inhibitor) but not methylene blue (a soluble guanylyl cyclase inhibitor) significantly lowered the inhibitory effect of the extract. This suggests that the extract stimulates nitric oxide release by synthase but not nitric oxide dependent soluble guanylyl cyclase pathway. Indeed, Nitric oxide-sensitive guanylyl cyclase has been shown to be dispensable for nitregic signaling and gut motility in intestinal

smooth muscle (Groneberg et al., 2011). In this study, L-Nitroarginine methyl ester and methylene blue increased the basal tone of spontaneous jejunal contraction; these findings are similar to those (Ragy and Elbassuoni, 2012) in which the same doses of the above blockers also caused an increase in the basal tone of rabbit jejunal contraction.

The β adrenergic receptors mediate their inhibitory effect on rabbit jejunum through cAMP and PKA (Cavalcante-Silva et al., 2016). Propranolol (a non-selective β adrenergic blocker) had no significant effect on the effect of the extract. This suggests that the myorelaxant effect of the extract is not mediated through activation of β adrenergic receptors. In this study, propranolol amplified the effect of the extract; this finding is similar to that of Naseri and Heidari in which propranolol amplified the myorelaxant effect of *Anethum graveolens* fruit extract on rat ileum (Naseri and Heidari, 2007).

Conclusion

The findings of this study show that *C. edulis* (Forssk.) Vahl has a significant myorelaxant effect on isolated rabbit jejunum. This effect appears to be mediated through stimulation on nitric oxide synthase. This supports its use by some African communities to manage hyperactive gut disorders such as abdominal colic and diarrhea.

Recommendations

Further studies are recommended in order to determine the extracts and other possible mechanisms of action as well as to identify the active compounds responsible for its effect.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATION

ARCE, Aqueous root bark extract of *Carissa edulis*.

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