



IN VITRO PAPAVERINE - LIKE ACTIVITY OF ALOE VERA GEL ON RABBIT ILEUM

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ABSTRACT

According to the pharmacological effect, Aloe vera has been reported to have various therapeutic properties, specifically to promote wound, burn and frost-bite healing, in addition to have anti-inflammatory, antifungal, hypoglycemic and gastro protective properties. In spite of all the advantages of this plant, its use is mainly limited to topical forms, giving no importance to other therapeutic uses. However no studies have been undertaken with regard to the antispasmodic activity of the leafy exudates. Accordingly: the aim of this study is *in vitro* to demonstrate for the first time the Papaverine like effect of Aloe vera on isolated rabbit ileum. This effect is due to the potential of using the whole leaf or inner fillet gel liquid preparation.

Keywords: Aloe vera, Anti-inflammatory, Antispasmodic, Papaverine, Rabbit ileum.

INTRODUCTION

Aloe vera or (Barbadensis) plant is considered to be out of Liliaceae and Aloeeaceae family which have numerous different species.¹ The leaf can be divided into two major parts, namely the outer green rind, including the vascular bundles, and the inner colorless parenchyma containing aloe gel which has been used since early times for a lot of curative purposes. This gel should be distinguished clearly from the bitter yellow exudates originating from the bundle sheath cells, which is used for its purgative effects.

The bitter yellow exudates contains Anthraquinones such as Aloe-emodin, aloetic-acid, anthranol, aloin A and B (or collectively known as barbaloin), isobarbaloin, emodin, ester of cinnamic acid, which are mainly used for their cathartic effects.^{2,3} In versus parenchyma tissue or pulp has been shown to contain proteins (Lectins, lectin-like substance), Miscellaneous organic compounds and lipids like Arachidonic acid, γ -linolenic acid, steroids (campesterol, holesterol, β -sitosterol), triglycerides, triterpenoid, gibberillin, lignins, potassium sorbate, salicylic acid, uric acid. Non-essential and essential amino acids: (Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine and valine. Vitamins (B1, B2, B6, C, β -carotene, choline, folic acid, α -tocopherol), enzymes (Alkaline phosphatase, amylase, carboxypeptidase, catalase, cyclooxygenase, cyclooxygenase, lipase, oxidase, phosphoenolpyruvate carboxylase and superoxide dismutase), inorganic compounds (Calcium, chlorine, chromium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium, zinc), small organic compounds and different carbohydrates like Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan, arabinogalactan, galactoglucoarabinomannan, pectic substance, xylan, cellulose. In addition to Polysaccharides

which make up most of the dry matter of the parenchyma.⁴⁻⁷

MATERIALS AND METHODS

Material

All drugs and chemicals used were of analytical grade. Papaverine is pharmaceutical product - NaCl – KCl extra pure from SDFCL India - CaCl₂ fused Qualikems fine chemicals Pvt Ltd - MgCl₂·6H₂O VWR international Ltd - NaH₂PO₄ POCLT SA poland - NaHCO₃ from HIMEDIA - D-glucose, and Barium chloride 2H₂O Merck- Kymograph : universal model from Harvard apparatus. Aloe vera were collected from AIU (Arab International University) botanical garden - Tyrode solution : (8 g of NaCl, 0.2 g of KCl, 0.2 g of CaCl₂, 0.1 g of MgCl₂ 6 hydrate, 0.05 g of NaH₂PO₄, 1 g of NaHCO₃, 1 g of d-glucose and distilled water to make 1000 mL).

Method

Plant material

5 to 6 Mature, healthy and fresh leaves of Aloe vera having a length approximately 2-3 feet were taken and washed with 40 ml of distilled water, dissected longitudinally and the colorless parenchymatous tissue was scraped out, liquefied in a food – blender and filtered to remove the fibers, then the result about 150 ml gel is used freshly.

Animal Tissue preparation

Male rabbits weighing 3.5 kg were sacrificed by a blow to the base of the skull. After fast excessive bleeding, 2 cm pieces of the ileum were removed.

From the end of ileum, piece was bound to tissue mounting hook of the holder and aeration tube and the other end was fixed by Plasticin on lever in which recording needle is fixed. The tissue was immersed in the 65-mL organ bath containing Tyrode solution at 37°C



which was aerated with bubbled air.⁸ The contraction movement was recorded on recording paper of kymograph cylinder.

Procedure

1. Effect of only Aloe vera gel on normal contractility of rabbit ileum

After stabilizing the ileum contraction, the base line of its contractility has been registered. With attention to wash after each step three times or more until returning back to base line, then increased volume of Aloe vera gel were added (as shown in table 1) in order to notice its effect on the ileum normal contractility. Results were provided in figure 1.

Table 1: Effect of Aloe vera on normal contractility

Aloe vera gel (ml)	Tyrode solution (ml)	% Inhibition for the normal contraction
0	65	0%
0.5	64.5	35.5%
1	64	47.2%
2	63	45.5%
3	62	45.5%
10	55	54.8%
15	50	64.51%
20	45	100%

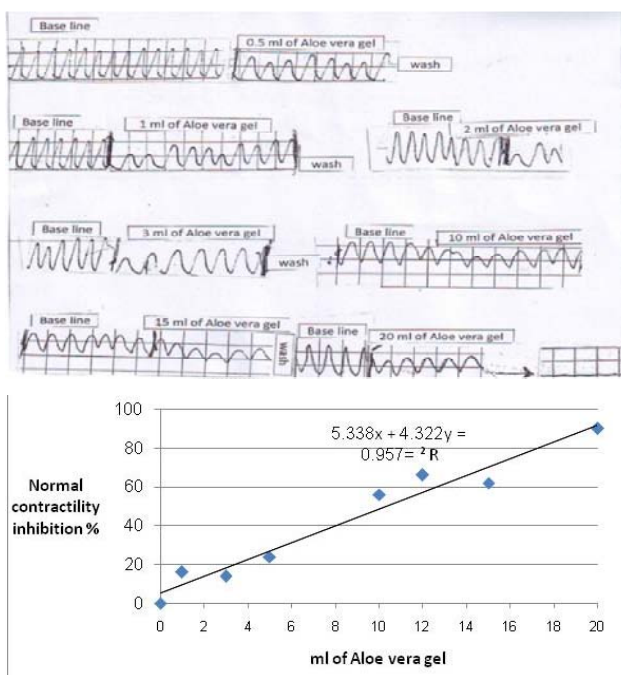


Figure 1: Effect of Aloe vera gel on normal contractility of rabbit ileum

2. Effect of barium chloride on normal contractility of rabbit ileum

The second step was to demonstrate the effective contracting dose of 1ml at 5 mg/ml, 1ml at 10 mg/ml, and 1ml at 20 mg/ml as shown in table 2 and in figure 2. The effective dose was 1 ml of barium chloride at 20 mg/ml which elicited the maximal spasmogenic effect. This dose

had been adapted to demonstrate the Aloe Vera relaxant activity.

Table 2: Effect of barium chloride on normal contractility

Barium chloride 5mg/ml	Barium chloride 10mg/ml	Barium chloride 20mg/ml	Tyrode solution (ml)	Contractility Amplitude (cm)
1	0	0	64	1.4
0	1	0	64	4
0	0	1	64	5

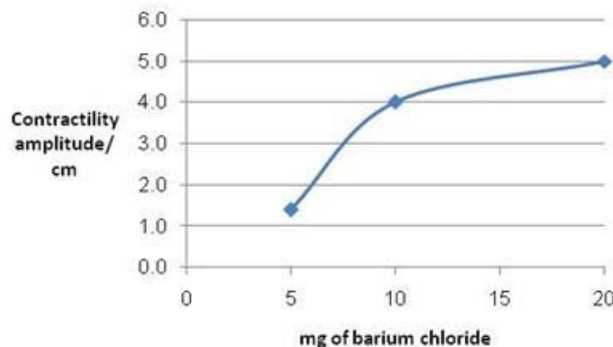


Figure 2: Effect of barium chloride on normal contractility of rabbit ileum

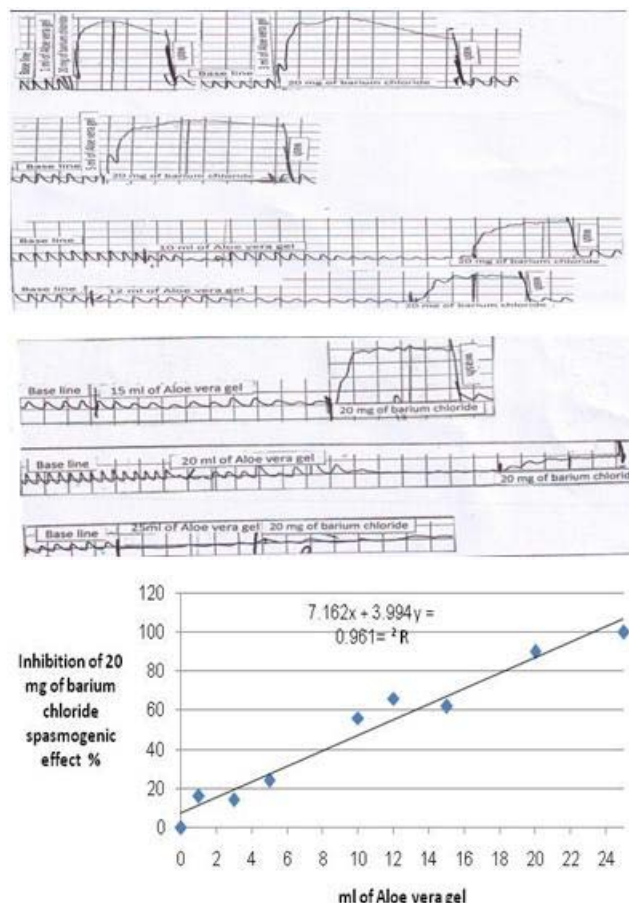


Figure 3: Aloe vera gel inhibitory effect on contraction induced by barium chloride in rabbit ileum

3. Effect of Aloe vera gel on contraction induced by barium chloride on rabbit ileum

The third step was to demonstrate antispasmodic activity of Aloe vera gel on the contracting effect of barium

chloride adapted dose at 20mg/ml (as shown in table 3 and figure 3).

Table 3: Effect of Aloe vera gel on contraction induced by Barium chloride

Aloe vera gel (ml)	Barium chloride adapted dose (ml)	Tyrode Solution (ml)	Adapted dose of Barium chloride for inhibition
1	1	63	16%
3	1	61	14%
5	1	59	24%
10	1	54	56%
12	1	52	66%
15	1	49	62%
20	1	44	90%
25	1	39	100%

4. Papaverine effect on contraction induced by barium chloride in rabbit ileum

Since Papaverine is considered as an inhibitor of barium chloride, the last step (as shown in table 4 and in figure 4) was to determine the inhibitory dose of Papaverine which oppose the adapted dose of barium chloride.

Table 4: Effect of Papaverine on contraction induced by adapted dose of Barium chloride

Barium chloride adapted dose (ml)	Tyrode solution (ml)	Papaverine (mg)	Adapted dose of Barium chloride for inhibition
1	63	1 ml of 1mg/ml	82%
1	62.5	1.5 ml of 1mg/ml	86%
1	62	2 ml of 1mg/ml	90%
1	63.5	0.5 ml of 5mg/ml	100%

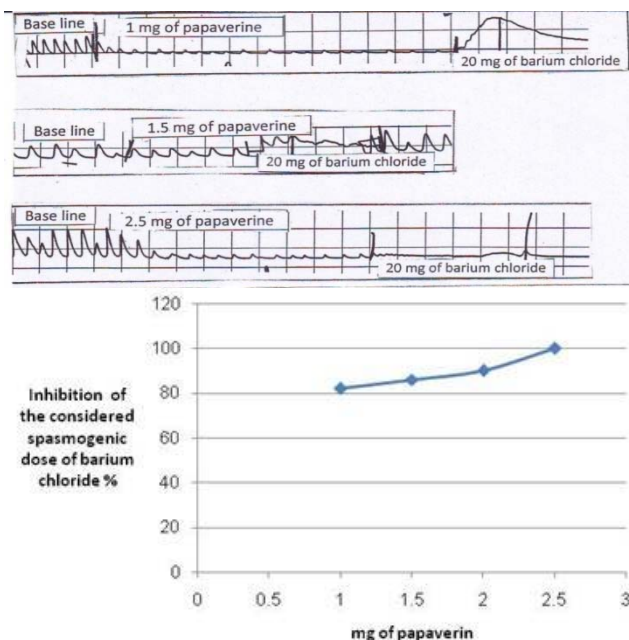


Figure 4: Papaverine inhibitory effect on contraction induced by barium chloride in rabbit ileum

RESULTS

Increased progressive volume of Aloe vera gel has achieved progressive abolishment of myogenic

spontaneous contraction of rabbit ileum which were completely abolished after adding 20ml of aloe gel. The inhibition was significant on the tone and amplitude of the contraction, whereas the inhibitory dose of Papaverine was 0.5ml of 5 mg/ml, which equals 2.5mg Papaverine. Returning back to Aloe vera effect that opposes spasmogenic activity of barium chloride, 25ml of Aloe gel has similar effect of 2.5mg of Papaverine. Repeating assay three times was producing same results.

DISCUSSION

From the plot of Aloe vera increasing volume versus the inhibition of normal contractility in Fig (1) we notice a liner relationship, with regression coefficient equal to 0.9574.

Also a liner relationship was found by plotting Aloe vera increasing volume versus the inhibition of the contraction induced with barium chloride in fig (3) with regression coefficient equal to 0.9611.

A statistical analysis using Khi square test proved that the two lines in fig (1) and fig (3) are imposable ($p = 0.0014 < 0.05$), which indicates that the relaxant activity of Aloe vera gel overcomes the spasmogenic effect of barium chloride on isolated rabbit ileum.

Our results demonstrate, for the first time, the muscle relaxant and Papaverine like effect of Aloe vera gel on spontaneous and induced contractions in rabbit ileum.

It is known that contraction of smooth muscle begins with an increase in the cytosolic concentration of Ca^{2+} , with the extra Ca^{2+} coming either from the extracellular medium or from the sarcoplasmic reticulum⁹. The inhibitory effect of the Aloe vera gel on spontaneous movements of the ileum may be due to interference either with release of calcium ions from the sarcoplasmic reticulum or with Ca^{2+} influx through voltage dependant Ca^{2+} channels. In fact the smooth muscle of urinary bladders, ileum or vas deferens (type B) is capable of generating action potential spontaneously, and thus produce spontaneous contraction¹⁰. On the other hand, Intracellular Ca^{2+} can be released by $BaCl_2$ in intestinal smooth muscle and is responsible for $BaCl_2$ -stimulated contraction^{11,12}. $BaCl_2$ can also induces intestinal secretion by releasing Ca^{2+} from intracellular stores which then combines with calmodulin to stimulate the secretory process.¹³

Papaverine is a non selective smooth muscle relaxant and several mechanisms of action were proposed to explain its action; 1- an intracellular cAMP or cGMP¹⁴ accumulation by inhibition effect of phosphodiesterase¹⁵. 2- inhibition of mitochondrial respiration. 3- effect on calcium movement. Papaverine can also inhibit contracting activity of high concentration of K^+ ion^{16,17}. On the other hand, Papaverine is able to produce significative influence on the opiate withdrawal *in vitro* and Papaverine was able to exert its effect both at μ and k opioid agonists.¹⁸

CONCLUSION

Our practical study suggests that action of Aloe vera may be due to one of Papaverine different mode of action. Supplementary assay in different medias with or without calcium, high concentration in K⁺ ion and assay of cAMP and cGMP can contribute to elucidate Aloe vera gel mode of action.

Isolation of the component responsible of this action can also conduct to discover a new muscle relaxant drug with some applications in cardio-vascular, bronchial and gastro-intestinal domains.

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