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Intestinal absorption, anti diarrheic activity of freeze dried aqueous extract from *Rhizophora mangle* L. Cytoprotective activity of polyphenolic compounds fractions on experimental gastric ulceration.

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ABSTRACT

The effect of the freeze aqueous extract from red mangrove bark on intestinal absorption was studied *in vitro* e *in situ* models in rats. Anti diarrheic activity of this extract was evaluated using a model of ricin oil in rats. The polyphenolic fractions were separated from the aqueous extract and its cytoprotective activity were studied on gastric ulceration induced by ethanol plus hydrochloric acid in rats. The freeze - dried aqueous extract from red mangrove bark was used in both models employed to determinate the intestinal absorption. The freeze dried extract of *R. mangle* shown a high absorption. It was superiority at 95% *in vitro* study and the constant apparent of absorption's rate was 2, 03 ± 0, 77. It was shown a high anti diarrheic effect of *R. mangle* in experimental model with ricin oil. The low molecular weigh polyphenolic fraction showed the highest level of gastric protection. Not additive effect was obtained by comparison between low and high molecular weigh polyphenols in this aqueous extract. The highest gastric protection was obtained by oral treatment with total of freeze aqueous extract at 500 mg/ kg body weight (b.w.). Probably the cytoprotective activity was resulted of other compounds presents in this extract joint at polyphenol substances.

Keywords: *Rhizophora mangle* L; Red mangrove; Rhizophoraceae; *in vitro e in situ* intestinal absorption models; cytoprotection; polyphenolic fractions.

INTRODUCTION

Tannins are major polyphenols in our diets and are characterized by their capacity to form non-specific complexes with proteins. Two types of tannins, hydrolyzable tannins and proanthocyanidins (PAs), can be distinguished according to their chemical structure. Hydrolyzable tannins are esters of a polyol, usually glucose, and a phenolic acid which can be gallic acid in gallotannins, or a more complex phenolic acid such as hexahydroxydiphenic acid in ellagitannins (1–2).

The better known property of tannins is their capacity to form complexes with proteins. Tannins are retained on the collagen when hide is transformed into leather. Interaction of tannins with salivary proteins or with mucous secretions in the oral cavity explains the pucker sensation felt in the mouth when tannin-rich foods and beverages are consumed. Tannin-protein complexation is explained by the formation of hydrogen bonds between phenolic residues and polar groups in the protein and by hydrophobic interactions involving the same phenolic residues and less polar groups in the protein such as prolyl

residues. Depending on the relative concentrations of both ligands, this phenomenon can be seen as a wrapping of the polar and soluble protein by a number of tannin molecules or as the formation of a molecular network through the simultaneous binding of each polyfunctional tannin molecule to several proteins. Tannins thus often cause precipitation of the protein or inhibition of enzymes. This property of tannins, commonly referred to as astringency, may have nutritional significance.

Tannins inhibit digestive enzymes *in vitro* but have limited effect *in vivo* unless they are ingested in excessive amounts. This is due to the existence of efficient adaptation mechanisms such as the induction of the secretion in the gut of bile acids and of proline-rich salivary proteins which alleviate their capacity to bind digestive enzymes or dietary proteins. Dietary tannins were also shown in various animals to stimulate the secretion of digestive enzymes. It is thus unlikely that the levels of PAs commonly ingested by humans affect digestion. Due to the limited absorption through the gut barrier of the PAs of high polymerization degree (see infra) and to the relatively weak affinity of Pas of low polymerization degree for proteins, it is also unlikely that the non-specific binding of Pas to proteins will influence their biological properties in inner tissues (1–3).

Increasing evidence for the possible effects of plant polyphenols on human health have been obtained in *in vitro* and *in vivo* systems. However, data on their absorption from the gastrointestinal tract are still scarce and, often, contradictory, although it is likely that the different biological effects of polyphenols are related to their absorption and bioavailability for target organs and tissues (4).

Rhizophora mangle L. (Rhizophoraceae), the red mangrove, has long been known as a traditional medicine in different Caribbean countries. Its bark has been used as astringent, antiseptic and haemostatic (5).

By this property as astringent in Cuba this plant is using in diarrhea disease in aqueous or in hydroalcoholic extract. In previous works, we report the cytoprotective effect of freeze - dried aqueous extract from red mangrove bark on gastric ulceration induced by ethanol – hydrochloric acid in rats and the presence of condensed and hydrolysable polyphenols in this extract (6). Also, we report the antiulcerogénico effect by other action's mechanism as antisecretor, inhibitor of depleting of PGE2 in the gastric mucous and antioxidant (7–8).

In preview studied performance with this extract, it had shown the presence polyphenolic structures (54.78%) and other structural components (45.22%). Polymeric tannins were the major polyphenolic component (80%) and 20% were hydrolysable tannins. Epicatechin, catechin, chlorogenic acid, gallic acid and ellagic acid were monomeric structures determined in this extract. Phytosterols (0.0285%): stigmasterol, β-sitosterol and likewise campesterol were present too (9). This extract present semivolatil compounds (10) fat acids and sugars (11), for also it represent a complex mixture of secondary metabolites.

The objective of the present study was to test the effect from freeze – dried aqueous red mangrove extract on two models of intestinal absorption *in vitro* e *in situ* in rats. The activity of this extract was to test on diarrhea produced by ricin oil in rats. Other proposal of the present work was to evaluate the cytoprotective activity of the major compounds, polyphenols and the possible synergic activity of low and high molecular weigh polyphenolic fractions.

MATERIAL AND METHODS

Plant material

Rhizophora mangle L. was collected in La Habana, Cuba, in November of 2006. The botanical identify of the plants was authenticated and a voucher specimen has been deposited (no. 6539, HAJB) in the Herbarium of National Botanical Garden, La Havana, Cuba.

Preparation of the extract

Fresh bark with distilled water in 1: 7.5 proportions was boiled for 20 minutes in lab reactor with 2 Liter of capacity. The plant material was separated by centrifugation and the aqueous extract was concentrated and freeze dried by Spray drier to preserve it.

Animals stock

This study was carried out in accordance with the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the National Institute of Health.

Male Sprague – Dawley rats weighing 200 – 220 g were obtained from the National Center for Lab Animals Production, CENPALAB, Cuba. Animals were fed on conventional diets and water ad libitum and they were maintained under standard conditions of humidity, temperature and light (12 h light: 12 h dark cycle).

The rats were randomly assigned to control and different treatments groups.

Intestinal absorption in vitro model

In the Experimental models of intestinal absorption in vitro were used small intestine segments from three animals.

Table 1. Doses of extract from *R. mangle* in the small intestine of rats.

Part of small intestine	Segment (cm.)	Doses(mg/Kg b.w.)
duodenum	6	250
	12	250
	18	250
JEJUNE	24	250
	30	500
	36	500
	42	500
	48	500
	54	250
ILEUM	60	250
	66	250

The administration of aqueous freeze dried of *R. mangle* was made as it was representing in table 1. For obtain the different doses the extract was weigh considered the weigh body of three rats and enough quantity for represent 250 and 500 mg/Kg body weigh and its were diluted in distillated water to obtain the doses. In each segment of small intestine were insert 0,2 ml of each solution, equivalent at each doses.

The selections of these doses were determinate by therapeutically answer as antiulcer effect.

The concentration of tannin insert in each segment of small intestine was 30,74 mg of tannins in 250 mg/ Kg b.w. (39,68 % of tannins in the freeze dried extract by Spray – Drier). The tannin determination was made by precipitation of protein methods (12).

In situ study to intestinal absorption of freeze dried extract of *R. mangle* in total segment of small intestine

The absorption model was to asses as a tool for describing passive absorption mechanism and to go deeply into the structures of the physiological absorbing barriers.

The methodology consist of establishing correlations between absorption rate constants and lipophilicity for a several homologous series of xenobiotics

Figure 1 shows the closed loop perfusion technique that was used to obtain intestinal absorption rate constants in rats. The Model considers passive intestinal absorption as the result of two simultaneous processes: diffusion

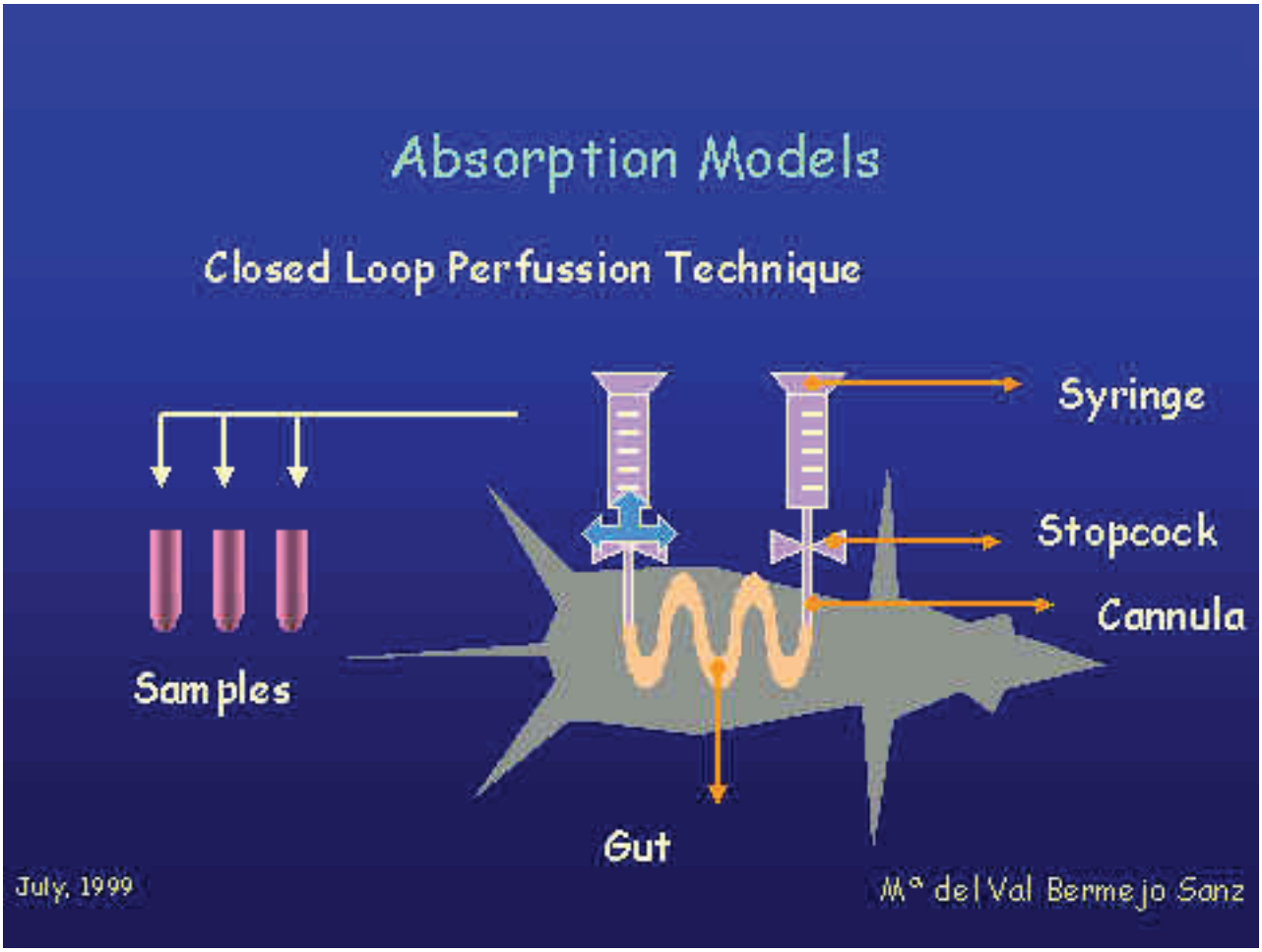

Figure 1. It shows the closed loop perfusion technique that was used to obtain intestinal absorption rate constants in rats.

through aqueous channels (porous or tight junctions) and diffusion through the lipophilic membrane. The absorption rate constant is the sum of the two parallel processes (13).

The Anti diarrheic effect of *R. mangle*. Accumulation of fluid in small intestine in rats

In this experiment were used Wistar rats weighing 150 – 180 g, with 12 hours faster. Three groups were made with 5 rats by group. First group was administered with 1 ml of vehicle after 30 minutes was administer 2 ml of ricin oil by oral administration. Groups 2 and 3 were treatment with 125 and 250 mg/Kg b.w. of extract from R. mangle (1 ml), after were administered 2 ml of ricin oil. After 30 minutes the rats were sacrificed. The small intestine were isolated by pyloric esfinger to ileocecal valvule and it were weight with intraluminal content, after the content of intestine were separated and it were weight another. The longitudes of intestines were measured. Intraluminal (enteropooling) fluid was calculated in mg/cm with the following $(W_1 - W_2)/L$, where W_1 is the weigh of small intestine, W_2 is the weigh of empty small intestine with intraluminal content and L is the intestine longitude.

Cytoprotective activity of polyphenolic compounds fractions on experimental gastric ulceration

The evaluation of gastric protection from polyphenolic fractions was made two experiments. In the first case we used the following doses, negative control with distilled water, one group with 500 mg/kg b.w. of freeze dried aqueous extract from *R. mangle*, one group with 100 mg/kg of high molecular weight polyphenols fraction, one group with 100 mg/kg of low molecular weight polyphenols fraction and one group using ranitidine (100 mg/kg b.w.) as positive control. In the second experiment we used the following doses, Distillated water as negative control, two groups treated by 200 and 400 mg/Kg b.w. from high molecular weigh polyphenols (HMWP); two groups treated by 200 and 400 mg/Kg b.w. from low molecular weigh polyphenols (LMWP); one group with 200 mg/Kg b.w. of HMWP and 60 mg/Kg b.w. of LMWP and one group treated with 500 mg/Kg b.w. of *Rhizophora mangle* L. (freeze – dried). In alls cases were given orally (gavage).

a- Gastric protection activity from polyphenolic compounds fractions.

The high and low molecular weight polyphenolic fractions separation of the freeze dried aqueous extract from *Rhizophora mangle* L bark were employed the scheme described in the Figure 2. These fractions were resolved in water by animal administration.

b- Gastric lesions induced by necrotizing agent

Animals in testing groups were given 1 ml of necrotic agent (60 ml ethanol + 1.7 ml hydrochloric acid + 38.3 ml water). Freeze dried *R. mangle* extract, polyphenolic

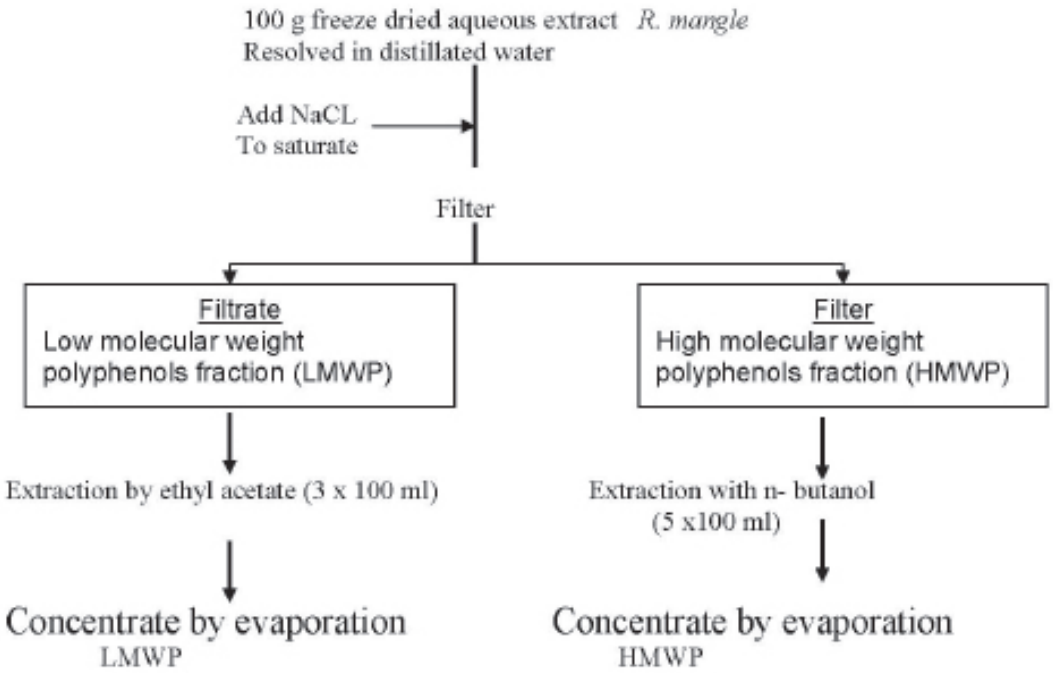

Figure 2. Low and High molecular weight polyphenols fractionation from an aqueous extract *Rhizophora mangle* L.

fractions and ranitidine were given 30 min before the necrotic agent. Animals were killed by cervical dislocation 1 h after treatment with the ulcerogenic agent. The stomach of each animal was excised and opened along the greater curvature. After washing with the normal saline, the gastric lesions were quantified using a binocular magnifier, the sum of length (in mm) to alls lesions in each animal was used as a lesion index. The number of erosions per stomach was assessed for the severity according to, (1) absence of lesion, vasodilatation or up to three pinpoint ulcers; (2) more than three pinpoint ulcers; (3) from one to five small ulcers (< 2 mm); (4) more than five small ulcers (< 2 mm); (5) one or more giant ulcers (≥ 2 mm).

c- Statistical analysis

The data are expressed as means ± S.E.M. (standard deviation of the means) Student's t-test was used for statistical analysis, P values < 0.05 were considered significant. Non – parametric data were analyzed by Wilcoxon Score test.

RESULTS

Intestinal absorption in vitro model

In the table 2 and Figure 3 it were shown a doses – answer results of the different doses employed, for also

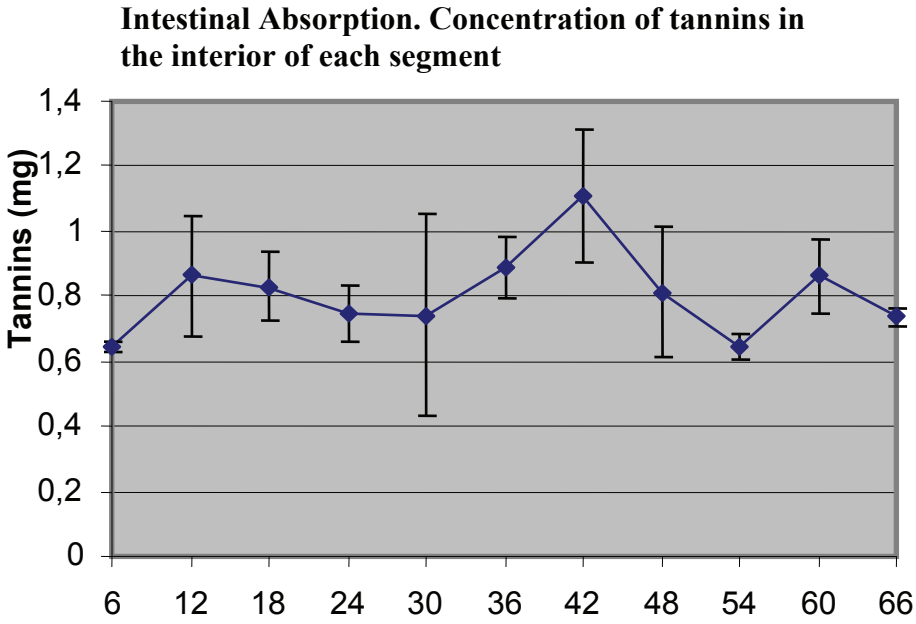

Figure 3. Concentration of residual's tannins in the interior of segment after the incubation of each segment during 5 minutes.

Table 2. Quantity of residual and absorb tannins by each intestinal segment to insert extract of *R. mangle* in buffer.

Part of intestine	Segment	Doses	Animal 1 Content of residual tannins (mg)	Animal 2 Content of residual tannins (mg)	Animal 3 Content of residual tannins (mg)	Media ± S.D. Content of residual tannins (mg)	Content of residual tannins (%).	Quantity absorb of tannins (%).
duodenum	6	250	0.654	0.626	0.648	0.6427 ± 0.014	2.091	97.99
	12	250	0.848	1.057	0.687	0.864 ± 0.185	2.811	97.19
	18	250	0.804	0.734	0.946	0.828 ± 0.108	2.693	97.30
	24	250	0.676	0.718	0.846	0.747 ± 0.088	2.430	97.57
jejune	30	500	0.657	0.481	1.090	0.743 ± 0.313	1.206	98.79
	36	500	0.837	0.829	1.002	0.889 ± 0.098	1.443	98.56
	42	500	0.996	0.982	1.349	1.109 ± 0.208	1.801	98.20
	48	500	0.626	0.795	1.021	0.814 ± 0.198	1.322	98.68
ILEUM	54	250	0.645	0.604	0.687	0.645 ± 0.041	2.098	97.90
	60	250	0.840	0.987	0.759	0.862 ± 0.116	2.804	97.20
	66	250	0.718	-	0.754	0.736 ± 0.025	2.394	97.61

to increases the doses increase the tannins absorption and the tannin's content residual is low. It was demonstrated that the content of residual tannins in 250 mg/kg b.w. is similar in duodenum, jejune and ileum. These results suggest that the absorption of tannins not depend of intestinal segment. The absorption in gut of tannins in the freeze dried extract of *R. mangle* is superiority at 97 %. These results suggest that the polyphenols present in the extract are polyphenols with high absorption, for also these compounds are structures with relative low molecular weigh structure (monomer, dimer or trimer) and don't present polymeric compounds.

In situ study to intestinal absorption of freeze dried extract of *R. mangle* in total segment of small intestine

The parameter representative of absorption process using was the constant apparent of absorption rate (k_{ap}) because it represents an index of the absorption rate intrinsic off solute. This constant was determinate within recirculation evading the aqueous layer of adjacent diffusion to the membrane.

It in situ method was choice because it plows considered in general reproducible with it lives reliable the absorption process whom occur in *vivo*. The perfumed volume stay all time to testing (30 Minutes) in contact with intestinal mucosa, for also the absorption yield is high than that the obtain by other techniques, where the contact fluid – mucosa is intermittent. In this method is it maintains blood flow and tisular integrity similar at in vivo models.

Table 3. Concentration of remanent tannins (mg/ml) in intestinal lumen at different time to sample taking alter the perfusion of extract from R. mangle at equivalent concentration to doses of 250 mg/Kg b.w. (0.8285 mg/ml total tannins).

Time(min)	Extract 250 mg/Kg m.c. C_0= 0.8285 mg/ml Remanent Concentrations in the lumen = C= mg/ml Experimental Animal					
	1	2	3	4	5	6
5	0.5528	0.1744	0.2449	0.2162	0.2945	0.6389
10	0.37014	0.1379	0.2053	0.2018	0.1444	0.5267
15	0.3023	0.1366	0.1679	0.1705	0.1340	0.5254
20	0.2632	0.1314	0.1666	0.1575	0.1131	0.4850
25	0.241	0.1275	0.1601	0.1366	0.0792	0.3988
30	0.1835	0.1066	0.1249	0.0909	0.074	0.1797
K_{ap} (h^{-1})	**Kap (h-1) 2,03100775**					
DE	**D.E. 0,76895328**					

$K_{ap:}$ constant of aparent rate of absorption.

In the table 3 shown the results of this experiment by freeze dried extract of R. mangle L. The constant apparent of absorption rate was 2, 03 ± 0, 77. For also, this study verify the high absorption of extract of *R. mangle* obtain in vitro.

The Anti diarrheic effect of *R. Mangle*. Accumulation of fluid in small intestine in rats

The accumulation of fluid in small intestine in rats is quick, qualitative and predictive of diarrhoea and it is useful to research agents that block this effect. The accumulation of fluid in small intestine in named "enteropooling". This accumulation is a sum of fluid secreted from blood to lumen and a portion of fluid of lumen that absorption

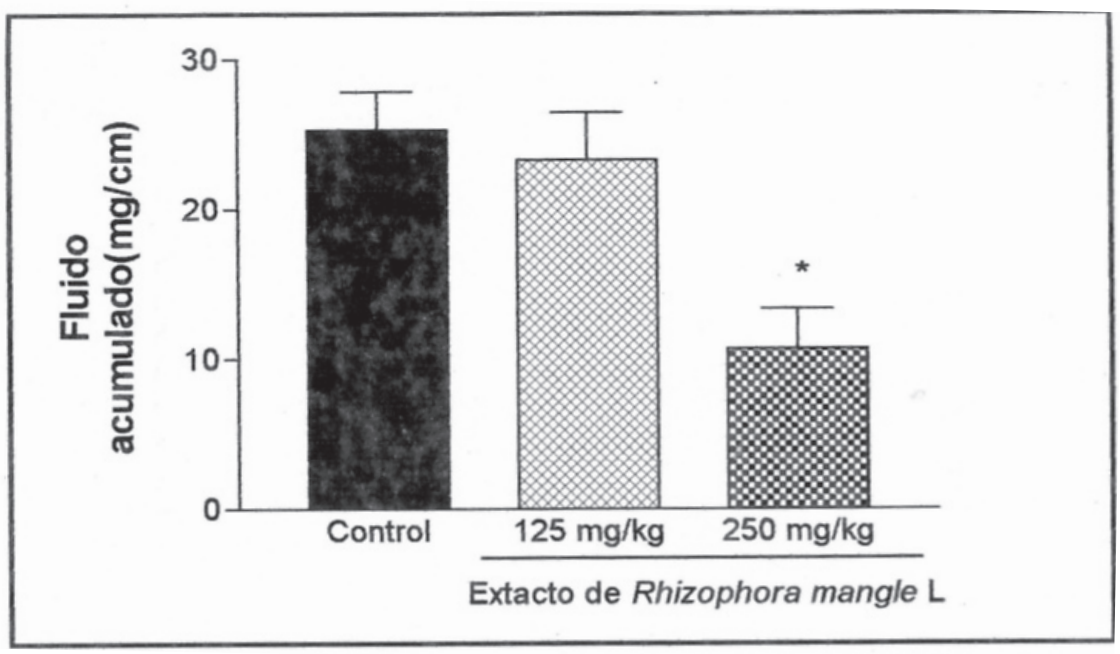

Figure 4. Accumulation of fluid induced by ricin oil. Activity of extract from *R. mangle*.

is inhibit by prostaglandin or other laxante agents as ricin oil hypertonic's solution that cause enteropooling too. Ricin oil increase a volume of intestinal fluid, this effect is maximum to 30 minutes, which it reduces the fluid accumulation. The Figure 4 showed the activity of aqueous extract of *R. mangle* on fluid accumulation, decreasing doses – dependence with significantly ($p < 0.01$) respect to control group.

Gastric protection activity from polyphenolic compounds fractions

First, we made a preliminary test of the cytoprotective effect of freeze dried aqueous extract of *Rhizophora mangle* L. in a acute ulcer model induced by ethanol plus chlorhidric acid (6). But, we not defined what compounds was responsible of this activity. This extract was different structural compounds: fatty acids, phytosterols, volatile compounds and polyphenols as major secondary metabolites.

In the first experiment with simple doses the low and high molecular weigh polyphenols fractions (100 mg/kg b.w.) we found a decreasing in lesion index respect to negative control but it was not statically significant (Table 4). The major activity was shown by total aqueous extract comparable with ranitidine. For also, the gastric protection shown by aqueous extract is possible a synergic effect of different compounds present in this extract and it effect was lost in a fractionation process.

In the study of gastric protection effect by low and high molecular weigh polyphenolic fractions using different doses level (experiment 2) was shown that low molecular weigh polyphenolic fraction had the major cytoprotector activity (Table 5). The best respond was obtained by LMWP in dose of 400 mg/kg b.w., by a significant $P < 0.05$. It was found a doses dependent decreasing in the lesion score in both cases (treatment with HMWP and LMWP). There is not additive effect between both fractions. It was confirmed in the group treated with 200 mg/kg HMWP more 60 mg/kg LMWP, where the action or LMWP was capable to decrease the lesion score.

Animal group treated by freeze dried aqueous extract of *R. mangle* (500 mg/Kg) was best answer than a group treated with a mixing of both fractions in the same proportion of calculated concentration of these substances in total extract. For also, we could to consider that the gastric protection activity of total aqueous extract was influence by other compounds present in this extract. These other compounds increase the gastric activity and this activity is lost in the fractionation process.

Table 4. Effect from low and high molecular weigh polyphenols in a simple dose on gastric protection.

Treatment	Lesion Index (Media ± E.S.M.) (mm)
Negative Control	42.4 ± 11.9
Freeze dried red mangrove 500 mg/Kg b.w.	13.2 ± 8.0**
HMWP 100 mg/Kg b.w.	37.5 ± 23.1
LMWP 100 mg /Kg b.w.	25.4 ± 17.8
Ranitidine 100 mg/Kg b.w.	10.8 ± 9.1**

P < 0.05, Wilcoxon Score, n = 5

Table 5. Low and high molecular weigh polyphenols effect on gastric protection using increasing doses.

Treatment	Lesion Score (Media ± E.S.M.) (mm)
Negative Control	4,2± 0,84
HMWP 200 mg/Kg b.w.	4± 0
HMWP 400 mg/Kg b.w.	3,4± 0,55
LMWP 200 mg /Kg b.w.	3,2± 0,84
LMWP 400 mg /Kg b.w.	1,8± 0,45**
HMWP 200mg/Kg + LMWP 60 mg /Kg b.w.	3,5± 1,00
Freeze dried red mangrove 500 mg/Kg b.w.	2,75± 0,50 *

P < 0.05, Wilcoxon Score, n = 5

DISCUSSION

Absorption is a complex problem, for also many works had been made to prediction of absorption potentiality in candidate to drugs, for this important in the biodisponibility of itself.

It is necessary ground in the knowledge of absorption mechanism and metabolism of substance clinically active. In vitro model have high complex and it not evaluated many factors in the absorption. In situ models in general reproduce with more dependability the absorption process that had been place in vivo (13–14). In the absorption process could be considered four important aspects: to step of digestive tube across esophagi, dissolution of the drugs in small particles; absorption to stomach level but principally in intestine and the drugs absorbed in blood circulation are distribute in the body and in different tissue (14).

Polyphenols – flavonoids, phenolic acids and tannins – are contained in several foods of plant origin (fruit and vegetables).Much evidence has been provided in recent years supporting a role for these compounds as protective agents against cardiovascular disease and certain forms of cancer, including breast, esophageal, gastrointestinal, lung and skin cancer. Increasing evidence for the possible effects of plant polyphenols on human health have been obtained in *in vitro* and *in vivo* systems. However, data on their absorption from the gastrointestinal tract are still scarce and, often, contradictory, although it is likely that the different biological effects of polyphenols are related to their absorption and bioavailability for target organs and tissues. Polyphenol bioavailability was evaluated using segments of the small intestine of rats. Two

polyphenolic compounds, tannic acid and catechin, were tested in our model system as representatives of high and low molecular weight polyphenols with gallic acid and flavonoid structures, respectively (15).

There have been several human studies that have investigated the absorption and bioavailability of flavonoids. Initially, absorption of flavonoids from the diet was believed negligible, given that the majority of food flavonoids are bound to glycosides. It was expected that the aglycones only could pass freely into the blood stream from the gut wall, because no enzymes are secreted in the gut that could cleave the glycosidic bonds. Recent studies, however, have demonstrated that he bioavailability of specific flavonoids is much higher than previously believed (16–17).

The bioavailability of polyphenols is reviewed, with particular focus on intestinal absorption and the influence of chemical structure (eg, glycosylation, esterification, and polymerization), food matrix, and excretion back into the intestinal lumen. Information on the role of microflora in the catabolism of polyphenols and the production of some active metabolites is presented. Mechanisms of intestinal and hepatic conjugation (methylation, glucuronidation, and sulfation), plasma transport, and elimination in bile and urine are also described (18).

Proanthocyanidins differ from most other plant polyphenols because of their polymeric nature and high molecular weight. This particular feature should limit their absorption through the gut barrier, and oligomers larger than trimers are unlikely to be absorbed in the small intestine in their native forms. In vitro experiments using single layers of Caco-2 cells as a model of absorption in the small intestine showed that only the dimers and trimers of flavanols are able to cross the intestinal epithelium. Procyanidin B2 is very poorly absorbed in rats, whereas procyanidin B3 is not absorbed. The possibility that procyanidin oligomers are hydrolyzed to mixtures of flavanol monomers and dimers in acidic conditions was suggested by

Spencer et al from in vitro experiments. However, purified procyanidin dimer B3, as well as grapeseed proanthocyanidins having a higher degree of polymerization, are not degraded to more readily absorbable monomers in rats. The stability of proanthocyanidins was investigated in humans by regular analysis of gastric juice sampled with a gastric probe after ingestion of a proanthocyanidin-rich cocoa beverage. This study confirmed that proanthocyanidins are not degraded in the acidic conditions of the stomach in vivo. A minor absorption of some procyanidin dimers seems possible in humans. The procyanidin dimer B2 was detected in the plasma of volunteers after ingestion of a cocoa beverage; however, the maximal plasma concentration that was reached 2 h after ingestion was much lower than that reached after a roughly equivalent intake of epicatechin (0.04 compared with 6.0_mol/L). Proanthocyanidins, which are among the most abundant dietary polyphenols, are very poorly absorbed and may exert only local activity in the gastrointestinal tract or activity mediated by phenolic acids produced through microbial degradation (18).

The freeze dried extract of *R. mangle* as active principle in antiulcer effect has more than 50 % of tannins identify principally proanthocyanidins (monomer and oligomer). For also the results obtain in this report about it absorption by *in vitro* and *in situ* models could be verify the report of other author in the field of absorption and bioavailability iof polyphenols in animals and in human.

Our found coincide by other report about the antiulcerogenic activity for some polyphenols (1, 19–21).

In conclusion, we demonstrated that the cytoprotective effect of this extract in peptic ulcer is a synergic effect of polyphenolic structures more other secondary metabolites present in the aqueous extract. The extract possessed anti diarrheic activity and it had a high absorption *in vitro* and *in vivo* models which warranty the biodisponibility of this extract in the body.

REFERENCES

1. Scalbert A.; Déprez S.; Mila I.; Albrecht A.M.; Huneau J.F. and Rabot S. Proanthocyanidins and human health: Systematic effects and local effects in the gut. *Mini-review. Biofactors* **13**, 115–120 (2000).
2. Scalbert A.; Morand C.; Manach C.; Rémésy C. Absorption and metabolism pf polyphenols in the gut and impact on health. *Biomed. Pharmacother.* **56**: 269–316 (2002).
3. Sols A.; Ponz F. A new method for the study of intestinal absorption. *Rev. Esp. Fisiol.* **56**: 269–316 (1947).
4. Carbonaro,M.; Grant,G.; Pusztai,A. Evaluation of polyphenol bioavailability in rat small intestine. *Eur. J. Nutr* **56**: 269–316 (2001).
5. Roig J.T. Plantas medicinales, aromáticas y venenosas de Cuba. Editorial Revolución y Progreso, La Habana, p. 745 (1974).
6. Sánchez Perera L.M.; Ruedas D.; Gómez B.C. Gastric antiulcer effect of *Rhizophora mangle* L. *Journal of Ethnopharmacology* **56**: 269–316 (2001).
7. Sánchez L.M.; Batista N.Y.; Rodríguez A.; Farrada F.; Bulnes C. Gastric and Duodenal Antiulcer Effects of *Rhizophora mangle* L. *Pharmaceutical Biology*. **42**(3):225–9 (2004).
8. Berenguer B.; Sánchez L.M.; Quilez A.; Lopez-Barreiro M.; de Haro O.; Galvez J.; Martin M.J. Protective and antioxidant effects of *Rhizophora mangle* L. against NSAID-induced gastric ulcers. *J. Ethnopharmacol.* **103**(2):194–200 (2006).
9. Sánchez Perera L.M.; Varcalcel L.; Escobar A.; Noa M. Polyphenol and phytosterols. Composition in an antibacterial extract from *Rhizophora mangle* L's bark. *Journal of Herbal Pharmacotherapy*. **6**: 5 (2006).
10. Pino J.A.; Marbot R.; Aguero J.; Sánchez L.M. Volatile components of red mangrove bark (*Rhizophora mangle* L.) from Cuba. *Journal of Essential Oil Research* Vol.**13**, No.2, pp.88–89 (2001).
11. Sánchez Perera L. M. Caracterización química y biológica del extracto acuoso de las cortezas de *Rhizophora mangle* L. Thesis. Centro Nacional de Sanidad Agropecuaria y Universidad Agraria de la Habana, La Habana (1998).
12. Travieso M.C.; Hechavarria O.; Betancourt A.; Frontela M.; Miranda I. Validación del método de determinación de taninos totales en productos

naturales vegetales. *Revista de Protección Vegetal*, Vol.**13**, No.3, pp.189–194 (1998).

13. González Álvarez M.A. *Estudio in situ e in vitro de los mecanismos de absorción y secreción intestinal del 3'metilciprofloxacino*. Doctoral Thesis Doctoral. Universidad de Valencia, Facultad de Farmacia, Valencia, España (2002).
14. Bustamante S.E. Absorción de fármacos. Apuntes docentes (2003).
15. Carbonaro M.; Grant G.; Pusztai A. Evaluation of polyphenol bioavailability in rat small intestine. *Eur. J. Nutr* **56**: 269–316 (2001).
16. Ross J.A. and Kasum C.M. Dietary flavonoids: Bioavalability, metabolic effects, and safety. *Annu. Rev. Nutr.* **56**: 269–316 (2002).
17. Gee J.M. and Jonson I.T. Polyphenolic Compunds: Interactions with the Gut and Implications for Human Health. *Current Medicinal Chemistry*. **56**: 269–316 (2001).
18. Manach C., Scalbert A.; Morand C. and Jiménez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr.* **56**: 269–316 (2004).
19. Esaki,N.; Kato, M.; Takizawa,N.; Morimoto,S.; Nonaka,G.I. and Nishioka, Itsuo. Pharmacological studies on *Linderae umbellatae* Ramus. IV. Effects of condensed tannin related compounds on peptic activity and stress-induced gastric lesions in mice. *Planta Medica.* 34–38 (1985).
20. Hung C.R. and Lee C.H. Protective effect of cimetidine on tannic acid – induced gastric damage in rats. *Pharm. Pharmacol.* **56**: 269–316 (1991).
21. Tebid T., Besancon P., Rovanet J.M. Effects of dietary grape seed tannins on rat cecal fermentation and colonic bacterial enzymes. *Nutrition Research* **16**(1): 105–110 (1996).