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Effect of Aqueous Extract of *Prasium majus's* L. Leaves on Water and Electrolytes Transport in Rat Intestine

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Abstract: The major aim of this investigation was to examine the effect of aqueous extract of *Prasium majus's* L. leaves (AEPML) on intestinal influx of water and electrolytes by Everted Gut Sac (EGS). Four segments of rat intestine were detected and used (duodenum, jejunum, ileum and colon). The extract of the plant was used with two concentrations, 8 and 16 g L^{-1}, respectively. The results obtained showed that AEPML provoke; firstly an inhibition of water secretion by different parts of EGS, but this decrease was only significant at the ileum ($p<0.05$). Secondly electrolytes fluxes were also sensitive to the addition of AEPML. On the other hand for Na^+ influx, we noted an inhibition of the secretion at the duodenum and the colon ($p < 0.01$) with the dose 8 g L^{-1}, while for Cl^-, influx this inhibition was only registered at the colon ($p<0.01$) with the dose 8 g L^{-1}. For K^+ influx, AEPML induced rather a significant reabsorption at the duodenum, the jejunum and the colon ($p < 0.01$) while at the ileum the reabsorption increased. This was only significant with the dose 16 g L^{-1}. It may be concluding that the heterogeneity of the gastrointestinal tract in response to numerous factors which are able to affect homeostasis of water and electrolytes. AEPML might have an antisecretory function on water Na^+ and Cl^- and an absorptive function on K^+.

Key words: Water influx, electrolytes, Everted Gut Sac, *Prasium majus* L., gastrointestinal tract

INTRODUCTION

Equilibrium of water and electrolytes is an important function for a well-functioning of cells.

Alteration of this equilibrium provokes perturbations of this functioning and may cause some pathophysiological disturbances. In physiological conditions, the mucosal surface of the gastrointestinal tract must be constantly hydrated by salt and water secretion[1] and the intestine is among the organs which have a primordial role in regulating salt and water homeostasis.

Membrane permeability, ingestion of food or drugs may affect variation of water and electrolytes flux. This variation has been the subject of several studies allowing to determinate or to clarify the mechanisms of transport of water and electrolytes[2]. Extracts of spices[3] were used to exhibit valuable activities of enzymes or substances involved in transport mechanisms of water and electrolytes. Extracts of medicinal plants were equally used to cure some intestinal illnesses as the use of seirogan crude extract of beech wood in rat intestine to reduce loss of water and electrolytes caused by diarrhoea[4].

In this study we report the effect of aqueous extract of *Prasium majus*'s L.[5] leaves (AEPML) on intestinal influx of water and electrolytes using the technique of Everted Gut Sac (EGS) in rat.

MATERIALS AND METHODS

Presentation of the plant: *Prasium majus* L. is a hairless climbing shrub of 0.5 to 1 m of height which belongs to the *Lamiaceae* family. This plant is spread in the Mediterranean regions. In Tunisia, it grows in south regions. The vernacular noun is *Kerchet arneb*. The leaves of this plant are used in popular medicine for their smoothing properties; they are also consumed as a raw food.

Animals: The experiments were achiveded on male Wistar rats. Animals were kept in the animal house at standard conditions. They had free access to water but food was withdrawn 24 h prior to the experiment. Their weight varied between 200 and 250 g. They were slaughtered by cervical dislocation. Four segments (4 cm length) were chosen (duodenum, jejunum, ileum and the colon). They were immediately extracted, stripped of adhering tissue

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cleaned with a ringer solution in mM L^{-1} as follows : NaCl: 0.154; KCl: 0.0034; HCO_3Na: 0.0024; $CaCl_2$: 0.0021.

All solutions were prepared daily. All chemical products used were obtained from Prolabo (France).

The aqueous extract of *Prasium majus* L. leaves (AEPML) was used with two concentrations 8 and 16 g L^{-1} and was supplied by the Laboratory of Organic Chemistry (Faculty of Sciences and Techniques of Monastir).

The Everted Gut Sacs (EGS): We prepared the everted bag according to the technique of Wilson and Wiseman[6]; that are filled with Ringer solution pH = 7.4. The sacs were hanged in an incubation medium containing AEPML for 60 min. For the control groups, incubation medium contained ringer solution half diluted (N/2).

The experiment was carried out at 37°C temperature. The EGS were constantly oxygenated during the incubation time.

Water and electrolytes quantification: Water influx is determined in the absence and the presence of (AEPML) weighing and the results are expressed in g of water per g of fresh intestine per hour.

Electrolyte dosage was determined by selective electrode of ion (ISE) with an COBAINTEGRA 400 Plus analyzer which determines quantitatively sodium, potassium and chloride. The results of electrolytic flux were expressed in mM L^{-1}

Statistical analysis: Comparison between controls samples (Ringer) and samples treated with AEPML were based on mean values derived from (6 to 8) EGS for each dose.

Statistical significance was evaluated using ANOVA test and differences were considered significantly when $p < 0.05$. The validity of present experimental model was previously controlled by many researchers[6,7] and we have confirmed this validity by a histological study (data not shown).

RESULTS

Investigation of water influx by EGS segments: During control conditions the results obtained showed that the movements of water are different from one segment to another (Table 1).

After 60 min of incubation time water influx is of -0.237±0.05 g of water g^{-1} of fresh intestine h^{-1} ,for the duodenum, -0.289 ± 0.05, for the jejunum, -0.362±0.069 for ileum and 0.119±0.38 for the colon, respectively.

Table 1: Effect of AEPML on the variation of water flux in rat intestine

	Duodenum	Jejunum	Ileum	Colon
Controls	-0.24±0.05	-0.29±0.05	-0.36±0.07	-0.12±0.04
PM 8 g L^{-1}	-0.12±0.03	-0.19±0.05	-0.35±0.11	-0.07±0.01
PM 16 g L^{-1}	-0.12±0.03	-0.16±0.03	-0.08±0.02	-0.05±0.01

Variation of the water flux in the Everted Gut Sacs of duodenum (N = 6 with ringer, N = 6 with AEPML at 8 g L^{-1} and N = 8 with AEPML at 16 g L^{-1}), jejunum (N = 6 with ringer, N = 6 with AEPML at 8 g L^{-1} and N = 7 with AEPML at 16 g L^{-1}) ileum (N = 6 with ringer, N = 6 with AEPML at 8 g L^{-1} and N = 6 with AEPML at 16 g L^{-1}) and colon (N = 7 with ringer, N = 10 with AEPML at 8 g L^{-1} and N = 13 with AEPML at 16 g L^{-1}) after 1 h in response to AEPML (Aqueous Extract of *Prasium majus* L. Leaves). Fluxes are reported in g of water per g of fresh intestine per hour. Everted Gut Sacs treated with ringer are considered as control group. Each value represents the (means±SEM) of N experiments

The addition of AEPML (Table 1) provokes a decrease of water secretion at the duodenum, jejunum and colon either with 8 or 16 g L^{-1}, but this decrease was not significant.

When the ileum was used, the addition of AEPML at the dose 8 g L^{-1} has practically no significant effect, whereas with the dose 16 g L^{-1} , water flux was significantly reduced ($p<0.05$).

Electrolytes influx: In basal conditions and without extract after 60 min of incubation time, we note a decrease of Na^+, Cl^- and K^+ uptake at the different parts of rat intestine (Table 2).

Na^+, influx were different in the duodenum, jejunum and ileum and in the colon and they 93.38±0.46, 68.98±3.4, 63.83±3.7, respectively and in the colon the uptake of Na^+, was 78.82±4.2 mM L^{-1}.

The colon presents 90% of the duodenum Na^+flux (Table 2). For the jejunum and the ileum, the Na^+, influxes were less important; they were, respectively 74 and 69% of the duodenal influx.

Chloride uptake was also studied and it was found that the influx was 84.78±4.05 mM L^{-1} by colon.

This influx was also important at the duodenum and it was 82.33±4.51 mM L^{-1} (Table 2). Lastly the influx of K^+ was investigate (Table 2).

The influx were respectively 2.14±0.13, 1.93±0.18, 1.92±0.23. mM L^{-1} at the duodenum, the jejunum and the ileum. The influx at the colon was 2.60±0.26 mM L^{-1}. The addition of AEPML at 8 and 16 g L^{-1} (Table 3). indicated a significant inhibition of Na^+ flux at the duodenum and the colon ($p<0.01$ and $p<0.05$). However, either at the jejunum or at the ileum, this addition has no significant effect on Na^+ flux.

When chloride uptake was studied, in the results there was significant variation of the flux at the duodenum, the jejunum and the ileum (Table 3). While at the colon AEPML induced a reduction of Cl^- secretion, this decrease was highly significant only with the dose 8 g L^{-1} ($p<0.01$).

Table 2: Electrolytes movement in rat intestine

	Duodenum	Jejunum	Ileum	Colon
Na^+	93.38±0.47	68.98±3.41	63.83±3.7	78.82±4.2
Cl^-	82.33±4.5	68.37±2.92	73.98±5.5	84.78±4.1
K^+	2.14±0.14	1.93±0.18	1.92±0.23	2.6±0.26

Sodium, chloride and potassium flux in the everted gut sacs of duodenum (N = 6), jejunum (N = 6), ileum (N = 6), and colon (N = 7), after 1 h (means±SEM) in presence of Ringer .Each value represents the mean of N experiments. Fluxes are reported in mM L^{-1}. Everted Gut Sacs treated with ringer are considered as control group

Table 3: Final concentration of electrolytes. Inside Everted Gut Sacs after 60 min of incubation time

	N	Na^+ (mM L^{-1})	Cl^- (mM L^{-1})	K+ (mM L^{-1})
Initial test solution		167.2	178.9	3.8
Duodenum				
Controls	6	93.38±0.47	82.33±4.50	2.14±0.14
PM8 g L^{-1}	6	70.78±3.12**	74.70±3.85	3.57±0.28
PM16 g L^{-1}	8	76.92±6.20*	79.50±6.50	6.10±1.1**
Jejunum				
Controls	6	68.98±3.41	68.37±2.92	1.93±0.18
PM8 g L^{-1}	6	68.2±4.90	68.42±5.0	3.46±0.66
PM16 g L^{-1}	7	72.4±3.40	77.90±3.4	4.60±0.60**
Ileum				
Controls	6	63.83±3.70	73.98±5.50	1.92±0.23
PM8 g L^{-1}	6	68.92±3.44	69.33±3.10	3.20±0.50
PM16 g L^{-1}	6	64.03±3.55	69.72±4.4	4.62±0.58*
Colon				
Controls	7	78.82±4.20	84.78±4.1	2.60±0.26
PM8 g L^{-1}	10	59.77±6.96**	61.10±7.3 0**	3.89±0.64
PM16 g L^{-1}	13	64.83±2.81*	68.60±2.76	5.03±0.58**

Variation of the electrolytes flux in the Everted Gut Sacs of duodenum (N = 6 with ringer, N = 6 with AEPML at 8 g L^{-1} and N = 8 with AEPML at 16 g L^{-1}), jejunum (N = 6 with ringer, N = 6 with AEPML at 8 g L^{-1} and N = 7 with AEPML at 16 g L^{-1}) ileum (N = 6 with ringer, N = 6 with AEPML at 8 g L^{-1} and N = 6 with AEPML at 16 g L^{-1}) and colon (N = 7 with ringer, N = 10 with AEPML at 8 g L^{-1} and N = 13 with AEPML at (N = 6), jejunum (N = 6) ileum (N = 6) and colon (N = 7) after 1 hour (means± SEM) in response to AEPML (Aqueous Extract of *Prasium majus* L. Leaves) with 2 doses 8 and 16 g L^{-1} Flux are reported in mM L^{-1} Everted Gut Sacs treated with ringer are considered as control group.

K^+ influx was affected by the presence of AEPML (Table 3). We noticed a reabsorption I of K^+ at the duodenum, the jejunum and the colon ($p<0.01$), at the ileum ($p<0.05$). These results were observed with the dose 16 g L^{-1}.

DISCUSSION

We confirm the validity of the choice of present experimental model which has been previously controlled[6,7]. Present results showed the heterogeneity of the gastrointestinal tract. This heterogeneity was previously elucidated by other techniques: ligatured loops or sheets on intestine mounted at the Ussing Chamber[8,9]. The validity of our model is also confirmed at three levels of rat intestine[10].

The use of EGS *in vitro* taken from different parts of rat intestine shows that the movement of water and electrolytes are different from one intestinal segment to another. In our experimental conditions, loss of water and electrolytes from serosa to mucosa side translates a mucosal secretion. Moreover determination of electrolytes transfer after 60 min of incubation time shows this secretion without addition of AEPML (Table 2).

A basal secretion of water and electrolytes (sodium chloride and bicarbonate) where previously described Charpin *et al.*[11] who have used the technique of *in situ* ligatured duodenal loops in the rat; although in this study the distension of the loop after liquid injection may play a role in this secretion which is absent in our experimental conditions. Equilibrium of fluid movement through gastrointestinal tract result from absorption and secretion phenomenon and aquaporines are channel proteins implied in this fluid transport[12]. Several types of aquaporine with a variable distribution and localization or location have been shown in the gastrointestinal tract[13]. This variability in distribution and localization may explain the differential water influxes observed through the different EGS considered in our data for control samples. Using rat ligatured loops in response to saline load has shown that maximum water absorption occurred at the duodenum than in the jejunum and the ileum[9]. This result may confirm the increase of secretion in our control samples where we noted the highest secretion at the ileum.

Water and electrolytes transfer through gastrointestinal tractus was studied in the presence of different products as plant extract, peptide, hormone, toxin and by several techniques *in vivo* or *in vitro*. Previous studies[9,10,14] show a heterogeneity of the gastrointestinal tract responses. However, *in vitro*, the results recorded were only in relation with the factors studied while *in vivo* effect of endogenous factors may influence the results recorded, these differences may explain the different results registered in our data.

The presence of toxins STX2[14] ST[15] and CT[16,17] in the gastrointestinal tract induces disturbance of fluid and ions transport frequently characterized by a secretion.

To elucidate the activities and the target cells of these toxins, some products known to have effects on the hydroelectrolytic balance were tested in presence of these toxins.

So, the perfusion of Arabic gum *in vivo* in rat jejunum in presence of CT[16] is at the origin of a reduction of water and ion secretion despite the activity of CT. *In vivo* in r at jejunal loops, the presence of heat stable *E. coli* enterotoxin provokes a secretion of water while the addition of loperamide with an antidiarrheal activity changes this secretion to a net absorption[18].

In the rabbit proximal colon, the endogenous production of SCFA (short chain fatty acid) in presence of CT induces a reduction of water and electrolyte

secretion[19]. The previous results showed that the presence of toxins in the gastrointestinal tract decrease absorption and increase secretion of water and electrolytes. However, an unexpected results were stipulated[15] and showed that ST in rat distal colon produced an increase of water and ion absorption by acting on selective secretory cells. These results are confirmed by immunohistochemical technique[15]. These results may explain the low flux of water secretion in the colon in our control samples. A low secretion of fluid in the distal intestine is also registered in ligaturated loops of porcine small intestine[17].

Besides toxin, hormonal factors equally modify the balance of water and ions: GH induces water and Na^+ Cl^- absorption and GH acts through fixation on specific receptors localized on rat intestine[20]. The implication of GH in the fluid homeostatic control was confirmed by the administration of GH on rat perfused jejunum, ileum and colon. In fact, GH induces water and ion absorption from proximal to distal decreasing pattern[21].

The finding of GH stimulation of water and electrolytes absorption agrees with our results in the jejunum and the ileum, but contrary in the colon where we have noticed a low rate of secretion.

GH was also tested on human intestinal cells. The finding confirmed increasing fluid and electrolytes absorption and equally cells growth[22]. GH may be used as a therapeutic factor in intestinal diseases (tissue damage, perturbation of fluid and ion movement).

Contrary to GH, ANP (atrial natriuretic peptide) inhibits water, Na^+ and glucose absorption in rat intestine and kidney[23]. It is in a variance of present results.

Gastrointestinal hormone may also modulate fluid movement and pentagastrine increased duodenal absorption of water and sodium and decreased ileal absorption whereas secretine and cholecystokinine induced a duodenal secretion and decreased ileal absorption. This study showed the heterogeneity of the response which is in relation with the segment under study and the hormone tested[24].

In comparison with the results already mentioned, the effect of AEPML favors a reduction of water influx secretion at the different EGS considered; however this secretion inhibition is significant at the ileum ($p < 0.05$).

AEPML may act by modification across aquaporines channels implied in the trans-epithelial permeability of water[13,25,26].

Present results agree with Arabic gum fibers which oppose to the loss of water and electrolytes through intestine exposed to toxins[16] and to the effect of seirogan[4] used to reduce loss of water and electrolytes caused by diarrhea.

We can emit the hypothesis that AEPML may present an antidiarrhoeal action as Arabic gum and seirogan.

Concerning electrolytes exchanges, the results showed different responses in controlled and treated EGS.

At the duodenum, jejunum, ileum and colon, we noticed a transfer of Na^+ from the serous side towards the mucous side in control samples (Table 3). However, addition of AEPML induced a significant reduction of the secretion at the duodenum and the colon ($p<0.01$), in the jejunum and the ileum, AEPML seems to be ineffective. A secretion decrease of water and Na^+ was noted in rat jejunum loops in presence of Arabic gum[16]. These results are in a variance of those registered in EGS rat jejunum where we note an active absorption of Na^+[10].

The variation of chloride influx is not significant ,the duodenum, the jejunum and the ileum seem to be insensible to the addition of AEPML. Contrary, at the colon, it produces a significant loss of Cl^- ($p<0.01$). Similar results are registered at slices of rat intestine incubated in the presence of nicotine and also at the Bruner's glands[27]. Moreover, *in vivo* the absorption of water mixed with nicotine during 10 days induces a decrease of chlorine concentration at the mouse intestine, the colon and at Bruner's glands[27]. This loss of Cl^- and Na^+ is also noted at the intestine and the kidney of the mouse in presence of two endogene peptides guanylin and uroguanylin. These two peptides are involved in the homeostatic regulation of salt and water[28-30].

The finding that AEPML addition is at the origin of a significant reabsorption of K^+ from the mucous side to the serous one at the duodenum, the jejunum, the ileum and the colon agrees with results registered at rat distal colon. This absorption increase may be activated by H^+ K^+ ATPase[31]. Present data is at a variance with results obtained in the mouse kidney and intestine where uroguanylin and guanylin provoke a secretion of K^+[28] and in rabbit colon, where SCFA, in presence of cholera toxin, does not significantly inhibit secretion of K^+[19].

This research will be pursed to study the active substance of extract *Prasium majus's* leaves and try to clarify the mechanism's action of this extract on the movement of water and electrolytes. However, more investigations are needed to make the exact mechanism clear.

Different techniques were used *in vivo* and *in vitro* (ligatured loops, sheets of intestine mounted through Ussing Chamber, EGS[8-10] to study the different factors involved in the homeostatic regulation of salt and water and the mechanisms implied in this regulation. Our data confirm previous studies which have shown the heterogeneity of the gastrointestinal tract to response to

the numerous factors to be able to affect homeostasis of water and electrolytes.

AEPML might have an antisecretory function on water, Na^+ and Cl^- and an absorptive function on K^+.

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