RESEARCH ARTICLE | DECEMBER 04 2023

Study the effect of some pharmaceuticals and alternative medicines on gastrointestinal (GI) motility in adult male rats

Manal N. Alhayder ✉; Nadheerah F. Neamah; Raghad I. Abed

AIP Conf. Proc. 2834, 020014 (2023)
https://doi.org/10.1063/5.0162045

Study the Effect of Some Pharmaceuticals and Alternative Medicines on Gastrointestinal (GI) Motility in Adult Male Rats

Manal N. Alhayder[1,a)], Nadheerah F. Neamah[1,b)], Raghad I. Abed[2]

[1] *Pharmacology &Toxicology Department, College of Pharmacy, University of Basrah, Basrah, Iraq*
[2]*Clinical Laboratory Sciences Department, College of Pharmacy, University of Basrah, Basrah, Iraq*

[a)] *Corresponding author: manal.abd-alhassan@uobasrah.edu.iq*
[b)] *Nadheerah.neamah@uobasrah.edu.iq*

Abstract. : Many medicines are used to treat diseases other than those related to the digestive system but they have effects on gastrointestinal (GI) motility. Thus, the present study aims to assessment the influence of some of common medications on bowel movement, by measuring the travel index of charcoal substance in the intestine. About145 Adult Male Albino Rats were used 5 rats/group. Charcoal used as GI motility indicator compered to control group (C) and castor oil group (Cs) and loperamide (Lo) as positive control. The results showed that evaluation of travel index (TI) of charcoal in the test medicines showed different responses. Lycopene, Allium ampeloprasum, Licorice, Ranitidine, MSG, Ciprofloxacin, Valsartan, and Syzigium aromaticum showed increased TI compare to Cs, only lycopene showed non-significant change on TI than in Cs. Medicines that decreased TI compared to C and Lo, the results showed significant decrease of Alhagi maurorum, Foeniculum vulgare, Vit C, Metamizole, and Diclofenac potassium TI compare to c, while Alhagi maurorum and Metamizole showed non-significant effects compare to Lo. Then medicines that reduced TI were evaluated with Cs induced diarrhea, the exhibit significant reduction on TI related to C+CS and LO+Cs groups, only orlistate+Cs exhibited non-significant alteration with Lo+Cs group.

Keywords: Intestinal motility, Transport index, Charcoal meal, Antidiarrheal effects, Castor oil

INTRODUCTION

Many pharmaceutical drugs and alternative medicine are used to treat diseases other than those related to the digestive system. So, the study aims to estimate the activity of some common medications on bowel movement. The study relied on measuring the travel index of charcoal substance in the intestine as an evidence of peristaltic movement and the effect of studied drugs that decrease TI on castor oil induced diarrhea. Gastrointestinal (GI) motility is controlled via neurons and GI hormones. Neurons include parasympathetic and sympathetic extrinsically; and enteric sensory and motor neurons intrinsically. GI motility hormones include; secretin; gastrin; gastrin releasing peptide; somatostatin and cholecystokinin. GI hormones are transduction molecules conduct information via blood stream from cell to another by stimulation of specific receptors [1]. This study tries to investigate the effects of some of pharmaceutical and traditional medicines that used for treatment of diseases rather than abnormal GI muscles contraction. The illness of diarrhea sits definitely both for morbidity and mortality. In fact it's the second worldwide gravest reason of children younger than 5years mortality. Diarrhea as GI syndrome, branded by

2nd International Conference of Mathematics, Applied Sciences, Information and Communication Technology
AIP Conf. Proc. 2834, 020014-1–020014-7; https://doi.org/10.1063/5.0162045
Published by AIP Publishing. 978-0-7354-4715-8/$30.00

variation in normal bowel motility, enhanced in the content of water, stools volume or frequency. It's frequently accompanied by perianal discomfort, pain, urgency, and incontinence. It's a sign, not a disease by itself, can be produced by several conditions [2]. Diarrhea and constipation can have many reasons which could be infective or non-infective. Commonly, five types of diarrhea are known; which are the secretary diarrhea, motility diarrhea, inflammatory diarrhea, osmotic diarrhea, and dysentery. It might as well be caused via undigested lactose, excessively magnesium, vitamin C, it might furthermore be caused as an effect of celiac disease or purgatives [3]. The existing article evaluates the activity of some pharmaceuticals as well folk medicines in motility diarrhea. Medicines such as; loperamide (Lo); reduced the bowels movement and castor oil (Cs) used as standard drug for induced GI motility.

Castor oil, an effective laxative is hydrolyzed to ricin oleic acid in the small intestine that can stimulate secretion of fluid, inhibit absorption of electrolyte and water, decrease sodium and potassium active absorption also; in the small intestine and colon Cs decreased Na, K-ATPase. Furthermore Cs produces changes in the permeability of the intestinal mucosa membrane, and increases the peristaltic activity. Besides, ricin oleic acid can lead to endogenous prostaglandins release, which have an important modulation in stimulating motility and secretion in the GIT [4].

Drugs affecting GI motility are valued in the controlling of several diseases. Drugs improved the material transit through the GIT, termed prokinetics include, dopamine antagonists, cholinomimetic drugs. While sign improvement in a different of motility disorders can be observed, those medicines have not revealed a selective value for a specific motility disturbance [5]. The spasmolytic drugs effect is frequently used for the decrease of extreme contractility of the smooth muscle, which responsible for discomfort and cramping in the abdominal area, caused by multiple conditions affecting the biliary genitourinary or GI tract. The oldest form of medicine known to mankind is Herbal Medicine, the mainstay of several early civilizations and nevertheless the most practiced form of medication worldwide nowadays. There is no doubt that supportive medicines have increasingly attracting attention and obtained wide acceptability from scientific community worldwide [6].

MATERIALS AND METHODS

Experimental Animals

The total number was 145 of Male Albino Rats aged between 5-6 weeks, weighing 135 – 150 g, they were obtained from the Veterinary Medicine College-University of Basrah. They kept for 2weeks for acclimatization at stainless steel cages at animal house of College of Pharmacy-Basrah University and treated in line with the guide and care for laboratory animals. All rats fed with free access standard pellet and clean water, room temperature was 31±2˚C and 12 light/12 h dark.

Extraction of natural test samples

The plants obtained from Al Aashar herbals market 500 g for each plant. After washing the plants materials, the water extract was prepared for each plant, by mixed each plant with boiling distilled water for 15 min and stirred on the hot plate, and then the filtrate was frozen and lyophilized (freeze-drying) in a lyophilizator at laboratories of college of pharmacy/ university of Basrah [7].

Normal gastrointestinal motility test using Charcoal meal

Rats were randomly divided into twenty nine groups, comprising of 5 rats each. The rats fasted for 18h with free-access drinking water before initiation the trial. Control group received 2 ml of distilled water (DW), while the rest groups were treated as described under grouping and dosing at Table 1[8], [9]. After 20 minutes, both the control and the experimental mice received orally 2 ml of 5 % activated charcoal. The rats were sacrificed after thirty minutes; the intestine from pylorus region up to ileocecal junction was removed. The distance traversed by the charcoal (as a marker) was measured and expressed as Peristalsis index. [10].

The results were stated as :

Peristalsis index or Travel index (%) =
(Distance travelled by charcoal meal cm /Length of small intestine cm)* 100

Preparation of 5% of Charcoal suspension

Drugs and Chemicals

Charcoal meal suspension was prepared through dissolving 2.5 g of Arabic gum powder and 2.5 g of activated charcoal in 50 ml of normal saline. [11]

TABLE1: Drugs and chemicals information's

Drugs and chemicals	Dose	Manufacturer
Loperamide	5 mg/kg	Torrent Pharmaceuticals. Ahmedabad, India
castor oil	10mg/kg	Amman Pharmaceutical, Jordan
Deactivated charcoal	2 ml of 5 % activated charcoal.	New India chemical enterprises. Kochi
Arabic gum powder	2.5g/ 50 ml of 5% activated charcoal	Xi'an Harmonious Natural Biotechnology Co., Ltd. Shaanxi, China
Metamizole	15mg/kg	Shaanxi pioneer Biotech. China
Ascorbic acid Vit C	100mg/kg	Shaanxi Sangherb Bio-Tech Inc china
Omeprazole	20mg/kg	Whiz Laboratory India private limited Punjab India
Diclofenac potassium	2mg/kg	Dipharma Italy
orlistat	2 mg/kg	Hikma pharmaceuticals – Aman-Jordan
Lycopene	50mg/kg	Shaanxi pioneer Biotech. China
Ranitidine	50 mg/kg	Uquifa/ USA
MSG	1mg/ Kg	Qingdao Trust Agri Chemical Co.,Ltd. China
Ciprofloxacin	10 mg/kg	Loghman pharmaceticals/ Tahran- Iran
Valsartan	2 mg/kg	Aburaihan pharmaceutical company
Erythromycin	5 mg/kg	Sana med pharmaceutical company

Effects on castor oil (Cs)-induced diarrhea

Forty five male of Wistar Strain Albino Rats were used divided in to 9 groups, Group 1 as negative control received 2 ml of DW orally via mouth gag. G2 as positive control received 0.2ml of Cs; while groups from 3 to 10 received test drug each, the dose were administered according to their body weight. After 20 minutes, G1 received 2ml of DW while the other groups received 0.2ml of Cs. than all rats received 1 ml of 5% of activated charcoal orally [12]. The rats sacrificed after 30 m, the distant that charcoal travelled was measured from pyloric region, and then the total distance from pyloric to ileocecal junction was measured. The results expressed as inhibition percentage of charcoal travelled, by means of the following formula [13].

Inhibition% = Mean distance travelled by the control (Dc)− Mean distance travelled by the test group (Dt) /Dc *100

$$\left(\text{Inhibition\%} = \frac{\text{Dc}-\text{Dt}}{\text{Dc}} \times 100 \right)$$

STATISTICAL ANALYSIS

The data values were presented as mean ± standard Deviation. The statistical tool applied for Comparison of data groups was ANOVA with level of significance $p < 0.05$.

RESULTS

Charcoal meal test

The results divided according to their effects on travel index (TI) into two groups; the first group of drugs decreases TI compared to control group and Loperamide as positive control. The second group of drugs increases TI in comparison with negative control and Castor oil as reference drug.

Drugs reduce TI

When the treatment groups TI compare with control as negative control and lipoamide as reference drug, the results showed that all test medicines that listed at Table 2, significant reduction of the travel index ($p<0.05$) than the control group, except orlistat treated group which has no significant effects on TI. While when comparing the drugs used in this part of the study with the deliberate drug, the results can be observed as follows; Alhagi maurorum and Metamizole were non-significant TI alteration with Lo treated group. The results of the rest of the treatments can be identified that a significant increase compared to Loperamide TI.

TABLE 2: Effects on Gastrointestinal Transit of Charcoal Meal in Wistar Strain Albino Rats

Group	Treatment (mg/kg)	Total length of intestine (cm)	Distance traveled by charcoal(cm)	travel index %
1	Control	83.857±6.335	20.155±1.73	17.09±4.324
2	Loperamide (3mg/kg)	79.90476±6.223	10.571±2.536	13.43±4.036*
3	Alhagi maurorum(200 mg /kg)	67.904±5.943	9.9.09±3.166	13.504±5.072*
4	Metamizole (15mg/kg)	80.61±5.149	11.523±1.87	14.49±2.88*
5	Vit C (100mg/kg}	86.28± 1.73	13.28±1.38	15.45± 1.83*•
6	Foeniculum vulgare(300 mg/kg)	108.42± 10.9	16.97±7.71	15.99± 8.37*•
7	Omeprazole	77.48±10.85	13± 2.13	15.53± 1.73*•
8	Diclofenac potassium	75.83±6.35	11.83±2.91	15.512±3.12*•
9	orlistat	82.25±13.73	16.063±2.62	19.53±8.82•

*=significant with C group, •= significant with Lop, significant means $p<0.05$

.

TABLE 3: the groups of test medicines that increase TI compare to control and castor oil group

Group	Treatment+ Charcoal	Total length of intestine (cm)	Distance traveled by charcoal(cm)	Travel Index%
1	Control	83.857±6.335	20.155±1.73	17.035±4.324
2	Castor oil	85±4.335	42.343±3.84	46.816±4.873*
3	Lycopene(50mg/kg)	68.518±9.816	27.032±5.84	39.452±6.352*
4	Allium ampeloprasum	80.333±5.7	28.212±3.535	29.119±4.511*•
5	Licorice	77.93±5.68	25.997±1.83	33.36±2.35*•
6	Ranitidine (50 mg/kg)	76.444±9.345	26.717±8.11	32.95± 10.07*•
7	MSG((1mg/Kg)	69.917±4.334	31.661±4.47	37.284±5.812*•
8	Ciprofloxacin (10 mg/kg)	72.285±7.271	23.968±4.17	33.159± 6.384*•
9	Valsartan	71.259±6.785	23.018±2.91	32.302±5.253*•
10	Syzigium aromaticum	78.3±3.7	26.12±3.0 3	27.12±2.5*•
11	erythromycin	85.142±5.01	21.63±2.25	25.64±2.66*•

*=significant with C group, •= significant with Lop, significant means $p<0.05$

Drugs increase TI

In comparison with the control group, all test groups revealed significant ($p<0.05$) enhanced in TI%. But when compared with Cs treated group; Allium ampeloprasum; Lycopene, Licorice, Ranitidine, MSG, Ciprofloxacin, Valsartan, *Syzigium aromaticum* and erythromycin group showed significant ($p<0.05$) decreased than Cs group. Only Lycopene reveals no important change in TI compare to Cs group.

Castor oil induced diarrhea

The Table 4 provides the inter-correlations among the test drugs TI compare with TI of DW as a negative control and castor oil as a reference drug. Also; compares the results obtained from the preliminary analysis of the test medicine and loperamide inhibition percentage as a positive control. From this data, we can see that TI% of all medicine that included in this part of the study were decreased significantly ($p<0.05$) than control-Castor oil TI%. However, all groups showed non- significant alteration related to Lo+ Cs treated group; only Orlistat+ Co showed significant increased than positive control. The inhibition percentage of charcoal travelled indicated that no significant differences among all studied medicines related to inhibition % of reference drug Lo except Foeniculum vulgare and orlistat showed significant reduction in compared with Lo.

TABLE4: Effects of test medicines on castor oil induced diarrhea and charcoal as travel indicator

	Treatment+ Charcoal+ Castor oil	Total length of intestine (cm)	Distance traveled by charcoal(cm)	Travel Index%	inhibition percentage of charcoal travelled%
1	DW+ Cs	118.5±7.42	56±5.77	47.355±4.901	--
2	Loperamide +Cs	119.857±8.64	15.857±3.52	13.434±3.73•	72.27±5.96
3	Alhagi maurorum +Cs	101.857±8.91	10.857±2.41	10.556±1.57•	69.83±3.95
4	metamizole+ Cs	83.85±6.33	15.87±2.91	15.539±2.88•	71.645±5.20
5	Vit C+ Cs	108.428±10.90	36.142±9.71	33.015±6.38•	65.24±4.16
6	Foeniculum vulgare + Cs	93.97±5.34	29.70±3.14	31.67±3.54•	45.95±5.61*
7	omperazole+ Cs	83.857±6.33	17.29±1.80	20.68±2. 36•	69.12 ±3.22
8	Diclofenac+ Cs	75.833±6.35	18.78±4.36	24.67±4.68•	66.45± 7.79
9	orlistat + Cs	82.25±13.73	30.063±2.62	36.5±5.76••	46.31±6.07*

*=significant compare to Cs group inhibition percentage, •= significant compare to Cs travel index, significant means $p<0.05$

DISCUSSIONS

Many pharmaceutical drugs and alternative medicine are used to treat diseases other than those related to the digestive system. Therefore, this study aims to evaluate the effect of some common medications on bowel movement. The study relied on measuring the travel index of charcoal substance in the intestine as evidence of peristaltic movement and the effect of studied drugs on peristaltic. First the effects on travel index for all medicines studied to evaluate their effects on traveling percentage of charcoal, then the results dividing into two tables; one consists the drugs increased TI in comparison with control. The drugs that reduced TI were listed at another table, also; the antidiarrheal effects against castor oil induced diarrhea were estimated.

Usually, folks use medicinal plants to be effective against diarrhea deprived of any scientific basis. Consistent with findings, Alhagi maurorum and Foeniculum vulgare reduced peristaltic, which may be due to a high absorption

rate of intestinal fluid as a result reduced intestinal motility and increased transit time [14]. Alhagi maurorum in a 200 mg/kg dose shows a significant anti-diarrheal effect against castor oil-induced diarrhea, in a recent study [15], the author found that Alhagi maurorum in low concentration >1.6 mg/ml increased the force of contraction, however in concentrations >3.2 mg/ml appears to be produced a rapid reduction in GI contraction. The author mentions that depressant effect seemed to be caused by blocking of calcium channel. Also, a review study [16] has revealed that Foeniculum vulgare oil has reduced GI motility but the mechanism for such action unknown. In another study; [17] showed that aqueous extract of the fruit had antidiarrheal activity. Diarrhea induction model by castor oil is commonly used for the assessment of anti-diarrheal effect of drugs. The ricinoleic acid is the most active constituent; it causes inflammation and irritation of the intestinal mucosa, which stimulates the small intestinal peristaltic movement, causing alterations in the electrolytic permeability. As sequence prostaglandins then released, this stimulates secretion and motility [18]. Licorice showed decreased in ulcer index due to presence of glycyrrhizins, which induced release of prostaglandins in the GIT. Prostaglandins increase mucus secretion and protect the epithelial cells; this may result in induced GIT motility [19]. Ciprofloxacin can induce GI motility, nausea and vomiting, as well as liver toxicity when used in a high dose or for long term treatments [20].

Little evidence is available about metamizole action on intestinal motility. Small intestinal peristalsis of guinea pig is not blocked by metamizole after in vitro exposure. In contrast, the drug had a myogenic spasmolytic effect at higher concentrations and it has confirmed that metamizole administration in rats delays gastric emptying. This effect mediates through norepinephrine [12].

Anti ulcerogenics drugs such as Proton pump inhibitor omeprazole, which significantly ($p<0.05$) reduced GI motility; this effect may relate to inhibition H^+- K^+ATPase enzyme at the parietal cells and prevent H^+ ion release as results decreases HCl formation. Low fluid secretion lead to reduce gastric emptying time and gastric retention of the meal [21]. Unlike omeprazole, Cimetidine basic anti ulcerogenic drug act by antagonized H_2 receptor in the gastric mucosa. Cimetidine increases GI motility and aids food absorption [22].

The findings of the current study are consistent with [23] who found that the extract of Syzigium aromaticum increased the propulsion of the gut muscle comparable to carbachol and metoclopramide (standard drugs). Also, the previous study showed the association of cholinergic mechanism, through prior administration of atropine [23]. Previous research has indicated that diclofenac sodium (50 mg three times a day) for a week, revealed no difference in gastric motility index and in amplitude of gastric antral contractions after NSAIDs with respect to the basal study and to the placebo group [24]. The data are relatively controversial, and there is no general agreement about reduced GI motility in rats at dose 2mg/kg body weight, the dose determined according to study done by [25] and the results similar to our results. Orlistat (tetrahydrolipstatin) is a gastrointestinal lipases reversible inhibitor that is commonly used in treatment of obesity. Besides, Orlistat increase gastric emptying, decreases pyloric pressure, and increases antro-pyloro-duodenal motility. In the recent study orlistat has non-significant effects TI compares with control groups and shows significant decline in TI than Lo TI, this result is contradictory that found by [26]. Castor oil increases peristaltic activity through the active metabolite ricinoleic acid. Ricinoleic acid also; changes permeability of the intestinal mucosal membrane to electrolytes and water through raises prostaglandin biosynthesis which consequences in inflammation and irritation of the intestinal mucosa as a result motility and secretion stimulated [12]. Erythromycin is a macrolide antibiotic has been identified to increase GI contractions in humans and rabbits in vitro. This fact when compared with in vivo studies there was little information about the effect on GI contractions [1], this stimulation mediated by extracellular Ca_2^+.influx [27]. Consistent with findings by [21], we found that conversely to omeprazole, Ranitidine is H_2 receptor antagonists increased ileum motility when compared with control group. Ciprofloxacin increased TI compared with control group, the result from the present study agrees relatively well with that from [28].

CONCLUSION

In conclusion, the results presented here showed that estimate of travel index (TI) of charcoal in the test drugs revealed different responses. Allium ampeloprasum, Licorice, Ranitidine, MSG, Ciprofloxacin, Valsartan, and Syzigium aromaticum showed increased TI compare to Cs, while the medicines that decreased TI compared to C and Lo, such as Alhagi maurorum, Foeniculum vulgare, Vit C, Metamizole, and Diclofenac potassium TI compare to c.

ACKNOWLEDGEMENTS

Authors offer our thanks and gratitude to University of Basrah for its constant help during this research.

REFERENCES

1. T. Kitazawa and H. Kaiya, Front. in endocrino. 2019;10: 278-278.
2. M.Y. Teferi, M .Abdulwuhab , J.S. Yesuf, J. of Evidence-Based Integr. M. 2019;24: 2515690X19833340.
3. A. Soforowa, Medicinal Plants and Traditional Medicine in Africa (John Wiley and Sons.Ltd. New York,2008),pp 200.
4. P.B. Shamkuwar , D.P. Pawar , Inter.J.of Pharmacog. and Phytoch.Res. ,2013; 5: 24-26
5. C. Scarpignato , *Diges.Disea*. ,1997;15(Suppl. 1): 112-136.
6. A. E. Al-Snafi ,Inter. J. of Pharmac. ,2015; 5(2):90-97.
7. F.M. Birdane, M. Cemek, Y.O. Birdane, I .Gülçin , M.E. Büyükokuroğlu, World j. of gastroenterology,2007 ;13(4): 607-611.
8. S. Kobuchi, T. Kabata, K .Maeda, Y .Ito , T. Sakaeda, Antibiotics (Basel), 2020;9(4).
9. S.R. Galaly, W.G. Hozayen, K.A. Amin , S.M. Ramadan, J. of Bas. and App.Sci., 2014;3(2): 93-105.
10. E. Tadesse, E. Engidawork, T. Nedi , G. Mengistu , BMC compl. and altern. Med., 2017; 17(1): 190-190.
11. A.Y. Shettima, A.F. Sanda, H. Ali, R.F. Bello, B. Modu, Y. Tijjani , *The Pharmaceut. and Chem. J*. ,2016;3(2):323-328.
12. F.C. Edgard, L.E. Troncon, J. of Medi. and Biolo. Res. ,2019; 52(2): 8103.
13. N.K. Ibrahim , G.A. Faris, Iraqi Journal of veterinary sciences,2020;26(suppl.II):47-53.
14. F.G. Shoba , M. Thomas , J Ethnopharmacol. ,2001;76(1): 73-76.
15. A.E. Al-Snafi , IOSR J Of Pharma. ,2018;8(6 Series-2): 53-66.
16. S.C. Heghes, O. Vostinaru, L.M. Rus, C. Mogosan, C.A. Iuga , L. Filip , A Review. Molecules,2019;24(9): 1675.
17. M. Tariq, A. Tariq, S. Mussarat, M. Adnan, E.F. Abd_Allah, A. Hashem, A.A. Alqarawi , R. Ullah ,Bio Med Res. Inter., 2015: 892947.
18. M.D. Nasir , S.H. Matti , Iraqi Journal of Veterinary Sciences,2020; 26(Suppl. II): 105-110.
19. Z.Y. Salih , Iraqi Journal of Veterinary Science ,2010;24(2): 137-141.
20. P.R. Dash, M. Nasrin, S.Z. Raihan , M.S. Ali, Int J Pharm Sci & Res. ,2014; 5(9): 3864-68.
21. E.O. Jimmy, J.G. Akpan , H.O. Mbagwu, World J of med. and medical sci. ,2015;3(1): 1-6.
22. G. Tougas, D.L. Earnest, Y. Chen, C. Vanderkoy , O. Rojavin , Pharmaco. & Therap.,2005; 1: 59-65.
23. E.O. Agbaje , Nig Q J Hosp Med., 2008;18(3): 137-141.
24. G. Bassotti, G. Bucaneve, P. Furno, A. Morelli , A. Del Favero, Dig Dis Sci. ,1998;43(6): 1172-1176.
25. J. Yuan, H. Ma, N. Cen, A. Zhou , H. Tao , Biomedical reports ,2017;7(2): 179-182.
26. F. Andic , M. Garipagaoglu, E. Yurdakonar, N. Tuncel , O. Kucuk, Nutrition and Cancer,2009;61(6): 784-788.
27. S. Kato, A. Takahashi, M. Takahashi, A. Shindo, T. Yoshida, K. Kawamura , Matsumoto, B. Matsuura , PLOS ONE,2019; 14(2):1-15.
28. J. Snel, M.E. Van Den Brink, M.H. Bakker, F.G. Poelma , P.J. Heidt ,Micr. Ecolo. in Heal. and Disease,2009;9(5): 207-21.