Ondansetron: A newer aspect of dose response relationship on ileal smooth muscles of rabbit

Ayesha Afzal[1], Bushra Tayyaba Khan[2] and Salman Bakhtiar[2]
[1]Pharmacology Department, Wah Medical College, Wah Cantt, Pakistan
[2]Department of Pharmacology, Army Medical College, Abid Majeed Road, Rawalpindi, Pakistan

Abstract: There are several life threatening deadly diseases in our world but 'Cancer' out powers them all in recent years. Chemotherapy may be used on its own or an adjunct to other forms of therapy. Despite the advancement in cytotoxic drug therapy and supportive treatment almost 70% of patient suffer from chemotherapy induced nausea and vomiting (CINV). Ondansetron, a 5-HT_3 receptor antagonist has now become a gold standard in the treatment of chemotherapy induced nausea and vomiting. The central actions of ondansetron are well established but its peripheral actions are not well recognized. The aim of our study was to explore the peripheral actions of ondansetron. Experiments were performed in five groups (n=6) and ileal smooth muscles activity was recorded on power lab (USA). The effects of increasing concentrations of acetylcholine, serotonin & ondansetron alone was observed in first three groups. In the next two groups effects of acetylcholine and serotonin pretreated with fixed concentration (1ml) of ondansetron (10^{-}ϖ M) were studied. The maximum response obtained by acetylcholine served as a control for our study. Maximum response with acetylcholine was taken as 100% and with serotonin was 177 percent of control. Cumulative dose response curve with ondansetron was triphasic. At 10^{-}ψM it was 28.8%, whereas with 10^{-}ξM the amplitude decreased to 16.87%, it reached to plateau at 10^{-}ϖ M. Response of acetylcholine & serotonin was decreased to 57% and 78% respectively in the presence of fixed concentration of ondansetron (10^{-}ϖ M). Ondansetron reduces the acetylcholine and serotonin induced gastrointestinal motility. Our study has indicated that ondansetron apart from having central action also has marked peripheral actions that play an important role in CINV and may act as a partial agonist.

Keywords: Serotonin, Ondansetron, Acetylcholine, Cancer Chemotherapy, Chemotherapy induced nausea and vomiting (CINV),

INTRODUCTION

Health and diseases are two very closely related issues that came into existence the moment the new life emerges on planet Earth. ALLAH Almighty has sent the 'cure' before the disease, so it is up to us to explore and find the cure. There are several life threatening deadly diseases in our world but 'Cancer' out power them all in recent years. Cancer is a disease characterized by uncontrolled multiplication and spread of abnormal forms of body's own cells. Approximately 70% of cancer deaths occur in low and middle-income countries. The WHO has envisaged that the number of cases of cancer would double in developing countries by the year 2020. The number of cancer patients in Pakistan is increasing by 8-10 per cent per year. There were more than 1.4 million cancer patients in Pakistan. More than 8,0000 people died each year due to this disease including 40,000 from breast cancer (Hajdu *et al*., 2011). It is one of the major causes of death in the developed nations as well. In 2009, cancer was the second leading cause of death in American population (Vita., 2012), one in three people will be diagnosed with cancer during life time. Chemotherapy may be used on its own or an adjunct to other forms of therapy (Richards, 2013). This chemotherapy leads to additional acute and chronic symptoms, which add agony to patient's life (Hawkin and Gunberg, 2009). Despite of advancement in cytotoxic drug therapy and supportive treatment almost 70% of patient suffer from chemotherapy induced nausea and vomiting (Rogers and Blackburn, 2010).

There are many neurotransmitters, which are involved in the pathopysiology of nausea and vomiting. The most common are serotonin, dopamine and substance P (Neurokinin 1). Chemotherapy damages the gastrointestinal tract and activates the abdominal vagal afferents. Under the influence of cytotoxic agents the enterochromoffin cells of gastrointestinal tract are stimulated, they inturn causes release of serotonin that binds to its receptors on vagal afferent neurons. Binding of serotonin to its receptors stimulate the chemoreceptor trigger zone (CTZ) and vomiting centre (VC) in the medulla. Vomiting center also modulate impulses to the abdominal muscles, diaphragm, and esophagus resulting in emesis (Thompson, 2006).

The main goal of anti emetic therapy in patients receiving chemotherapy is to prevent acute emesis as this is the main causative agent responsible for therapeutic failure of chemotherapy. Anti emetics should be prescribed before chemotherapy and patient should be protected for uptill

**Corresponding author:* e-mail: dr.ayeshaafzal@hotmail.com

24 hours after initiation of therapy, as this improves quality of life in cancer patients, they become involved in household task, maintain daily functioning and help them in completing the chemotherapeutic treatment (Celio *et al*., 2008).

Chemotherapy induced nausea and vomiting is categorized into acute and delayed type. Acute onset emesis is due to release of serotonin and occur within 24 hours of chemotherapy, whereas delayed onset emesis is due to release of substance P (neurokinin 1) occurring within 24 hours to several days of initial treatment (Basch *et al*., 2010: Aapro *et al*., 2005). Moreover CINV leads to dehydration, electrolyte imbalance and malnutrition, which affect the quality of life. So, control of nausea and vomiting from day first is important for successful completion of chemotherapy (Bajetta *et al*., 2009).

Clinical and basic research in the past twenty five years enable us to control CINV, especially with the development of serotonin antagonist in the early 1990 was the most significant advancement in the field of cancer chemotherapy (Hawkin, 2009). It was hypothesized that acute protection from CINV was provided largely by attenuation of peripheral serotonin receptors located on vagal afferents. Current guidelines recommend the use of 5-HT_3 receptor antagonist either alone or in combination with dexamethasone and neurokinin 1 antagonist should be prescribed for both acute and delayed onset of emesis (Trigg., 2008). The central actions of ondansetron are well established but their peripheral actions are not well established. The current study was designed to explore the peripheral actions of ondansetron.

MATERIAL AND METHODS

Animals and housing conditions

Thirty healthy local rabbits (*Oryctalagus cuniculus*) both male and female (non-gravid)) of 1-2Kg weight were used for the current study and was purchased from local market. The animals were kept in animal house Army Medical College, Rawalpindi at room temperature undergoes 12hrs light and 12hrs dark cycles and were fed with a standard diet consisting of carrots, choker and grains along with tap water ad libitum.

Chemical for study

Acetylcholine Chloride (Sigma Chemical Co. USA), Serotonin Carnitine Sulfate (Sigma Chemical Co. USA), Ondansetron Hydrochloride (Werrick Pharmaceuticals Pak) were purchased. All the solutions and dilutions were prepared fresh in distilled water at the time of experiments (Noor *et al.*, 2011). The molar solutions used for all the drugs including acetylcholine, serotonin and ondansetron in strength ranging from $10^{-\psi}$ to $10^{-\varpi}$ M were made.

Preparation of tissue

Overnight fasting rabbit was sacrificed. The small intestine was taken out by recognizing the ceacum and cut down into two inch pieces (Jabeen. Q *et al*., 2007). The intestinal tissue was thoroughly washed with normal saline and fecal content was removed, fatty tissues dissected. The isolated tissue was then transferred to isolated organ bath of 50-milliliter capacity containing tyrode's solution (in mM: NaCl, 136.8mM; KCl, 2.7mM; $MgCl_2$, 0.5mM; $CaCl_2$, 1.3mM; NaH_2PO_4, 0.14mM; $NaHCO_3$, 12.0mM, Dextrose, 5.5mM) and aereated continuously with 100% oxygen. The cumulative dose respose curve was made by using power lab (USA). One end of the ileal strip was attached to the bottom of oxygen tube in tissue bath and the other end was connected to a research grade Force Displacement transducer DT-475 (USA) by means of a thread. iWorx/214(USA) was conected to the computer. Plug the DIN conector on the cable of the DT-475 Displacement transducer into channel 3 of the iWorx/214 unit. The tissue was allowed a period of equilibration of 15 minutes and physiological solution was changed 2 times. The isotonic ileal smooth muscle activity was recorded through the Displacement Transducer (Chetty *et al*., 2006; Tanko *et al*., 2012).

Construction of cumulative dose response curve

Cumulative dose response curves for ACh,5-HT and ondansetron alone was constructed using 1ml of concentrations ranging from $10^{-\psi}$M to $10^{-\varpi}$M. Cumulative response curves were also constructed for ACh and 5-HT in the presence of fixed concentration of ondansetron$10^{-\varpi}$M (3.65µM). The antagonist was allowed a period of 15 min and then cumulative dose response curve with increasing concentrations of ACh and 5-HT was made. Each tissue was used for only one discrete concentration-response curve to prevent desensitization and their responses was compared with the response of ACh $10^{-\varpi}$M (1.46µM) which was taken as 100% response (Chetty *et al*., 2006).

STATISTICAL ANALYSIS

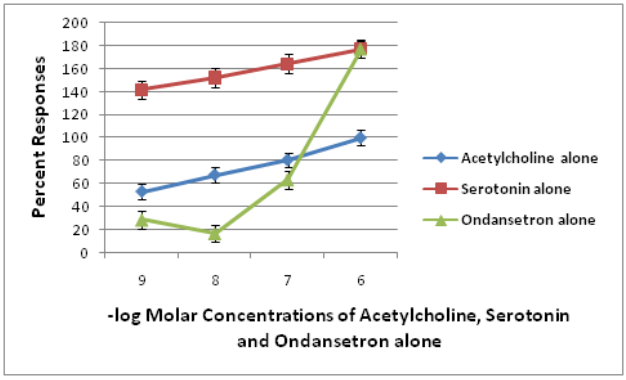
The results were expressed as Means± Standard Error of Means (SEM). The arithmetic means and SEMs were calculated using one way analysis of variance (ANOVA) using SPSS version 20, Post Hoc Tukey was applied when three drugs are compared and Student't' test was applied when two drugs were compared. The difference between observations was considered as significant if the *p* value is less than 0.05. Percent deviation was also calculated and compared.

RESULTS

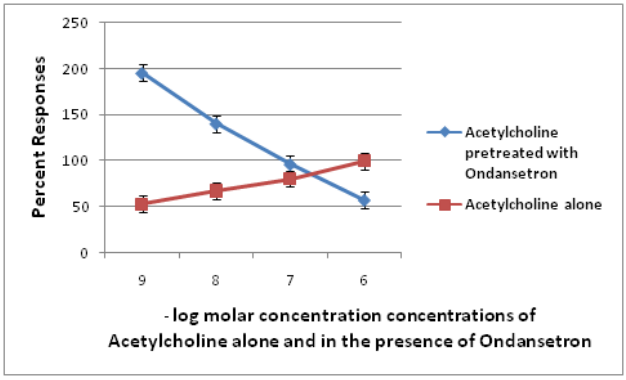
Response to acetylcholine (control)

Cumulative dose response curve of acetylcholine was obtained using 1ml concentration of $10^{-\psi}$ (0.00146µM),

$10^{-\xi}$ (0.0146µM), $10^{-\omega}$ (0.146µM) and $10^{-\varpi}$M (1.46µM). Each new concentration of acetylcholine was added after achievement of maximal response from the former concentration. The mean ±SEM values were 13.2±1, 16.8±1.6, 20±1.5 and 24.8±1.22 respectively (n=6). Percent responses were calculated for all the concentrations mentioned above, taking the response of $10^{-\varpi}$ M of acetylcholine as hundred percent. With other concentrations the percent responses were 53.21 percent, 67.73 percent and 80.64 percent respectively (graph A).



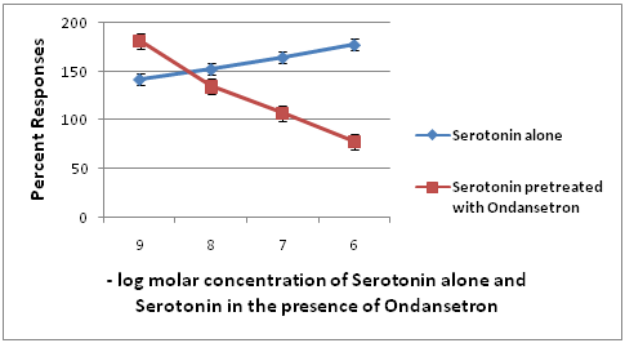
Graph A: Comparison of semi log concentration response curve of acetylcholine, serotonin and ondansetron on isolated ileal smooth muscles of rabbit (n=6). Data is represented as mean ± standard error of means (SEM)



Graph B: Comparison of semi log concentration response curve of acetylcholine alone and acetylcholine + ondansetron on isolated ileal smooth muscles of rabbit (n=6). Data is represented as mean ± standard error of means (SEM)

Response to serotonin

Serotonin produced a dose dependant, reversible contraction of ileal smooth muscle of rabbit. Aseries of six experiments were performed and the mean ±SEM values of responses to various concentration of serotonin from $10^{-\psi}$M (0.00176µM) to $10^{-\varpi}$M (1.76µM) were 35.2±0.594, 37.8±0.494, 40.8±0.494 and 44±0.527 mm respectively. The percent responses for different concentrations of serotonin were 141.8percent, 152.3 percent, 164.4 percent and 177.4 percent respectively as compared to control group (graph A).



Graph C: Comparison of semi log concentration response curve of serotonin and serotonin + ondansetron on isolated ileal smooth muscles of rabbit (n=6). Data is represented as mean ± standard error of means (SEM)

Response to ondansetron

Cumulative dose response curve of ondansetron was obtained using1m concentration of $10^{-\psi}$ (0.00365µM) to $10^{-\varpi}$M (3.65µM). The mean ±SEM values of responses to various concentration of ondansetron were 7.2±1.21, 4.2±0.816, 15.8±18.55 and 44±14.66 mm respectively. The percent responses for different concentrations of ondansetron were 28.8 percent, 16.87 percent, 63.5 percent and 177 percent respectively (n=6) as compared to acetycholine control (graph A).

Effect of ondansetron on ACh induced contractions

There was marked shift of dose response curve towards right in the presence of ondasetron. Effect of acetylcholine was studied on isolated ileal smooth muscle of rabbits by adding the different doses of acetylcholine in the presence of fixed concentration of ondansetron $10^{-\varpi}$M. The mean ±SEM values of responses to various concentration of acetylcholine were 48.5±11.23, 34.83±7.73, 23.83±6.21 and 14.16±3.92 respectively (n=6). The percent responses were 195.5 percent, 140.4 percent, 96.08 percent and 57.09 percent respectively (graph B). The result was statistically significant (p< 0.05).

Effect of ondansetron on serotonin induced contractions

Ondansetron 1ml was added in the organ bath and allowed an equilibration of 15 minutes**,** cumulative dose response curve was made with increasing concentration of serotonin (n=6). Ondansetron produced a significant change in serotonin-induced contraction of isolated ileal smooth muscle of rabbit and caused a marked rightward shift of dose response curve. The mean ±SEM values of responses to various concentration of serotonin were 45±0.526, 33.4±0.96, 26.6±0.69 and 19.4±0.64 mm respectively. Percent responses were calculated for all the concentrations mentioned above, taking the response of $10^{-\varpi}$ M of acetylcholine as hundred percent and was 181.42 percent, 134.66 percent, 107.25 percent and 78.22 percent respectively (graph C) where as the mean percent deviation was 16.30 percent (p< 0.05).

Table 1: Amplitude of contractions and percent responses of acetylcholine, serotonin and ondansetron

Concentration (M)	Amplitude of contractions (Mean ±SEM) mm Acetylcholine n=6	Percent (%) response Acetylcholine	Amplitude of contractions (Mean ±SEM) mm Serotonin n=6	Percent (%) response Serotonin	Amplitude of contraction (Mean ±SEM) mm Ondansetron n=6	Percent (%) response Ondansetron
10 ψ	13.2±1	53.21	35.2±0.544	141.8	7.2±1.211	28.8
10 ξ	16.8±1.6	67.73	37.8±0.494	152.3	4.2±0.816	16.87
10 ω	20±1.5	80.64	40.8±0.494	164.4	15.8±18.55	63.5
10 ϖ	24.8±1.22	100	44±0.527	177.4	44±14.66	177
P-value	< 0.05*					

P-value * Significant
P-value Non Significant
Mean ±SEM (standard error of mean) mmmillilitre

DISCUSSION

The effect of acetylcholine alone, on gastrointestinal ileal smooth muscles of rabbit was used as a control and maximum response was taken as 100%. This study was carried out in five groups with each group containing six animals. In the first group of experiments the effect of different concentrations of acetylcholine was studied on isolated ileal smooth muscles. The acetylcholine caused a concentration dependent increase in the amplitude of contractions by acting on the muscarinic receptors (M_3). Activation of M_3 produced an inositol triphosphate (IP_3) mediated release of intracellular calcium, the release of diacylglycerol (which inturn activate protein kinase C), causes contraction of smooth muscles. The maximum amplitude of contraction with acetylcholine was taken as 100%. This group served as a control for our study and all other groups were compared with the effect of acetylcholine (Chetty *et al*., 2006).

In the second group, the effect of increasing concentrations of serotonin was studied on isolated ileal smooth muscles of six different rabbit. Serotonin produced a dose dependent and sustained contractile response, which was 177 percent of acetylcholine mediated response on ileal smooth muscles of rabbit.

This result is in close agreement with the previous findings of Mujezinduic and his colleagues who postulated that serotonin has diverse motor and sensory functions in the gastrointestinal tract through sub mucosal and myenteric neurons. Serotonin influences the gastrointestinal motility by acting directly through 5-HT_4 receptors on enterocytes and indirectly via 5-HT_3 receptors on mucosal nerves and vagal afferents (Mujezinduic *et al*., 2011).

Our finding is also in correlation with the findings of Pithadia along with his co- workers, who also observed increase in contractile response of serotonin as compared to acetylcholine. 5-HT_4 receptors are G- protein coupled receptors and are located on both cholinergic interneurons and motor neurons. The stimulation of 5-HT_4 receptors causes an increase in the acetylcholine release which in turn increases the intestinal motor activity (Pithadia *et al*., 2009).

According to Chetty and his fellows, serotonin produces an increase in contractile response because of its ability to stimulate 5-HT_3 receptors in both cholinergic and non-cholinergic neurons (Chetty., 2006). However the increase in response by serotonin was best explained in the past by Robbin Spiller. He concluded that, 5-HT_3 receptors are ligand gated cationic channel, on binding, serotonin opens the channels allowing the entry of inward rectifier potassium current as well as calcium leading to depolarization. Whereas, 5-HT_4receptors are G-protein coupled, ligand binding activates an increase in cAMP via adenylcyclase causing activation of protein kinases, inhibiting potassium channels preventing hyper polarization thereby enhancing excitability of the cells. The actions of 5-HT_4 is longer lasting as compared to 5-HT_3 effects and is well suited in neuro modulatory role causing an increase in serotonin release more than acetylcholine (Robbin., 2002).

In-group 3, concentration response curves for ondansetron were constructed. The response was biphasic showing increase response at dose of 10^{-}ϖM and decrease respone at 10^{-}ω, 10^{-}ξ and 10^{-}ϖM. This triphasic response with ondansetron was peculiar to our work. Literature search revealed no such varying response at low and high concentrations. The decrease in response is related to the ability of the drug to antagonise 5-HT_3 receptors. However, the increase in response at high doses needs further evaluation (Tsukaamoto *et al*., 2007). The central effects of ondansetron, which is a 5-HT_3 antagonist, are well established but its peripheral actions were not well elucidated (Camilleri *et al*., 2002). To explore the peripheral actions of ondansetron, we also observed the

Table 2: Amplitude of contractions and percent responses of acetylcholine and serotonin in the presence of fixed concentration of ondansetron $10^{-\varpi}$ M

Concentration (M)	Amplitude of contractions (Mean ±SEM) mm ACh Control n=6	Amplitude of contractions (Mean ±SEM) mm ACh + Ondansetron n=6	Percent (%) response Ach +Ondansetron	Amplitude of contractions (Mean ±SEM) mm Serotonin +Ondansetron n=6	Percent (%) response Serotonin +Ondansetron
10 ψ	13.2±1	48.5±11.23	195.55	45±0.526	181.42
10 ξ	16.8±1.6	34.83±7.73	140.44	33.4±0.96	134.66
10 ω	20±1.5	23.83±6.21	96.087	26.6±0.69	107.25
10 ϖ	24.8±1.22	14.16±3.92	57.096	19.4±0.64	78.22
P– value	< 0.05*				

P-value * Significant
P-value Non Significant
Mean ±SEM (standard error of mean) mmmillilitre

responses of acetylcholine and serotonin in the presence of fixed concentration of ondansetron ($10^{-\varpi}$M).

When the concentration response curves were plotted with increasing concentration of acetylcholine in the presence of fixed concentration of ondansetron ($10^{-\varpi}$M), ondansetron produced a 57 percent decrease in the motility of the intestinal smooth muscles of rabbit as compared to group 1 in which acetylcholine alone was used as a control. This finding though consistent was surprising, because ondansetron alone at same concentration enhanced the motility of intestinal smooth muscles. This finding can only be explained if ondansetron has either some partial agonistic activity on 5-HT receptor at this concentration or it has some activity on some of the other receptors present in the ileal smooth muscles namely cholinergic or histaminic receptors. This aspect needs to be further investigated.

In-group 5, the contractile effects of serotonin on ileal smooth muscles of rabbit in the presence of fixed concentration of ondansetron ($10^{-\varpi}$M) also decreased the serotonin induced prokinetic response. However, the magnitude of this decrease in response was significantly less than the decrease produced by ondansetron in acetylcholine-induced contraction. This result is consistent with the findings of Tuladhar *et al* 2002, which showed a more or less parallel shift of concentration response curve of serotonin to the right in the presence of ondansetron. This result strengthens the concept that apart from having central actions ondansetron also has significant peripheral actions on gastrointestinal smooth muscles.

CONCLUSION

Our study has indicated that ondansetron apart from having central action by blocking 5-HT_3 receptors also has marked peripheral actions as well. Where, it reduce the acetylcholine and serotonin induced gastrointestinal motility. But, the fact that at higher concentration ondansetron alone can enhance the motility of intestinal smooth muscles indicate that it either has a partial agonistic activity at 5-HT_3 receptors or it enhances the motility by virtue of its interaction with other receptors present in the intestinal smooth muscles. This aspect of ondansetron can be investigated in future studies (Tsukamoto *et al*., 2007).

ACKNOWLEDGEMENT

I am grateful to NUST for its financial support

REFERENCES

Aapro, MS, Molassiotis A and Oliver I (2005). Anticipatory nausea and vomiting. *Support care cancer,* **13**: 117-121.

Bajetta E, Puscedd S, Guadalupi V, Ducceschi M and Celio L (2009). Prevention of acute chemotherapy induced nausea and vomiting. The role of palanosetron. *Cancer Manag Reo*., **1**: 89-97.

Barrack S (2011). Selective serotonin reuptake inhibitors (SSRIs). *J. Neurology Version,* volume 29(8): 954-956.

Basch E and Abernethy AB (2011). Supporting clinical practice decisions with real-time-patient-reported outcome. *J Clin Oncol* **29**(8): 954-956.

Camelleri M (2002). Serotonergic modulation of visceral sensation: Lower gut. *Gut.,* **51**(Supp1): 181-186.

Chetty N, Irving RH and Coupar MI (2006). Actiation of 5-HT_3 receptors in the rat and mouse intestinal tract: A comparative study. *Br. J. Pharmacol.,* **148**: 1012-1021.

Devita VT Jr and Rosenberg SA (2012). Two Hundred Years of Cancer Research. *N. Engl. J. Med.,* **366**(23): 2207-2214.

Hajdu SI, Thun MJ, Hannan LM and Jemal A (2011). A note from history: Landmark in history of cancer. *Cancer,* **117**(5): 1097-102.

Hawkin's R and Gunberg S (2009). Chemotherapy induced nausea and vomiting. Challenges and opportunities for improved patient outcome. *Clin. J. Oncol. Nurs*., **13**: 54-64.

Jabeen Q, Aziz N, Afzal Z and Gilani HA (2007). The spasmogenic and spasmolytic activities of lavandula Stoechas are mediated through muscarinic receptor stimulation and calcium channel blockade. *Int. J. Pharmacol*., **3**(1): 61-67.

Mujezinovic I, Cupic V, Samajlovic A and Muminovic M (2011). Identification of serotonergic (5-H_{1A}-Type) receptor in broiler small intestine by application of its serotonin and antagonist. *Vet. glasnick*., **65**(2): 51-59.

Pithadia BA and Jain MS (2009). 5-Hydroxytryptamine receptor subtypes and their modulation with therpeutic Potentials. *J. of clin. Med Res.*, **1**: 72-80.

Richards R (2013). Is chemotherapy always necessary for breast cancers? Ehow Health (newsletter) National Cancer Institute, National Institutes of Health.

Rogers and Blackburn (2010). Use of neurokinin-1 receptor antagonists in patients receiving moderately or highly emetogenic chemotherapy. *Clinical J ONS 14*, 500–504. doi: 10.1188/10.CJON.500-504

Spiller R (2002). Serotonergic modulating drugs for functional gastrointestinal diseases. *Br. J. Pharmacol.*, **54**: 11-20.

Tanko Y, Alladey O, Ahmad KM, Muhammad A and Musa KY (2012). The effect of methanol leaves extract of Ficus Glumosa on gastrointestinal motility and castor oil induced diarrhoe in laboratory animals. *Scholar research library*, **2**(3): 360-367.

Thompson AJ and Lummis SC (2006). 5-HT_3 receptors. *Curr. Pharm.*, Vol 12(26): 3615-3630.

Trigg ME and Inverso DM (2008). Nausea and vomiting with high-dose chemotherapy stem cell rescue therapy: A review of antiemetic regimens. *Bone Marrow Transplant*, **42**: 501-506.

Tsukaamoto K, Ariga H, Mantyh C, Pappas NT, Yanagi H, Yamamura T and Takahashi T (2007). Luminally released serotonin stimulates colonic motility and accelerates colonic transit in rats. *Am. J. PhysiolIntegr., Comp. Physiol*., **293**: R64-R69.

Tuladhar RB, Costall B and Naylor JR (2002). Modulation of 5-HT_4 receptor functionin the rat isolated ileum by fluoxetine: The involvement of endogenous 5-Hydroxytryptamine. *Br. J. Pharmacol*., **136**: 150-156.