Author's response to reviews

Title: Anti-emetic mechanisms of Zingiber officinale against cisplatin induced emesis in the pigeon; behavioral and neurochemical correlates

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Author's response to reviews: see over
Response to reviewer’s comments:

Reviewer: Latifa Bulbul

1. Analytical protocols for phytochemical investigation are not reported.

Response:

We would like to thank the referee for his/her comment regarding phytochemical investigations of *Zingiber officinale* (Ginger).

Ginger has been extensively screened for its phytochemical composition and report for the presence of pungent constituents collectively known as “gingerols” for its pharmacological profile, where the literature is already providing evidences about phytochemical composition of ginger extracts [1-3]. In current study, we selected acetone fraction by following the study of Sharma and co-workers [4] and we extended the study to find out its impact on neurotransmitters (and metabolites) implicated in the act of vomiting.

2. Lines 137. Why you did not mention Fig-1

Response:

We thank the referee for his/her comments.

“Figure 1” provide an overview for the extraction procedure and the same have been mentioned in the section “Extraction of *Zingiber officinale*”
3. The real biological effects are due to a particular compound present in the extract?

Response:

We thank the referee for his/her comment regarding the presence of particular compound responsible for real biologic/pharmacologic effects.

Ginger finds massive use in many of the world’s different medicinal systems and has been traditionally used for the treatment of many disorders. The major pharmacologic/biologic activity of ginger appears to be from the active ingredients collectively known as “Gingerols” (Figure) which have the effects of antiemetic, analgesic, antipyretic, antitussive, antiulcer, hypotensive and cardiodepressant. Gingerols suppress gastric contraction but increase gastrointestinal mobility [5-6] and spontaneous peristaltic activity. They have also the antiserotonin effect and scavenging activity against the free radicals causing emesis [7]. The standardization of ginger extract is reporting the quantities of gingerols in concentration ~ 60 mg/g of extract [8]. In our study, we used acetone fraction of ZO based on the study of Sharma and Co-workers [4], where acetone fraction is reported to be more effective to attenuate cisplatin induced emesis in dogs.

Figure: Gingerol
4. Author didn’t clarify extraction procedure & how to isolate active compound?

Response:

We thank the referee for his/her comments.

Maceration method was used for the extraction of active components (gingerols).

To address the reviewers concern, the following paragraph has been added in the methods chapter “Extraction of Zingiber officinale” in the revised version of the Manuscript.

“A total of 0.5 kg of fresh ginger was bought from the central vegetable market in Mardan, Pakistan. A sample was deposited at the Herbarium of the Department of Botany, The University of Peshawar, Peshawar, with the voucher number (voucher No 20017 - pup). Ginger was washed for any contaminants and then sliced to expose the inner part. It was then soaked in 2 L of acetone and kept for a total of 3 days, thrice. The combined filtrate was concentrated in a rotary evaporator to obtain a thick extract with a yield of 4.72 % (Figure 1)”

5. The subject of the article is potentially interesting but the manuscript is not well written and organized. The scientific content of the manuscript fits with the general scope of the journal. Conclusions part is not well written and anti-emetic action is peripheral or CNS didn’t mention.

Response:

We thank the referee for appreciation of the Manuscript and concluding that the Manuscript falls in the general scope of the journal.

We have reviewed the manuscript and got more input from co-authors to improve the write up and presentation.
We have also revised Conclusions chapter and modified it according to the reviewer’s concern especially mentioning central and peripheral anti-emetic mechanisms. The following conclusion paragraph is now inserted in the revised version of the manuscript.

“In conclusion, ZO acetone fraction attenuated cisplatin induced vomiting in the pigeon which is mediated by both central and peripheral anti-serotonergic and anti-dopaminergic components in a blended manner at the two different time points. At the acute time point (3\textsuperscript{rd} hr), dominantly the anti-serotonergic effects were seen centrally in the area postrema and brain stem as well as peripherally at the level of intestine, while at the delayed time point (18\textsuperscript{th} hr) anti-serotonergic effects were observed centrally in the brain stem and peripherally in the intestine where centrally in the area postrema there was anti-dopaminergic aftermath”

6. In the introduction section, the aim of the current study should clearly be presented.

Response:

We thank the referee for his/her suggestion regarding the clear presentation of “Aim of the current study”.

To address the reviewer’s concern we have modified the statements for clarity of the “Aim of the current study” and are presented as follows

“Keeping in view the gastroprokinetic and 5HT\textsubscript{3} receptor antagonist property, the present study was designed to screen the intrinsic anti-emetic activity of *Zingiber officinale* (ZO) against cisplatin induced emesis in pigeon. Furthermore, considering the relevance of serotonin and dopamine in emesis, this study was extended to evaluate the participation of these monoamine neurotransmitters and their metabolites in cisplatin induced vomiting, and to examine the impact of *Zingiber officinale* (ZO) on serotonin, dopamine and their metabolites centrally in specific brain areas involved in the act of vomiting and peripherally in the intestine in pigeon”
7. Figure-2 is obscure.

Response:

We thank the referee for his/her comments.

The resolution of the “Figure 2” has been enhanced in the revised version of the Manuscript.

8. There are several research articles published on the same plant and with the same activities & ways but with different procedure and animal (1. S SHARMA, V KOCHUPILLAI, S GUPTA, S SETH, Y GUPTA. Antiemetic efficacy of ginger (Zingiber officinale) against cisplatin-induced emesis in dogs. Journal of Ethnopharmacology – J ETHNOPHARMACOL, 1997; 57(2):93-96.


Response:

We appreciate the reviewer for finding out related articles on the chemotherapy induced vomiting in different animal models.

In research on vomiting, there are important species differences. In general, vomiting phenomenon is not so important for survival but is highly advantageous for the body to get rid of toxic substances which have been ingested. The animals which are commonly used in laboratory research including rats, mice, rabbit, guinea pig and hamster are lacking the vomiting reflex
therefore, the alternative options are cats [9], dogs [10], pigeon [11], Suncus murinus [12], least schrew [13] and some other species capable of vomiting response.

The article

“Sharma, S.; Kochupillai, V.; Gupta, S.; Seth, S.; Gupta, Y. Antiemetic efficacy of ginger (Zingiber officinale) against cisplatin-induced emesis in dogs. Journal of ethnopharmacology, 1997, 57(2), 93-96” is a very interesting study and is an article of interest for our emesis research group. This said article is the behavioral study using vomit model of Dog and they have concluded the intrinsic anti-emetic activity of various fractions of Zingiber officinale against cisplatin induced vomiting and they found the acetone fraction to be highly effective to attenuate cisplatin induced vomiting which is imperative for the presence of gingerols in enough quantities in the acetone fraction. In the current study, we followed Sharma and Co-workers to test acetone fraction against cisplatin induced vomiting in the Pigeon model and correlated our behavioral findings with the neurotransmitter and their metabolites involved in the act of vomiting, centrally at the level of brain stem and peripherally in the intestine. Our study has provided evidences for the involvement of anti-serotonergic and anti-dopaminergic mechanisms for the mediation of anti-emetic effect by ginger acetone fraction in the pigeon.

The article by N Takeda and Co-workers titled “Effects of antiemetic drugs on cisplatin-induced pica in rats”

And another similar study

are the studies conducted in rodents which are based on the “Pica behavior”. “Conditioned Taste Aversion (CTA)” is another paradigm used.

There are reports that motion or poison induces pica behavior (eating of non-nutritive substances such as Kaolin etc) in rats. This pica in rats (non-vomiting specie) is considered analogous to vomiting in the species that vomit.

CTA is also a paradigm used in rats to assess anti-emetic potential of chemical entities/molecules and is considered as prodromal sign of emesis in species which vomit.


*Suncus murinus* (*S. murinus*, House musk shrew, family, *Soricidae*) locally known as Chuchunder, has been used in emesis research since 1980s [12], to study the mechanisms of chemotherapy induced vomiting. *S. murinus* is an acceptable animal model for the study of vomiting induced by chemotherapy [14]. The vomiting response can be observed with emetic challenge by motion [15], X-irradiation [16], copper sulphate, nicotine and cancer chemotherapeutic agents [14, 17].

*Cryptotis purva* (least shrew) is also used now in emesis research and is an acceptable model for the study of cisplatin induced acute and delayed sickness [18-20].
Minor Essential Revisions

Since the authors used animals it is required to state whether the experimental protocol has been reviewed and approved by the institutional review board.

Response:

The experimental protocol/procedures were already been reviewed by the Ethical committee Department of Pharmacy, University of Peshawar.

The same have already been mentioned in the Chapter: Material and methods, but to address reviewer’s concern about the approval from institutional review board, the following paragraph is added in the Chapter: Material and methods:

“All the experimental procedures were approved by the Ethical Committee of the Department of Pharmacy, University of Peshawar (Ref. No 5/EC/Pharm; Dated: June 15, 2011) and are in accordance with the UK Animal Scientific Procedure Act, 1980”

Discretionary Revisions comments

Introduction section doesn’t report a phytochemical introduction for the titled species. Please provide sentences that report paper and/or studies on this plant.

Response:

We thank the referee for his/her comments.

To address the reviewer’s concern the following Para has been added in the introduction section:

“The major components present in ginger extract which is a mixture of homologues having 10, 20 and 14 carbon atoms in side chain that are designated as gingerols [21]. Gingerols, in particular 6-gingerol has been identified as the major active constituent”
References:


Response to reviewer’s comments:

Reviewer: Ralf Regenthal

1. The authors should comment on the limitations of the study, i.e. that the actions on behaviour and neurotransmitters are restricted to the total acetonic extract.

Response:

We thank the referee for his/her comments.

Gingerol is the generic term for pungent constituents in ginger, which has been reported to be effective for inhibiting vomiting. Ginger juice and ethanol extraction significantly inhibited the emesis induced by cisplatin, copper sulfate and motion sickness. Gingerol, has been reported to inhibit contraction of isolated guinea-pig ileum by acting on 5-HT3 receptors and this is presumed to be related to its antiemetic activity [1]. Gingerol s extraction has been reported with solvents like pure acetone, ethanol and ethanol + aqueous (50:50) [2]. All the extracts were found effective to attenuate cisplatin induced vomiting in dogs as reported by Sharma and Co-workers [2], but the acetone fraction showed more efficacy as compared to other fractions tested. Following the study of Sharma and Co-workers [2], we proceeded with acetone fraction of ginger to find out its intrinsic antiemetic activity against cisplatin induced vomiting in relation to its impact on central and peripheral neurotransmitters (and metabolites) involved in the act of vomiting.

To address the reviewer’s concern we have added the following text in the discussion section of the revised manuscript

“Ginger acetone extract was selected to find out its intrinsic antiemetic activity in line with its impact on central and peripheral neurotransmitters (and metabolites) involved in the act of...
vomiting, because the said fraction was found to be more effective to attenuate cisplatin induced vomiting in dogs as reported by Sharma and co-workers [2].

2. I would advice a more exact title like: “Anti-emetic actions of *Zingiber officinale* against cisplatin induced acute emesis in the pigeon – behavioural…” as rather time-related different neurotransmitter modulations are studied in comparison to MCP than “mechanisms” can be derived.

**Response:**

We would like to pay thanks to the referee for suggestions in the title. Neural correlations are very complex especially talking about cisplatin induced vomiting, as cisplatin induces bi-phasic (acute and delayed phase) vomiting in human [3] and in vomit models of dog [4] and ferret [5] and both the phases are mechanistically different from each other. In the Pigeon model we are providing evidences for the involvement of different neurotransmitters at the two different time points, which are refractory for the presence of two phases, although it needs more studies with regard to neuropeptides like substance P to establish the split among acute and delayed sickness in the Pigeon model. We are happy with the title “Anti-emetic mechanisms of *Zingiber officinale* against cisplatin induced emesis in the pigeon; behavioral and neurochemical correlates” as in the current study we are focusing on mechanisms and have provided evidences for the involvement of serotonergic and dopaminergic components in a blended way. The mention of “acute emesis in the Pigeon” in the title will not be so appropriate as we are looking at the two different time points i.e. 3rd hour and 18th hour; for involvement of different neurotransmitters and their metabolites to find out the split among the acute and delayed sickness in the Pigeon model as
Tanihata and Co-workers have suggested [6], although to conclude the split among acute and delayed phase of cisplatin induced vomiting in the pigeon model need further investigations.

3. Method Chapter Tissue Sampling for neurotransmitter analysis: The authors should make clear, that that brain samples were cleaned from vessels and meninges before freezing and consecutive analyzing, as this may influence the results.

Response:

We thank the referee for his/her comments with regard to cleaning of tissue samples from vessels and meninges etc. In the current study off course we have gone through proper cleaning of tissue samples whether of brain or intestine and the dissection of tissue samples was followed by cleaning from vessels and meninges (brain samples), fecal matter and mesenteries (intestinal samples) and were then stored at -80°C until analysis.

To address the reviewer concern, the following paragraph with regard to proper cleaning of tissue samples have been incorporated in the Section “Tissue sampling for neurotransmitters analysis” in the revised version of the manuscript.

“The collected brain tissue samples were first cleared of vessels and meninges, while fecal matter and mesenteries were carefully removed from intestinal samples, were weighed and stored at -80°C until analysis.”
4. The Result Chapter “Effect of ZO-ActFR or MCP on cisplatin induced jerks and weight loss” can be omitted, as these parameters are only secondary; they do not contribute to the main results and are not subject of discussion.

**Response:**

We are thankful to the referee for his opinion regarding parameters like jerks and weight loss.

We agree with the referee that the Result Chapter “Effect of ZO-ActFr or MCP on cisplatin induced jerks and weight loss” are secondary, but they have importance in determination of antiemetic potential of chemical entities and plant extracts. Jerks/Jerking* behavior reflects the vomiting intensity and one vomiting episode may comprised of 2 – 80 Jerks whereas weight loss is also important to be considered as the persistent vomits and diarrhea results in weight loss and the subsequent significant reduction in weight loss is in support of the anti-emetic profile.

*I failed to attach the video clip (20.3MB) of the experimental recordings to clarify the jerking episodes as the online submission system did not uploaded it as supplementary data.

To address the reviewer’s concern we have added the following text in the discussion section of the revised version of the manuscript

“ZO-ActFr (25 mg) also resulted in the decrease in jerks (indicative of vomiting intensity) and reduced the weight loss significantly. The reduction in jerks and weight loss are the secondary parameters supporting the antiemetic profile of ginger acetone extract”

**Discretionary Revisions**

1. Introduction is coherent and conclusive.

**Response:**

We thank the referee for appreciation of the Introduction Chapter.
2. Method Chapter Drugs and Chemicals: Please correct: sodium dihydrogen orthophosphate, only.

Response:
We apologize for the mistake in the methods Chapter.
sodium dihydrogen orthophosphate is now corrected in the revised version of the Manuscript.

3. Method Chapter Determination of neurotransmitters: Additionally to refer to a reference, please give a brief method validation with essentials like limit of quantification (LOQ), inter-assay and intra-assay reproducibility, recovery of analytes and the parameters which was used for quantification e.g. peak height or peak area. These issues are often highly problematic in electrochemical detection of neurotransmitters.

Response:
We thank the referee for suggesting the incorporation of method validation and other parameters related with HPLC analysis of neurotransmitters.
The method for determination of neurotransmitters and their metabolites was developed by our laboratory and has been published [7-8] and considering your this comment in “discretionary revision” section we would like to say that the mention of above parameters in the current Manuscript will be unnecessary repetition of already published work.
4. Result Section: Chapter “Effect of standard MCP or ZO-ActFR on the level of neurotransmitters …at 3rd hour of cisplatin administration” – The 2. Paragraph which begins with “The treatment with standard MCP …” is only true, if “in comparison with saline control” is added. Further in the text, “5HT reduction in BS/intestine” is so not indexed in table 3. Chapter: “Effect of ….at 18th hour of cisplatin administration”: In the 4th paragraph the reviewer would advice the words pre-treatment instead of treatment and diminished instead of decreased.

**Response:**

We thank the referee for his/her comments.

The manuscript is modified accordingly.

5. Discussion: 4th paragraph: Please consider…nonetheless longer, but moderate protection. The last paragraph remains unclear in meaning and content – please prove the content.

**Response:**

We thank the referee for his/her comments.

The manuscript is modified accordingly.

6. References: Ref. 17 and 18 are incorrectly cited and need a verification.

**Response:**

We thank the referee for his/her comments.

The references are verified and modified accordingly.
References:


Response to reviewer’s comments:

Reviewer: Muhammad Zia-Ul-Haq

1. The study although novel is very limited and supported by only 1 animal model. I therefore don't recommend its publication in journal.

Response:

We understand the discretion of the reviewer not to recommend the manuscript for publication although the reviewer admits the present study as novel.

In emesis research most of the articles have been published where the studies are conducted on only one animal model and are published in reputable international journals. For example


Potent inhibition of both the acute and delayed emetic responses to cisplatin in piglets treated with GR205171, a novel highly selective tachykinin NK$_1$ receptor antagonist. *British journal of pharmacology*, **2009**, 124(8), 1643-1650.

Hereby, we are in line with previous studies and agree with our Manuscript in the one vomit model of Pigeon providing mechanistic evidences for the antiemetic action of *Zingiber officinale*.

2. Models/manufacturers of instruments like HPLC are not mentioned in methods section.

Models/manufacturers of instruments like HPLC are already mentioned in the methods section as mentioned below.

“Neurotransmitters and their metabolites were analyzed using High Performance Liquid Chromatography system (HPLC, Shimadzu, Japan) coupled with Electrochemical Detection (ECD, ESA Coulochem III model 5300), a pump (model LC-20AT), and analytical column (Teknokroma 3 x 150, 3um)”

3. Even ZO authentication has not been provided. No pic of histology has been provided.

“The plant rhizome were purchased from a local market at Mardan and was authenticated by Prof. Dr. Muhammad Ibrar, Department of botany, University of Peshawar”

“A sample was deposited at the Herbarium of the Department of Botany, The University of Peshawar, Peshawar, with the voucher number (voucher No 20017 - pup)”

The above paragraphs are already been present in the methods section in the Manuscript and clearly indicate the authentication of *Zingiber officinale*. 