Phytotherapeutic Approach in the Management of Cisplatin Induced Vomiting; Neurochemical Considerations in Pigeon Vomit Model

Ihsan Ullah,1 Fazal Subhan,2 Muhammad Shahid,2 Nisar Ahmad,3,4 Rehmat Shah,5 Javaid Alam,6 Ikram Ul Haq,7 Rahim Ullah,8 Muhammad Ayaz,9 and H. C. Ananda Murthy10,11

1Department of Pharmacy, University of Swabi, Swabi, Pakistan
2Department of Pharmacy, Institute of Integrative Biosciences, CECOS University of IT and Emerging Sciences, Peshawar, KP, Pakistan
3Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
4Department of Pharmacy, Islamia College of Pharmacy, Sialkot, Pakistan
5Pharmacist, Health Department, Khyber Pakhtunkhwa, Pakistan
6Drug and Herbal Research Center, Faculty of Pharmacy, University Kebangsan, Malaysia
7National Institute of Health, Islamabad, Pakistan
8Sarhad University of Science and Information Technology, Peshawar, Pakistan
9Department of Pharmacy, Faculty of Biological Sciences, University of Malakand, Chakdara, 18000 Dir (L), KP, Pakistan
10Department of Applied Chemistry, School of Applied Natural Science, Adama Science and Technology University, P O Box 1888, Adama, Ethiopia
11Department of Prosthodontics, Saveetha Dental College & Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai 600 077, Tamil Nadu, India

Correspondence should be addressed to Ihsan Ullah; ihsanmkd@gmail.com, Muhammad Ayaz; ayazuop@gmail.com, and H. C. Ananda Murthy; anandkps350@gmail.com

Received 11 July 2022; Revised 29 August 2022; Accepted 2 September 2022; Published 13 September 2022

Academic Editor: Tarique Hussain

Copyright © 2022 Ihsan Ullah et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Cisplatin induced vomiting involves multiple mechanisms in its genesis and a single antiemetic agent do not cover both the phases (acute & delayed) of vomiting in clinics; necessitating the use of antiemetics in combination. Cannabis sativa (CS), Bacopa monniera (BM, family Scrophulariaceae), and Zingiber officinale (ZO, family Zingiberaceae) in combinations against vomiting induced by highly emetogenic anticancer drug-cisplatin in pigeons. We have analysed the neurotransmitters which trigger the vomiting response centrally and peripherally. Electrochemical detector (ECD) was used for the quantification of neurotransmitters and their respective metabolites by high performance liquid chromatography in the brain stem (BS) and area postrema (AP) while peripherally in the small intestine. Cisplatin (7 mg/kg i.v.) induced reliable vomiting throughout the observation period (24 hrs). CS-HexFr (10 mg) + BM-MetFr (10 mg)–Combination 1; BM-ButFr (5 mg) + ZO-ActFr (25 mg)–Combination 2; ZO-ActFr (25 mg) + CS-HexFr (10 mg)–Combination 3; and CS-HexFr (10 mg) + BM-ButFr (5 mg)–Combination 4; provided ~30% (30 ± 1.1), 70% (12 ± 0.4; P < 0.01), 60% (19 ± 0.2; P < 0.05) and 90% (05 ± 0.1; P < 0.001) protection, respectively, against cisplatin induced vomiting as compared to cisplatin control. Standard MCP (30 mg) provided ~50% (23 ± 0.3) protection (P > 0.05). CS Hexane fraction (10 mg/kg), BM methanolic (10 mg/kg) and bacoside rich n-butanol fraction (5 mg/kg) and ZO acetone fraction (25 mg/kg) alone provided ~62%, 36%, 71%, and 44% protection, respectively, as compared to cisplatin control. The most effective and synergistic combination 4 was found to reduce 5HT and 5HIAA (P < 0.05 – 0.001) in all the brain areas area postrema (AP), brain stem (BS) and intestine at the 3rd hour of cisplatin administration. In continuation, at the
1. Introduction

Nausea and vomiting are the two important adverse effects faced by the patients undergoing cancer chemotherapy [1, 2]. These adverse effects may often result in noncompliance to the chemotherapy but it may result to the refusal of patients to undergo emetogenic chemotherapy cycles. It is known that the cancer is second in the United States which resulted in more deaths; and the studies advocate the increase in the number of cancer patients and, more importantly, breast, lungs, head and neck, colorectal, and stomach carcinomas [3–5].

The emetogenicity of antineoplastics varies and that is why they are also classified based on its emetogenic propensity. The high emetogenic class contains all the platinum analogues including cisplatin. Cisplatin has the unique aspect that induces vomiting in two phases; the first phase which stays up to 24 hours is known as acute phase while the phase after 24 hours is called delayed phase and it is believed today that it remains up to 7 days after the initiation of chemotherapy cycle. Mechanistically, the vomiting caused by cisplatin is multifactorial with respect to acute and delayed phases. The acute phase is triggered by serotonin; the primary neurotransmitter to be considered while neurokinin 1 receptor antagonists (Apprepitant) in combination with dexamethasone. In clinical setups, the combination of antiemetics is used to control both the phases of vomiting induced by cisplatin by following the international guidelines, but still this is a clinical challenge, as the considerable proportion of patients undergoing cancer chemotherapy faces the problem of vomiting [8, 9] making it a need for a time to look for new antiemetic having a broad spectrum so it has the capability to control both the phases of vomiting.

The natural plants and the phytochemicals isolated from them are proved to be very important for ailing community and also provide structural templates for the new compounds to be developed [10–15]. The drugs like Quinine etc. have their source from plants and have still significance in the management of various diseases [16–22]. The scientific community is still involving in the isolation and characterization of active phytochemicals against various pathologies [23–25]. Recently, the standardization of extracts/fractions/isolates is getting much more attention to identify the active moiety responsible for the therapeutic response [26–30]. Keeping in view the biphasic vomiting response and the multimechanisms behind the two phases established the use of antiemetics in combination and provides the platform to search for a cost effective combination of herbal origin which may provide good control over the acute and delayed phases of vomiting in clinics. The current study is focusing on Bacopa monniera (BM), Cannabis sativa (CS), and Zingiber officinale (ZO) to see their impact on cisplatin induced vomiting either alone or in combination in the pigeon emesis model. Pigeon emesis model is good for preliminary screening of chemical entities/compounds/extracts/fractions for the antiemetic potential and has been used by the scientific community for the said purpose. Pigeon demonstrates a robust and very clear vomiting response as compared to Suncus murinus to almost all the emetogens.

The literature is rich enough to advocate the antiemetic effect of Cannabis sativa and Zingiber officinale as antiemetic, while the antiemetic activity of Bacopa monniera is reported by our laboratory for the first time. The major chemical moiety of Cannabis extract i.e. Δ9-tetrahydocannabinal (Δ9-THC) has been shown to be antiemetic and also shown promising results in clinics [31, 32]. Furthermore, the cannabis preparations have shown superior antiemetic activity as compared to Dopamine receptor blockers [33]. The identification of endocannabinoids and cannabinoid receptors [34, 35] revolutionized the research in cannabinoids for the two decades.

Zingiber officinale is well known for its use as spice and flavouring agent and also been used for the treatment for vomiting and anorexia [36]. Gingirol is reported as the active component responsible for its activities along with some other moieties as well [36, 37]. Sharma and his coworkers have reported the antiemetic activity of ginger against cisplatin induced vomiting in dogs [38] and against cyclophosphamide induced vomiting in the Suncus murinus [39].

Bacopa monniera is well known for its significance in the management of memory impairments [40] and cognitive disorders [41]. The literature is indicating bacosides as the major and important chemical constituent responsible for its activities along with some other constituents [42]. Our laboratory also did the HPLC fingerprinting of bacopa extracts [43] indicating the bacosides as major constituents. Our studies also provided evidences for the mechanisms behind the antiemetic activity of bacosides against cisplatin induced vomiting in pigeon. Based on the previous reports as its antidopaminergic aspect and our findings the bacoside rich fraction is included in the current study.

The antiemetic activity of CS, ZO, and BM is well investigated alone. Keeping in view the mechanistically multifactorial phenomenon associated with cisplatin induced vomiting, we hypothesize that the combinations of these safe and tolerable plant extracts may exhibit a broad spectrum antiemetic activity which may be helpful to cover all the phase of vomiting caused by cisplatin in clinics.
2. Materials and Methods

2.1. Animals. Pigeons of both gender (male and female) and of all species (mix breed at the breeding facility of the Department) having weight in range of 250–350 g were used (n = 8). The light/dark cycle was kept as 12 hours and the food and water was available as usual. All the procedures to be done on experimental animals were first approved by the Ethical committee of the Department having the reference No S/pharm and are according to the animal scientific procedure ACT, 1986 (UK).

2.2. Drugs and Chemicals. Methanol, acetonitrile, 1-octane naphosphonic acid (HPLC grade, Fisher scientific), EDTA, and sodium dihydrogen orthophosphate (Merck), Metoclopramide (GSK, Pakistan). The neurotransmitter standards (noradrenaline, dopamine, and serotonin) and their metabolites (DOPAC, 5HIAA, and HVA) were purchased from Acros Organics (Belgium). Cisplatin was gifted by the Korea United Pharm (Korea). Bacosides were gifted by the University of Mississippi USA. N-butanol, n-hexane, and acetone were from Haq Chemicals Pakistan.

2.3. Extraction and Fractionation of Bacopa monniera. Bacopa monniera (BM) was carefully collected near the locality of Quid-e-Azam University, Islamabad. A specimen was identified by taxonomist Prof. Dr. Muhammad Ibrar from University of Peshawar and the same was submitted to the Department of Botany herbarium for future reference (V # 7421). The plant required parts were collected, shade dried, and then treated to the herbarium with voucher number 20017. The ginger rhizomes were washed and crushed in a way to expose its inner part. Maceration procedure was used for extraction of active phytochemicals (yield 4.72%) [49].

2.4. Extraction of Cannabis sativa. Cannabis sativa (CS) was collected at District Malakand KP, Pakistan at its flowering stage and later on authenticated by Prof. Dr. Muhammad Ibrar and a specimen was submitted to the Department herbarium with voucher number 8717. The required plant parts were collected, dried under shade, and then ground. The ground material was extracted as reported by our lab [46–48].

2.5. Extraction of Zingiber officinale. 500 grams of the ginger rhizomes were purchased from local market at Mardan, Pakistan. A specimen was identified and the same is submitted to the herbarium with voucher number 20017–pup. The rhizomes were washed and crushed in a way to expose its inner part. Maceration procedure was used for extraction of active phytochemicals (yield 4.72%) [49].

2.6. Drug Formulation. The emetogenic drug cisplatin was dissolved in normal saline by gentle heating up to 60°C. The n-butanol (bacoside rich fraction) was dissolved in distilled water for administration. The n-hexane fraction of CS was dissolved in mixer of ethanol, emulsifier, and distilled water in ratio of 5:5:90, respectively [50, 51]. Ginger acetone fraction was dissolved in distilled water and sonicated for complete dissolution.

2.7. Drug Administration. Intramuscular route (Chest muscle) was used for administration of test extracts, standard, and vehicle, while intravenous route was used for administration of cisplatin. After cisplatin administration the animals were put back in confining cages and the behaviour was observed up to 24 hours. Standard antiemetic–metoclopramide, respective vehicles, and test extract combinations were administered 30 minutes before the administration of cisplatin. At the end of the experiment the body weight was noted to calculate body weight loss and the animals were euthanized.

2.8. Antiemetic Assay. Cisplatin was administered (7 mg/kg) and the behaviour of the animals was recorded for 24 hours [48, 52]. Food and water were available to the experimental animals as usual. The one vomiting episode was considered with or without the expulsion of stomach contents and the relaxed posture among the two episodes was considered the separation marker [53]. Further in the studies, cisplatin was used at the dose of 7 mg/kg to induce vomiting and to evaluate the antiemetic effects of various extracts alone or in combination.

2.9. Tissue Sampling for Neurotransmitters Analysis. The brain areas: (1). Area postrema and (2). Brain stem were collected at the end of experiment by following the Atlas [54, 55], which were later on processed for quantification of neurotransmitters and their metabolites. Intestinal samples were also collected 10 cm from the pylorus for HPLC-ECD analysis.

2.10. Determination of Neurotransmitters and Their Metabolites. The brain and intestinal samples were first cleared using cold saline and then homogenized in cold 0.2% perchloric acid at 5000 rpm using Teflon glass homogenizer, centrifuged at 12000 g/minute (4°C), filtered using 0.45 μ filter. High performance liquid chromatography was used along with electrochemical detector for quantification of neurotransmitter and their metabolites in brain areas and intestine as reported in our previous studies [56].

2.11. Statistical Analysis. One-way analysis of variance was applied as a tool for group comparison and Student t-test/tukey’s multiple comparison test/Dunnett’s test was used as post hoc tests by using GraphPad Prism (Version 8). P value less than 0.05 was considered as statistically significant. The animal which showed no vomiting response is excluded from latency calculations.

3. Results

3.1. Antiemetic Effect of CS Hexane Fraction (10 mg/kg), BM Methanolic (10 mg/kg), and Bacoside Rich N-Butanol Fraction (5 mg/kg), and ZO Acetone Fraction (25 mg/kg) Alone and in Combinations. To see for any possible
synergistic combination among the selected plant extracts against cisplatin induced vomiting. The combinations tested were

1. CS-HexFr+BM-MetFr
2. BM-ButFr+ZO-ActFr
3. ZO-ActFr+CS-HexFr
4. CS-HexFr+BM-ButFr

Furthermore, all the fractions of CS, BM, and ZO most effective doses were also tested for their antiemetic effects alone as well.

The vomiting response of ~45 episodes with latency of ~66 minutes was recorded for cisplatin control, where all the animals showed reliable vomiting response up to the observation period (Table 1). Standard antiemetic metoclopramide suppressed the vomiting response to ~23 episodes (50%) and increased the latency up to 248 min (P > 0.05) as compared to cisplatin control. Combination 4 proved to be a synergistic combination as calculated by limpel equation [57] and it provided protection up to 89% (P < 0.001, Table 1) against the vomiting induced by cisplatin during the observation period. Combination 4 significantly increased the latency to first vomit as well (P < 0.01). Combination 2 reduced the vomiting episodes up to 12 (73%), while combination 3 provided up to 58% protection (~19 episodes) against cisplatin induced vomiting (P < 0.05, Table 1). Furthermore, Combination 1 also attenuated the vomiting response but nonsignificantly. Only combination 4 significantly (P < 0.01) increased the latency to first vomit while others failed to do so. The combination 4 provided enhanced protection and proved to be synergistic where it provided complete remission of vomiting response in one animal, although it attenuated the vomiting response to a maximum degree and increased the latency as well. Combination 4 in comparison to other combinations lowered the vomiting episodes but the difference was found to be statistically nonsignificant (Table 1, Figures 1–3).

The most effective doses of the CS (10 mg/kg), BM methanolic (10 mg/kg), BM n-butanol (5 mg/kg), and ZO (25 mg/kg) alone provided up to 62%, 36%, 71%, and 44% attenuation of vomiting as compared to cisplatin control (Table 1, Figures 1–3).

3.2. Effect of CS Hexane Fraction (10 mg/kg), BM Methanolic (10 mg/kg) and Bacoside Rich N-Butanol Fraction (5 mg/kg), and ZO Acetone Fraction (25 mg/kg) Alone and Combination 1, 2, 3, and 4 on Cisplatin-Induced Jerks and Weight Loss. Animals in control group (cisplatin treated) lost their body weight up to 15%, while the combination 1 and 3 showed the reduction in weight loss significantly (P <0.05–0.01, Table 1). In continuation, combination 2, 4, and standard metoclopramide failed to do so. No combination reduced the jerking episodes any significantly.

3.3. Effect of Standard MCP and Combination 4 on Basal Neurotransmitters Cum Metabolites in the Brain Areas and Intestine. Metoclopramide significantly reduced the concentration of 5 hydroxy indole acetic acid (5HIAA) in the area postrema and brain stem significantly (P < 0.05 and P < 0.001, respectively) as compared to basal level. Furthermore, the homovanillic acid (HVA) was also decreased significantly when compared with basal HVA concentration (Table 2). Combination 4 only reduced the concentration of 5HIAA in the brain stem as compared to basal level (P < 0.05, Table 2).

3.4. Effect of Metoclopramide and Combination 4 on Neurotransmitters Cum Metabolites in the Brain Areas and Intestine at 3rd Hour of Cisplatin Treatment. The concentration of 5-hydroxy tryptamine was significantly increased (P < 0.001) in the brain stem at the level of intestine as compared to vehicle treated, while in the area postrema a nonsignificant increase was observed (Table 3). Metoclopramide (30 mg/kg) did not change the concentration of all the neurotransmitters and their metabolites in the brain areas and intestine but only reduced the concentration of 5-hydroxy tryptamine in the brain stem and intestine (P < 0.001) as compared to cisplatin control (Table 3). In continuation, metoclopramide also decreased the concentration of 5HIAA in the brain stem, area postrema, and intestine (P < 0.01 – 0.001, Table 3).

Combination 4 significantly (P <0.05–0.001) decreased the concentration of 5HT and its metabolite 5-hydroxy indole acetic acid (5HIAA) in the brain stem, area postrema, and intestine (Table 3). However, no significant effects were observed on the other neurotransmitters in the brain areas and intestine except dihydroxy pheny acetic acid (DOPAC) which was found significantly increased in intestine (P < 0.05, Table 3).

3.5. Effect of Metoclopramide or Combination 4 on Neurotransmitters Cum Metabolites in the Brain Areas and Intestine at 18th Hour of Cisplatin Treatment. The concentration of neurotransmitter–Dopamine was significantly (P < 0.001) increased in the area postrema while a nonsignificant trend was observed in the brain stem and intestine (Table 4). 5HT concentrations were also noted to be increased in area postrema, brain stem, and intestine with significance of P < 0.01, P < 0.001, and P < 0.001, respectively, and did not affect the levels of others (DOPAC, HVA, 5HIAA, and NA) (Table 4). Metoclopramide decreased the dopamine surge significantly in area postrema (P < 0.001). In addition, the decrease in the concentration of 5HT was also observed in the area postrema (P < 0.01), brain stem (P < 0.001), and intestine (P < 0.001) as compared to cisplatin control (Table 4). Furthermore, 5HIAA concentration was also decreased in area postrema and 5HT in the brain stem and intestine (Table 4) as compared to cisplatin control. No significant changes were noted by Combination 4 on any of the neurotransmitter and their metabolites (Table 4).

4. Discussion

The current study is expedited to investigate the antiemetic effects of Cannabis sativa (CS), Zingiber officinale (ZO), and Bacopa monniera (BM) alone or in combination against
**Table 1:** Effect of CS Hexane fraction (10 mg/kg), BM methanolic (10 mg/kg) and bacoside rich n-butanol fraction (5 mg/kg) and ZO acetone fraction (25 mg/kg) alone and in combinations on cisplatin induced R+V in pigeons.

| Drug treatment                 | Dose & route          | Pigeons n/vomited | R + V Mean ± sem | Latency (min) mean ± sem | Jerks Mean ± sem | Wt loss (%) mean ± sem |
|--------------------------------|-----------------------|-------------------|------------------|--------------------------|-----------------|-----------------------|
| Saline+cisplatin               | 0.2 ml/kg i.m. + 7 mg/kg i.v. | 6/6               | 45 ± 1.9         | 66 ± 8.4                  | 542 ± 84        | 15.5 ± 1.8            |
| MCP+cisplatin                  | 30 mg/kg i.m. + 7 mg/kg i.v.  | 7/7               | 23.5 ± 0.3       | 248 ± 95                  | 411 ± 112       | 10.8 ± 1.6            |
| CS-HexFr+cisplatin             | 10 mg/kg i.m. + 7 mg/kg i.v.  | 6/6               | 16.5 ± 2.7 **    | 258 ± 113                 | 226 ± 84        | 7.5 ± 1.8             |
| BM-MetFr+cisplatin             | 10 mg/kg i.m. + 7 mg/kg i.v.  | 6/6               | 29 ± 4.3         | 243 ± 172                 | 570 ± 138       | 8.9 ± 1.3             |
| BM-ButFr + cisplatin           | 5 mg/kg i.m. + 7 mg/kg i.v.  | 8/8               | 13 ± 2.1 ***     | 137 ± 24                  | 330 ± 95        | 9.1 ± 2.1 *           |
| ZO-ActFr + cisplatin           | 25 mg/kg i.m. + 7 mg/kg i.v.  | 8/8               | 25 ± 1.8         | 139 ± 21                  | 223 ± 81        | 8.7 ± 1.4 *           |
| (CS-HexFr + BM-MetFr) + cisplatin | (10+10 mg/kg i.m.) + 7 mg/kg i.v.  | 6/6                 | 30 ± 1.1         | 131 ± 16                  | 672 ± 124       | 5.1 ± 2.5 **          |
| (BM-ButFr + ZO-ActFr) + cisplatin | (5+25 mg/kg i.m.) + 7 mg/kg i.v.  | 6/6                 | 12 ± 0.4 **      | 69 ± 21                   | 598 ± 194       | 9.6 ± 2.4             |
| (ZO-ActFr + CS-HexFr) + cisplatin | (25+10 mg/kg i.m.) + 7 mg/kg i.v.  | 7/7                 | 19 ± 0.2 *       | 85 ± 12                   | 415 ± 108       | 7.3 ± 1.9 *           |
| (CS-HexFr + BM-ButFr) + cisplatin | (10+5 mg/kg i.m.) + 7 mg/kg i.v.  | 6/5                | 05 ± 0.1 ***     | 369 ± 123 **              | 99 ± 47         | 10.6 ± 1.7            |

Effect of CS Hexane fraction (CS-HexFr), BM methanolic fraction (BM-MetFr), bacoside rich n-butanol fraction (BM-ButFr), and ZO acetone fraction (ZO-ActFr) alone and in combinations on cisplatin induced vomiting and jerking during a 24 hr observation period. Standard metoclopramide (MCP; 30 mg/kg) is also shown. Values significantly different compared to cisplatin control are indicated as *P<0.05, **P<0.01, and ***P<0.001 (ANOVA followed by Tukey post hoc test). Combination 1 (CS-HexFr 10 mg + BM-MetFr 10 mg), Combination 2 (BM-ButFr 5 mg + ZO-ActFr 25 mg), Combination 3 (ZO-ActFr 25 mg + CS-HexFr 10 mg), and Combination 4 (CS-HexFr 10 mg + BM-ButFr 5 mg).
cisplatin induced vomiting in pigeon. CS, ZO, and BM extracts exhibited prominent antiemetic activity. Preparations containing the active phytochemical from CS were found effective against cisplatin induced vomiting (7 mg/kg) [58]. In continuation, our previous study reported CS antiemetic activity in pigeons where the hexane fraction proved to be very effective against cisplatin induced vomiting at the dose of 10 mg/kg single and twice daily dosing provided up to 58.5% (17 ± 3.4 episodes) and 65.6% (14.1 ± 2.9 episodes) protection, respectively [47]. In the current study, CS-HexFr 10 mg provided up to 62.2% (17 ± 2.7 episodes) protection against cisplatin induced vomiting (Table 1). The hexane fraction of CS extract contains all the nonpolar compounds and the active component Δ9-THC. The CS major component Δ9-THC has been reported to have its clinical significance in the management of cisplatin induced vomiting [59]. CB1 receptors which are present presynaptically are involved in the mediation of antiemetic effect of THC, whose stimulation results in the inhibition of neurotransmitters which trigger the act of vomiting [60, 61]. The dose of 7 mg/kg was selected based on our previous study [45] which produced reliable vomiting response during the observation period and also not resulted in mortality.

BM belongs to family Scrophulariaceae and is present abundantly in Pakistan [62]. BM extracts are subjected to standardization in previous studies and our laboratory also did the standardization of plant extracts for quantification of bacosides by HPLC finger printing. The findings authenticate that the butanolic fraction contains the highest concentration of bacosides [43]. Clinical trials on bacosides for the management of memory enhancement establish the safety and tolerability of these phytochemicals and available currently in various herbal preparations alone or in combination. BM exhibit prominent antioxidant activity [63] and attenuate the dopamine receptor mediated hyperactivity [64]. The reports by our lab advocate that bacosides in a potent manner (~700 μg/kg) suppress the vomiting induced by cisplatin up to 24 hours in pigeons [51] so it will be a good candidate to be used alone or in combination for the CIV management in clinics.

In this study, BM-MetFr 10 mg and BM-ButFr 5 mg attenuated cisplatin induced vomiting up to 35.6%
Figure 2: Antiemetic effect of combination of CS Hexane fraction (10 mg/kg), BM methanolic (10 mg/kg) and bacoside rich n-butanol fraction (5 mg/kg) and ZO acetone fraction (25 mg/kg).

Figure 3: Number of vomiting episodes observed after treatment with combination of CS Hexane fraction (10 mg/kg), BM methanolic (10 mg/kg) and bacoside rich n-butanol fraction (5 mg/kg) and ZO acetone fraction (25 mg/kg). Each column represents mean vomiting episodes after every 4 h ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to control, two-way repeated measures ANOVA followed by Tukey’s post hoc test.
Tukey post hoc analysis).

### Table 2: Effect of metoclopramide (MCP) or combination (CS-HexFr 10 mg + BM-ButFr 5 mg) on basal level of neurotransmitters and their metabolites at the brain level of Area postrema (AP), Brain stem (BS), and intestine in pigeons.

| Treatment                              | NA     | DOPAC  | DA     | 5HIAA  | HVA     | 5HT     |
|----------------------------------------|--------|--------|--------|--------|---------|---------|
| **Area Postrema**                       |        |        |        |        |         |         |
| Saline                                 | 0.590 ± 0.011 | 0.470 ± 0.011 | 0.610 ± 0.139 | 0.175 ± 0.106 | 0.887 ± 0.083 | 0.059 ± 0.041 |
| MCP 30 mg                               | 0.031 ± 0.004 | 0.020 ± 0.001 | 0.032 ± 0.021 | 0.006 ± 0.011* | 0.120 ± 0.056* | 0.041 ± 0.002 |
| (CS-HexFr 10 mg + BM-ButFr 5 mg)       | 1.491 ± 1.382 | 0.408 ± 0.276 | 0.225 ± 0.088 | 0.100 ± 0.044 | 1.096 ± 0.507 | 0.147 ± 0.095 |
| **Brain stem**                          |        |        |        |        |         |         |
| Saline                                 | 0.089 ± 0.021 | 0.063 ± 0.070 | 0.193 ± 0.067 | 0.071 ± 0.031 | 0.059 ± 0.020 | 0.012 ± 0.001 |
| MCP 30 mg                               | 0.120 ± 0.041 | 0.034 ± 0.004 | 0.050 ± 0.019 | 0.006 ± 0.010*** | 0.073 ± 0.040 | 0.020 ± 0.020 |
| (CS-HexFr 10 mg + BM-ButFr 5 mg)       | 0.160 ± 0.115 | 0.031 ± 0.000 | 0.428 ± 0.157 | 0.012 ± 0.003* | 0.104 ± 0.042 | 0.020 ± 0.006 |
| **Intestine**                           |        |        |        |        |         |         |
| Saline                                 | 0.187 ± 0.063 | 0.074 ± 0.010 | 0.087 ± 0.056 | 0.083 ± 0.049 | 0.071 ± 0.031 | 0.054 ± 0.013 |
| MCP 30 mg                               | 0.129 ± 0.047 | 0.063 ± 0.014 | 0.063 ± 0.021 | 0.012 ± 0.010 | 0.207 ± 0.012 | 0.071 ± 0.010 |
| (CS-HexFr 10 mg + BM-ButFr 5 mg)       | 0.248 ± 0.040 | 0.123 ± 0.045 | 0.056 ± 0.001 | 0.029 ± 0.010 | 0.119 ± 0.115 | 0.063 ± 0.021 |

Effect of combination of CS-HexFr (10 mg) with BM-ButFr (5 mg) administered 30 minutes before saline administration, on the basal level of neurotransmitters and their metabolites (ng/mg tissue wet weight) at the brain level of AP and BS and Intestine in pigeons at \( t = 3 \) hr \((n = 6 – 8)\). Standard MCP is also shown. Values significantly different compared to basal level are indicated as \(*P < 0.05, **P < 0.01, ***P < 0.001\) (ANOVA followed by Tukey post hoc analysis).

### Table 3: Effect of standard metoclopramide (MCP), or combination of CS-HexFr (10 mg) with BM-ButFr (5 mg) on neurotransmitters and their metabolites at the brain level of area postrema (AP) and brain stem (BS) and intestine at 3rd hour of cisplatin treatment.

| Treatment                              | NA     | Dopac  | DA     | 5HIAA  | HVA     | 5HT     |
|----------------------------------------|--------|--------|--------|--------|---------|---------|
| **Area Postrema**                       |        |        |        |        |         |         |
| Saline                                 | 0.701 ± 0.271 | 0.199 ± 0.010 | 0.763 ± 0.200 | 0.091 ± 0.040 | 0.900 ± 0.173 | 0.131 ± 0.050 |
| Cisplatin                              | 1.704 ± 1.401 | 0.408 ± 0.170 | 0.091 ± 0.270 | 0.379 ± 0.001# | 0.607 ± 0.109 | 0.314 ± 0.110 |
| MCP 30 mg                               | 0.116 ± 0.078 | 0.142 ± 0.050 | 0.310 ± 0.137 | 0.026 ± 0.006** | 0.040 ± 0.021 | 0.030 ± 0.005* |
| (CS-HexFr 10 mg + BM-ButFr 5 mg)       | 0.166 ± 0.139 | 0.192 ± 0.088 | 0.339 ± 0.144 | 0.046 ± 0.019** | 0.443 ± 0.181 | 0.048 ± 0.022* |
| **Brain stem**                          |        |        |        |        |         |         |
| Saline                                 | 0.117 ± 0.031 | 0.041 ± 0.020 | 0.260 ± 0.130 | 0.020 ± 0.010 | 0.070 ± 0.023 | 0.016 ± 0.001 |
| Cisplatin                              | 0.113 ± 0.040 | 0.185 ± 0.046 | 0.040 ± 0.010 | 0.057 ± 0.001### | 0.032 ± 0.002 | 0.153 ± 0.011### |
| MCP 30 mg                               | 0.041 ± 0.021 | 0.039 ± 0.003 | 0.013 ± 0.002 | 0.021 ± 0.001*** | 0.023 ± 0.001 | 0.008 ± 0.000*** |
| (CS-HexFr 10 mg + BM-ButFr 5 mg)       | 0.089 ± 0.007 | 0.011 ± 0.001 | 0.119 ± 0.069 | 0.003 ± 0.002*** | 0.018 ± 0.003 | 0.006 ± 0.002*** |
| **Intestine**                           |        |        |        |        |         |         |
| Saline                                 | 0.416 ± 0.037 | 0.092 ± 0.010 | 0.129 ± 0.024 | 0.041 ± 0.000 | 0.107 ± 0.052 | 0.051 ± 0.001 |
| Cisplatin                              | 0.301 ± 0.047 | 0.024 ± 0.002 | 0.037 ± 0.004 | 0.304 ± 0.030### | 0.043 ± 0.005 | 0.689 ± 0.104### |
| MCP 30 mg                               | 0.109 ± 0.040* | 0.029 ± 0.001 | 0.246 ± 0.183 | 0.031 ± 0.006*** | 0.067 ± 0.030 | 0.041 ± 0.005*** |
| (CS-HexFr 10 mg + BM-ButFr 5 mg)       | 0.266 ± 0.104 | 0.047 ± 0.275* | 0.399 ± 0.232 | 0.003 ± 0.001*** | 0.004 ± 0.002 | 0.007 ± 0.006*** |

Effect of combination of CS-HexFr (10 mg) with BM-ButFr (5 mg) administered 30 mins before cisplatin challenge, on the level of neurotransmitters and their metabolites (ng/mg tissue wet weight) at the brain level of AP and BS and Intestine of pigeons at \( t = 3 \) hr of cisplatin administration \((n = 6 – 8)\). Standard MCP is also shown. Values significantly different compared to cisplatin control are indicated as \(*P < 0.05, **P < 0.01, ***P < 0.001\), while values significantly different compared to basal level are indicated as \(*P < 0.05, ###P < 0.001\) (ANOVA followed by Tukey post hoc analysis).
The standardization of ginger extracts has the potential of NK2 receptors [68]. Our previous study [49] and our previous published work [48] observed with the dose of 25 mg [49]. There are so many operative nausea and vomiting is well managed by Ginger where up to 60 mg/g of gingerols are present in extract [66]. Post-operative nausea and vomiting is well managed by Ginger and a single antiemetic fails for control both the phases of vomiting. The International guidelines also recommend the use of 5HT3 blockers, NK1 receptor antagonists, and dexamethasone in the management of both the phases of vomiting. Various combinations of plant extracts were tested in this study and one combination (No 4) was found to be synergistic and provided very nice remission of vomiting response (Table 1).

The protection observed for CS-HexFr 10 mg alone was ~62.2% (Current study) and 55.45% in our previous published work [48] while for BM-ButFr 5 mg the protection observed was 71.1% (Current study) and 55.45% in our previous published work [48]. Combination therapy which is reported to be gingerol rich fraction was based on a previous study [70]. Serotonin receptor blockers have showed intrinsic emetic activity in pigeons (Unpublished data) so the 5HT3 antagonist drugs were not used as standard antiemetic.

The multi mechanisms behind the vomiting induced by cisplatin resulted in the use of antiemetics in combination and a single antiemetic fails for control both the phases of vomiting. The international guidelines also recommend the use of 5HT3 blockers, NK1 receptor antagonists, and dexamethasone in the management of both the phases of vomiting. Various combinations of plant extracts were tested in this study and one combination (No 4) was found to be synergistic and provided very nice remission of vomiting response (Table 1).

In continuation, the protection observed for CS-HexFr 10 mg alone was ~62.2% (Current study) and 55.45% in our previous published work [48] while for BM-ButFr 5 mg the protection observed was 71.1% (Current study) and 55.45% in our previous published work [48]. Combination 2 was also found to be effective though less significant (P < 0.01) to combination 4 (Table 1).

### Table 4: Effect of standard metoclopramide (MCP) or combination of CS-HexFr (10 mg) with BM-ButFr (5 mg) on neurotransmitters and their metabolites at the brain level of area postrema (AP) and brain stem (BS) and intestine at 18th hour of cisplatin treatment.

| Treatment | Area Postrema | Brain stem | Intestine |
|-----------|---------------|------------|-----------|
|           | NA | Dopac | DA | 5HIAA | HVA | 5HT | NA | Dopac | DA | 5HIAA | HVA | 5HT | NA | Dopac | DA | 5HIAA | HVA | 5HT |
| Saline    | 0.507 ± 0.054 | 0.299 ± 0.129 | 0.520 ± 0.117 | 0.207 ± 0.020 | 0.863 ± 0.130 | 0.012 ± 0.011 | 0.091 ± 0.004 | 0.083 ± 0.013 | 0.081 ± 0.041 | 0.193 ± 0.037 | 0.032 ± 0.020 | 0.010 ± 0.000 | 0.317 ± 0.160 | 0.120 ± 0.060 | 0.193 ± 0.050 | 0.010 ± 0.001 | 0.041 ± 0.010 | 0.062 ± 0.013 |
| Cisplatin | 0.307 ± 0.056 | 0.021 ± 0.001 | 6.898 ± 1.300## | 0.205 ± 0.048 | 0.584 ± 0.106 | 0.153 ± 0.040## | 0.012 ± 0.002 | 0.005 ± 0.001 | 0.021 ± 0.028 | 0.015 ± 0.003 | 0.097 ± 0.048 | 0.020 ± 0.001### | 0.265 ± 0.029 | 0.013 ± 0.001 | 0.230 ± 0.031 | 0.340 ± 0.054 | 0.073 ± 0.005 | 0.506 ± 0.107### |
| MCP 30 mg | 0.250 ± 0.081 | 0.076 ± 0.041 | 0.125 ± 0.030 *** | 0.020 ± 0.010 ** | 0.383 ± 0.129 | 0.005 ± 0.002 ** | 0.471 ± 0.174 | 0.166 ± 0.066 | 0.504 ± 0.362 *** | 0.072 ± 0.012 | 1.388 ± 0.370 | 0.107 ± 0.029 | 0.277 ± 0.094 *** | 0.007 ± 0.002 | 0.074 ± 0.074 | 0.014 ± 0.002 | 0.022 ± 0.020 | 0.022 ± 0.004 *** |
| (CS-HexFr 10 mg + BM-ButFr 5 mg) | | | | | | | | | | | | | | | | | | | |

### Effect of combination of CS-HexFr (10 mg) with BM-ButFr (5 mg) administered 30 mins before cisplatin challenge, on the level of neurotransmitters and their metabolites (ng/mg tissue wet weight) at the brain level of AP and BS and Intestine of pigeons at t = 18hr of cisplatin administration (n = 6-8). Standard MCP is also shown. Values significantly different compared to cisplatin control are indicated as ***P < 0.01 **P < 0.001, while values significantly different compared to basal level are indicated as ##P < 0.01 ###P < 0.001 (ANOVA followed by Tukey post hoc analysis).
In the current study, treatments with combination 4 do not changed basal neurotransmitters level and their metabolites any significantly. Furthermore, the decrease in the concentration of 5HIAA by MCP (30 mg) and combination 4 was observed at the brain stem (Table 2). The combination of CS-HexFr (10 mg) with BM-ButFr (5 mg) reduced 5HT and 5HIAA in the brain areas (AP and BS) and intestine (Table 3, \( P < 0.05 - 0.001 \)). These findings are supportive for the antiemetic activity of Combination 4 for the 3rd hour (at the acute vomiting response). Similar effect was also observed by metoclopramide. Combination of CS-HexFr 10 mg with BM-ButFr 5 mg suppressed the dopamine concentration in the brain area of AP as compared to cisplatin control while no significant dopaminergic suppression was seen in the BS and intestine (Table 4) at 18th hour of cisplatin treatment. In continuation, Combination 4 (CS-HexFr 10 mg + BM-ButFr 5 mg) significantly (\( P < 0.001 \)) reduced 5HT concentration at the level of BS and intestine (Table 4). The standard MCP (30 mg) also presented almost the same picture of neurotransmitter suppression in the brain area of AP, BS, and intestine. The antiserotonergic along with antidopaminergic effects noted of combination 4 in the current study is supporting the synergistic and prolongs protection provided against the vomiting induced by cisplatin in pigeons as compared to metoclopramide (Table 1).

5. Conclusions

In conclusion, the combination 4 provided a synergistic protection and our neurotransmitter quantification supports the involvment of antiserotonergic and antidopaminergic effects in an overlapping mode at the two different time points. At the acute time point (3rd hour), dominantly the antiserotonergic effects were observed. Moreover, antidopaminergic and antiserotonergic effects were observed at the 18th of cisplatin administration. These neurochemical findings advocate the promising antiemetic effect of combination 4 against cisplatin induced vomiting in pigeon. The combination may be useful alone or as adjunct in the management of cisplatin induced vomiting in clinics as cannabis preparations (Nabilone etc.) and preparations of bacopa (Bacomind®) are already available in the market and have safety and tolerability profile.

Abbreviations

| Abbreviation | Full Form |
|--------------|-----------|
| CS           | Cannabis sativa |
| BM           | Bacopa monniera |
| ZO           | Zingiber officinale |
| HPLC         | High performance liquid chromatography |
| ECD          | Electrochemical detector |
| 5HT          | 5-Hydroxy tryptamine |
| DA           | Dopamine |
| HVA          | Homovanillic acid |
| Dopac        | Dihydroxy phenyl acetic acid |
| 5HIAA        | 5 Hydroxy indole acetic acid |
| CS-HexFr     | Cannabis sativa (hexane fraction) |
| ZO-ActFr     | Zingiber officinale (acetone fraction) |
| BM-ButFr     | Bacopa monniera butanolic fraction |
| AP           | Area Postrema |
| BS           | Brain stem |
| HEC          | Highly emetogenic chemotherapy |
| 5HT3         | 5 hydroxy tryptamine type 3 |
| NK1          | Neurokinin type 1 |
| CIV          | Chemotherapy induced vomiting |
| THC          | Tetrahydrocannabinol |
| MCP          | Metoclopramide |
| R+V          | Retching+vomiting |

Data Availability

Data presented in the current manuscript belongs to the PhD work of Dr Ihsan Ullah and is not published anywhere. Data is available to researchers upon request.

Ethical Approval

All the experimental procedures were approved by the Ethical committee, Department of Pharmacy, University of Peshawar (Ref. No 5/pharm) and are in accordance with the animal scientific procedure ACT, 1986 (United Kingdom).

Conflicts of Interest

The authors declare to have no conflict of interest.

Authors’ Contributions

Ihsan Ullah (IU) is responsible for the data creation, analysis, and manuscript writing. Fazal Subhan (FS) is responsible for the project design, supervision, and data analysis. Muhammad Shahid (MS) is responsible for the data analysis and manuscript writing. Nisar Ahmad (NA) is responsible for the manuscript write up and revision. Javid Alam (JA) is responsible for the data creation, project design, and manuscript write up. Ikram Ul Haq (IUH) is responsible for the project design, analysis, and manuscript write up. Rahim Ullah (RU) is responsible for the manuscript write up, and Muhammad Ayaz (MA) and Hanabe Chowdappa Ananda Murthy (HCAM) is responsible for the manuscript write up, data analysis, and revision of the manuscript draft. Dr. Ihsan Ullah, PhD scholar at the Department of Pharmacy, University of Peshawar conducted the research work there.

Acknowledgments

Authors are grateful to Department of Pharmacy, University of Peshawar for providing laboratory facilities to conduct the research.

References

[1] R. Navari, “Management of chemotherapy-induced nausea and vomiting,” *Drugs*, vol. 73, no. 3, pp. 249–262, 2013.
[2] A. T. Khalil, M. Ovais, J. Iqbal et al., "Microbes-mediated synthesis strategies of metal nanoparticles and their potential role"
in cancer therapeutics,” in Seminars in cancer biology, Academic Press, 2021.

[3] D. M. Parkin, “Global cancer statistics in the year 2000,” The Lancet Oncology, vol. 2, no. 9, pp. 533–543, 2001.

[4] M. H. Mahnashi, Y. S. Alqahtani, B. A. Alyami et al., “Phytochemical analysis, α-glucosidase and amylase inhibitory, and molecular docking studies on Persicaria hydropiper L. Leaves essential oils,” Leaves Essential Oils: Evidence-Based Complementary and Alternative Medicine, vol. 2022, article 7924171, 11 pages, 2022.

[5] C. Patra, I. Ahmad, M. Ayaz, A. T. Khalil, S. Mukherjee, and M. Ovais, Biogenic Nanoparticles for Cancer Theranostics, Elsevier, 1st edition, 2021.

[6] G. Higgins, G. Kilpatrick, K. Bunce, B. Jones, and M. Tyers, “5HT3 receptor antagonists injected into the area postrema inhibit cisplatin induced emesis in the ferret,” British Journal of Pharmacology, vol. 97, no. 1, pp. 247–255, 1989.

[7] L. Grelot, J. Dapzol, E. Estève et al., “Potent inhibition of both the acute and delayed emetic responses to cisplatin in piglets treated with GR205171, a novel highly selective tachykinin NK1 receptor antagonist,” British Journal of Pharmacology, vol. 124, no. 8, pp. 1643–1650, 1998.

[8] M. Markman, “Progress in preventing chemotherapy-induced nausea and vomiting,” Cleveland Clinic Journal of Medicine, vol. 69, no. 8, pp. 609-610, 2002.

[9] D. G. Pfister, D. H. Johnson, C. G. Azzoli et al., “American society of clinical oncology treatment of unresectable non–small–cell lung cancer guideline: update 2003,” Journal of Clinical Oncology, vol. 22, no. 2, pp. 330–353, 2004.

[10] M. Ovais, M. Z. Hoque, A. T. Khalil, M. Ayaz, and I. Ahmad, “Mechanisms underlying the anticancer applications of bio-synthesized nanoparticles,” in Biogenic Nanoparticles for Cancer Theranostics, pp. 229–248, Elsevier, 2021.

[11] N. T. Mir, U. Saleem, F. Anwar et al., “Lawsonia inermis markedly improves cognitive functions in animal models and modulate oxidative stress markers in the brain,” Medicina, vol. 55, no. 5, p. 192, 2019.

[12] M. H. Mahnashi, Y. S. Alqahtani, B. A. Alyami et al., “Cyotoxicty, anti-angiogenic, anti-tumor and molecular docking studies on phytochemicals isolated from Polygonum hydropiper L,” BMC Complementary Medicine and Therapies, vol. 21, no. 1, pp. 1–14, 2021.

[13] A. Sani, D. Hassan, A. T. Khalil et al., “Floral extracts-mediated green synthesis of NiO nanoparticles and their diverse pharmacological evaluations,” Journal of Biomolecular Structure and Dynamics, vol. 39, no. 11, pp. 4133–4147, 2021.

[14] M. F. Akhtar, M. O. Mehal, A. Saleem et al., “Attenuating effect of Prosopis cineraria against paraquat-induced toxicity in pre-pubertal mice, Mus musculus,” Environmental Science and Pollution Research, vol. 29, no. 10, pp. 15215–15231, 2022.

[15] M. Ayaz, A. Nawaz, F. Naz, F. Ullah, A. Sadiq, and Z. U. Islam, “Phytochemicals-based therapeutics against Alzheimer’s disease: an update,” Current Topics in Medicinal Chemistry, vol. 27, 2022.

[16] D. J. Newman, G. M. Cragg, and K. M. Snader, “The influence of natural products upon drug discovery,” Natural Product Reports, vol. 17, no. 3, pp. 215–234, 2000.

[17] M. S. Butler, “The role of natural product chemistry in drug discovery,” Journal of Natural Products, vol. 67, no. 12, pp. 2141–2153, 2004.

[18] T. Zohra, A. T. Khalil, F. Saeed et al., “Green nano-biotechnology: a new sustainable paradigm to control dengue infection,” Bioinorganic Chemistry and Applications, vol. 2022, Article ID 3994340, 21 pages, 2022.

[19] M. Q. Nasar, M. Shah, A. T. Khalil et al., “Ephedra intermedia mediated synthesis of biogenic silver nanoparticles and their antimicrobial, cytotoxic and hemocompatibility evaluations,” Inorganic Chemistry Communications, vol. 137, article 109252, 2022.

[20] M. Ayaz, F. Subhan, A. Sadiq, F. Ullah, J. Ahmed, and R. D. E. Sewell, “Cellular efflux transporters and the potential role of natural products in combating efflux mediated drug resistance,” Frontiers in Bioscience, vol. 22, no. 4, pp. 732–756, 2017.

[21] S. Ahmad, F. Ullah, M. Ayaz, A. Ahmad, A. Sadiq, and S. N. U.-H. Mohani, “Nutritional and medicinal aspects of Rumex hastatus D. Don along within vitroanti-diabetic activity,” International Journal of Food Properties, vol. 22, no. 1, pp. 1733–1748, 2019.

[22] A. T. Khalil, M. D. Khan, S. Razzaque et al., “Single precursor-based synthesis of transition metal sulfide nanoparticles and evaluation of their antimicrobial, antioxidant and cytotoxic potentials,” Applied Nanoscience, vol. 11, no. 9, pp. 2489–2502, 2021.

[23] M. Ayaz, A. Nawaz, S. Ahmad et al., “Underlying anticancer mechanisms and synergistic combinations of phytochemicals with cancer chemotherapeutics: potential benefits and risks,” Journal of Food Quality, vol. 2022, Article ID 1189034, 15 pages, 2022.

[24] M. Mukim, A. Kabra, C. Hano et al., “Rivea hypocrateriformis (desr.) choisy: an overview of its ethnomedicinal uses, phytochemistry, and biological activities and prospective research directions,” Journal of Chemistry, vol. 2022, Article ID 9099672, 11 pages, 2022.

[25] F. A. Khan, G. Ali, K. Rahman et al., “Efficacy of 2-Hydroxyxylanovane in rodent models of pain and inflammation: involvement of opioidergic and GABAergic antinociceptive mechanisms,” Molecules, vol. 27, no. 17, p. 5431, 2022.

[26] M. Ayaz, M. Junaid, F. Ullah et al., “GC-MS analysis and gas-protective evaluations of crude extracts, isolated saponins, and essential oil from Polygonum hydropiper L,” Frontiers in Chemistry, vol. 5, p. 58, 2017.

[27] M. Ovais, M. Ayaz, A. T. Khalil et al., “HPLC-DAD finger printing, antioxidant, cholinesterase, and α-glucosidase inhibitory potentials of a novel plant Olax nana,” BMC Complementary and Alternative Medicine, vol. 18, no. 1, pp. 1–13, 2018.

[28] T. Zohra, M. Ovais, A. T. Khalil et al., “Bio-guided profiling and HPLC-DAD finger printing of Atriplex lasiantha Boiss,” BMC Complementary and Alternative Medicine, vol. 19, no. 1, pp. 1–14, 2019.

[29] A. Zeb, “A reversed phase HPLC-DAD method for the determination of phenolic compounds in plant leaves,” Analytical Methods, vol. 7, no. 18, pp. 7753–7757, 2015.

[30] M. S. Islam, A. M. Al-Majid, E. N. Sholkamy et al., “Synthesis, molecular docking and enzyme inhibitory approaches of some new chalcones engrafted pyrazole as potential antialzheimer, antidiabetic and antioxidant agents,” Journal of Molecular Structure, vol. 1269, article 133843, 2022.

[31] M. Ware, P. Daenick, and V. Maida, “A review of nabilone in the treatment of chemotherapy-induced nausea and vomiting,” Therapeutics and Clinical Risk Management, vol. 4, no. 1, pp. 99–107, 2008.
of the New York Academy of Sciences, vol. 191, no. 1, pp. 3–14, 1971.

[47] I. Ullah, F. Subhan, J. Alam, M. Shahid, and M. Ayaz, "Suppression of cisplatin-induced vomiting by Cannabis sativa in pigeons: neurochemical evidences," Frontiers in Pharmacology, vol. 9, p. 231, 2018.

[48] I. Ullah, F. Subhan, K. Rauf, A. Badshah, and G. Ali, "Role of gastrointestinal motility/gastric emptying in cisplatin-induced vomiting in pigeon," African Journal of Pharmacy and Pharmacology, vol. 6, no. 35, pp. 2592–2599, 2012.

[49] I. Ullah, F. Subhan, M. Ayaz et al., "Anti-emetic mechanisms of Zingiber officinale against cisplatin induced emesis in the pigeon; behavioral and neurochemical correlates," BMC Complementary and Alternative Medicine, vol. 15, no. 1, pp. 34–42, 2015.

[50] J. Feigenbaum, S. Richmond, Y. Weissman, and R. Mechoulam, "Inhibition of cisplatin-induced emesis in the pigeon by a non-psychochotropic synthetic cannabinoid," European Journal of Pharmacology, vol. 169, no. 1, pp. 159–165, 1989.

[51] I. Ullah, Evaluation of some selected medicinal plants and their combinations in cisplatin induced vomiting in vomit model(s); behavioral neurochemical correlates, (Dissertation), University of Peshawar, 2013.

[52] S. Tanihata, H. Igarashi, M. Suzuki, and T. Uchiyama, "Cisplatin-induced early and delayed emesis in the pigeon," British Journal of Pharmacology, vol. 130, no. 1, pp. 132–138, 2000.

[53] P. Preziosi, M. D’Amato, R. Del Carmine, M. Martire, G. Pozzoli, and P. Navarra, “The effects of 5-HT3 receptor antagonists on cisplatin-induced emesis in the pigeon,” European Journal of Pharmacology, vol. 221, no. 2-3, pp. 343–350, 1992.

[54] H. J. Karten and W. Hodos, A stereotaxic atlas of the brain of the pigeon: (Columbia Livia), vol. 696, Johns Hopkins University Press Baltimore, MD, 1967.

[55] H. M. Duvernoy and P. Y. Risold, "The circumventricular organs: an atlas of comparative anatomy and vascularization," Brain Research Reviews, vol. 56, no. 1, pp. 119–147, 2007.

[56] K. Rauf, F. Subhan, G. Ali, and M. Ayaz, "Effect of acute and sub chronic use of Bacopa monnieri on dopamine and serotonin turnover in mice whole brain Khalid Rauf1," African Journal of Pharmacy and Pharmacology, vol. 6, no. 39, pp. 2767–2774, 2012.

[57] L. Limpel, P. Schulte, and D. Lamont, "Weed control by dimethyl tetrachloroterephthalate alone and in certain combinations," In: Proceedings of 27th Northeast Weed Control Conference, vol. 16, pp. 48–53, 1962.

[58] R. Mechoulam and J. Feigenbaum, "5 towards cannabinoid drugs," Progress in Medicinal Chemistry, vol. 24, pp. 159–207, 1987.

[59] S. Sallan, N. Zinberg, and E. Frei, "Antiemetic effect of delta-9-tetrahydrocannabinol in patients receiving cancer chemotherapy," New England Journal of Medicine, vol. 293, no. 16, pp. 795–797, 1975.

[60] N. Darmani, "Δ9-tetrahydrocannabinol and synthetic cannabinoids prevent emesis produced by the cannabinoid CB1 receptor antagonist/inverse agonist SR 141716A," Neuropsychopharmacology, vol. 24, no. 2, pp. 198–203, 2001.

[61] N. Darmani, J. Janoyan, N. Kumar, and J. Crim, "Behaviorally active doses of the CB1 receptor agonist SR 141716A increase brain serotonin and dopamine levels and turnover," Pharmacology Biochemistry and Behavior, vol. 75, no. 4, pp. 777, 2003.
[62] R. Qureshi and G. Raza Bhatti, “Ethnobotany of plants used by the Thari people of Nara Desert, Pakistan,” *Fitoterapia*, vol. 79, no. 6, pp. 468–473, 2008.

[63] S. Bhattacharya, A. Bhattacharya, A. Kumar, and S. Ghosal, “Antioxidant activity of Bacopa monniera in rat frontal cortex, striatum and hippocampus,” *Phytotherapy Research*, vol. 14, no. 3, pp. 174–179, 2000.

[64] K. Balakrishna, G. Veluchamy, S. N. Devaraj, and T. Sumathi, “Inhibitory effect of Bacopa monniera on morphine induced pharmacological effects in mice,” *Natural Product Sciences*, vol. 13, no. 1, pp. 46–53, 2007.

[65] I. Ullah, F. Subhan, J. A. Rudd et al., “Attenuation of cisplatin-induced emetogenesis by standardized Bacopa monnieri extracts in the pigeon: behavioral and neurochemical correlations,” *Planta Medica*, vol. 80, no. 17, pp. 1569–1579, 2014.

[66] S. Rai, K. Mukherjee, M. Mal, A. Wahile, B. P. Saha, and P. K. Mukherjee, “Determination of 6-gingerol in ginger (Zingiber officinale) using high-performance thin-layer chromatography,” *Journal of Separation Science*, vol. 29, no. 15, pp. 2292–2295, 2006.

[67] E. Ernst and M. Pittler, “Efficacy of ginger for nausea and vomiting: a systematic review of randomized clinical trials,” *British Journal of Anaesthesia*, vol. 84, no. 3, pp. 367–371, 2000.

[68] Q. Qiu-hai, Y. Wang, C. Wen-hui, Y. Zhi-hong, L. Zhan-tao, and W. Yao-xia, “Effect of gingerol on substance P and NK1 receptor expression in a vomiting model of mink,” *Chinese Medical Journal*, vol. 123, no. 4, pp. 478–484, 2010.

[69] M. H. Al-Zubaidy and F. K. Mohammad, “Metoclopramide-induced central nervous system depression in the chicken,” *BMC Veterinary Research*, vol. 1, no. 1, pp. 2–6, 2005.

[70] R. Coronas, L. Pitarch, and J. Mallol, "Blockade of reserpine emesis in pigeons by metoclopramide," *European Journal of Pharmacology*, vol. 32, no. 2, pp. 380–382, 1975.