

1 ORIGINAL ARTICLE

2 Effects of *Pluchea lanceolata* Root Extract on
3 Cisplatin--induced Nausea and Vomiting in Rat Pica
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9 ABSTRACT

10 Cisplatin is an effective chemotherapeutics against a wide range of cancers. However, it causes
11 significant nausea and vomiting which limit its usefulness. In the present study, the effects of methanolic
12 root extract of *Pluchea lanceolata* (DC.) C. B. Clarke, asteraceae (*MPL*) was investigated against
13 cisplatin-induced nausea using a rat pica model. In rat pica model, rats react to cisplatin (emetic/nausea
14 stimuli), with altered feeding habits, manifested by increased consumption of kaolin. The pica in rats was
15 measured to quantify cisplatin-induced nausea, and to evaluate the protective effect of pretreatment with
16 *MPL* given orally. Cisplatin at 3 mg/kg (i.p.) induced significant pica indicated by reduced food intake and
17 increased kaolin consumption, suggesting the presence of nausea/emesis. Cisplatin-induced pica
18 decreased significantly when animals were pretreated with *MPL* at doses of 400 mg/kg p.o. ($p < 0.05$).
19 *MPL* pretreatment decreased cisplatin-induced kaolin intake in the rat model of simulated nausea,
20 suggesting that *MPL* and/or its active constituent(s) may play a therapeutic role as protective against
21 chemotherapy-induced emesis.

22 **Keywords:** *Cisplatin, Pica, Pluchea lanceolata, Asteraceae*

23 Chemotherapy regimens for the treatment of cancer 41 and vomiting 1 to 2 hours after receiving chemotherapy
24 are unfortunately better known for their toxicity than for 42 [4]. At approximately 18 to 24 hours, the emesis
25 their efficacy. Although some of the toxic effects may 43 typically subsides, only to recur and reach a second
26 be life-threatening, patients are often most fearful of the 44 peak at approximately 48 to 72 hours after receipt of the
27 nausea and emesis caused by chemotherapy, which are 45 agent [5]. On the basis of the cisplatin model, emesis
28 generally self-limited and seldom life-threatening [1]. 46 occurring within the first 24 hours has been defined as
29 Nausea and vomiting has been commonly reported by 47 'acute', and emesis occurring more than 24 hours later
30 patients ever since chemotherapeutic agents were first 48 as 'delayed' [6]. The incidence of 'anticipatory emesis',
31 used to treat cancer [2]. The severity and pattern of 49 a third emetic syndrome, has decreased in recent years.
32 chemotherapy-induced emesis depend on the specific 50 'Anticipatory emesis' represents a learned response
33 agents used, the dose, and the regimen. Cisplatin (cis- 51 conditioned by the severity and duration of previous
34 diaminedichloroplatinum), a platinum-containing 52 emetic responses to chemotherapy [7]. As strategies for
35 anticancer drug, is one of the most commonly used 53 controlling emesis have improved, the frequency of
36 cytotoxic agents in the treatment of a variety of solid 54 anticipatory emesis has decreased.

37 malignant tumors [1] and is associated with profound 55 Cisplatin-induced nausea and vomiting can be
38 nausea and vomiting [3]. 56 disruptive to a person's life in various ways. It can
39 In the absence of effective antiemetic prophylaxis, 57 negatively affect a patient's functional, nutritional,
40 virtually all patients receiving cisplatin will have nausea 58 psychological, social, physical and economical quality

of life. The pathophysiology of these symptoms has been partly attributed to oxidant injury to the intestinal epithelium [8,9]. The mucosal injury results in excessive serotonin release from the enterochromaffin cells that could mediate the gastrointestinal adverse effects of chemotherapy and radiotherapy [10-14]. Since oxidant injury to the gut may be the primary event responsible for the gastrointestinal symptoms following chemotherapy or radiotherapy, we hypothesized that pretreatment with an antioxidant should ameliorate these symptoms.

Despite advances in antiemetic therapy, nausea and vomiting remain among the most feared adverse events associated with chemotherapy. Herbal medicines may represent an alternative new class of low-cost antiemetic agents for the treatment of chemotherapy-induced nausea/vomiting. In present paper, the efficacy of a

methanolic extract of *Pluchea lanceolata* (DC.) C. B.

Clarke, asteraceae, for protection against cisplatin-

induced nausea/vomiting was evaluated using rat pica

model of simulated emesis, where emetic stimuli is

reflected by increasing consumption of non-nutritive

substances such as clay or kaolin [15-18]. Cisplatin

induces significant nausea and vomiting in humans and

causes pica behavior in rats [19-20]. In present study,

effect of pretreatment with *MPL* on pica behavior was

determined in cisplatin-treated rats.

NISCAIR, New Delhi, India [Ref. no. NISCAIR/RHMD/Consult/-2009-10/1290/93]. A voucher specimen (PP-569) was deposited in the Department of Pharmaceutical Science, Guru Jambheshwar University of Science and Technology, Hisar. The plant material was further size reduced and stored until further use in an air tight container. The powdered material (200 g) was extracted with petroleum ether using a Soxhlet apparatus. The defatted material was air-dried, then extracted with 70% methanol using a Soxhlet apparatus. The extract was filtered through Whatman No. 1 filter paper and the supernatant was evaporated using rotary evaporator at 45°C and the final liquid suspension was lyophilized to get a reddish brown powder with 6.2% yield, hereafter referred as *MPL* (Methanolic extract of *Pluchea lanceolata*).

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125 filtered through Whatman No. 1 filter paper and the

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127 45°C and the final liquid suspension was lyophilized to

128 get a reddish brown powder with 6.2% yield, hereafter

129 referred as *MPL* (Methanolic extract of *Pluchea*

130 *lanceolata*).

131 **Kaolin preparation**

132 Kaolin was prepared based on earlier reported

133 method [21]. Briefly, pharmacological grade kaolin

134 (hydrated aluminum silicate) and gum acacia (Gum

135 Arabic) were mixed at a ratio of 99:1. A thick paste of

136 this mixture was prepared using distilled water. The

137 paste was rolled and cut into pieces similar to regular rat

138 chow pellets. The pellets were dried at room

139 temperature for 72 h.

140 **Experimental design**

141 The rats were randomly assigned to six groups of six

142 animals each. Group I and II treated with vehicle

143 (distilled water) was kept as normal and control group

144 respectively. Group III and IV were administered with

145 *MPL* (200 and 400 mg/kg body wt; p.o.) for 7 days.

146 Group V and VI were also administered with *MPL* (200

147 and 400 mg/kg body wt; p.o.) for 7 days. Group II, III

148 and IV were injected with a single dose of cisplatin (03

149 mg/kg body weight; i.p.) on day 4, to induce the pica

150 behavior. On each experimental day (next five

151 consecutive days), kaolin intake (g), food intake (g), and

152 body weight (g) were measured. To measure kaolin and

153 food intake, the remaining kaolin and food from the day

154 prior was collected including that spilled outside the

155 containers. The collected kaolin and food were dried for

156 72 h to obtain dry weight (g).

157 **Statistical analysis**

158 The statistical significance of differences among

159 values of individual parameters was evaluated by using

160 the Student's *t* test. All the values are expressed as mean

161 \pm SD. The significance was set at $p < 0.05$.

MATERIALS AND METHODS

Drugs and Chemicals

Cisplatin injection (Cipla, Ltd., India), Kaolin and Methanol (SD Fine-Chem Ltd, India) and all other chemicals were of analytical grades.

Animals

Male Wistar strain rats (150-250 g, 3-4 months of age) were procured from the disease-free small animal house of CCS Haryana Agriculture University, Hisar, Haryana, India. The animals were housed at 24 \pm 1°C temperature, 45 \pm 5% humidity, 12-h light-dark cycle, and left to acclimatize for 1 week before the experiments. Rats were allowed free access to water, standard laboratory rat chow and kaolin, placed in separated containers continuously available throughout the experiment. Experiments were carried out between 09:00 and 17:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee, GJUS&T, Hisar, Haryana and the care of the laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

Preparation of extracts of *Pluchea lanceolata*

The shade dried roots of the plant *Pluchea lanceolata* (DC.) C. B. Clarke, asteraceae, was collected from waste land of Dist. Hisar and Sirsa, Haryana (India), in October 2009 and authenticated by Raw Materials, Herbarium and Museum division of

RESULTS

Kaolin intake (pica) was measured in rats of various groups under study. Fig 1 demonstrates that *MPL* pretreatment significantly reduced kaolin intake induced by cisplatin. Cisplatin induced a significant increase in kaolin consumption in the animals of group II at 24, 48,

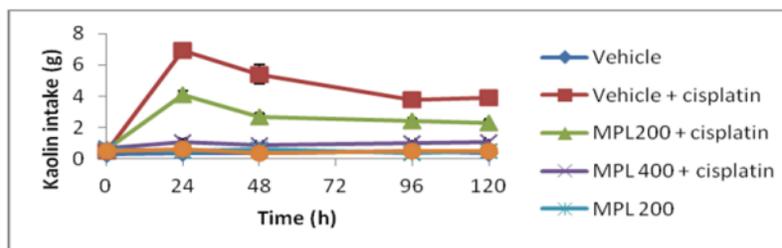


Fig 1. Effect of cisplatin (3 mg/kg) and cisplatin plus *MPL* (200 and 400 mg/kg) on kaolin intake. Values are expressed as mean \pm SD. ^a $p < 0.05$ with respect to normal, ^b $p < 0.05$ with respect to control.

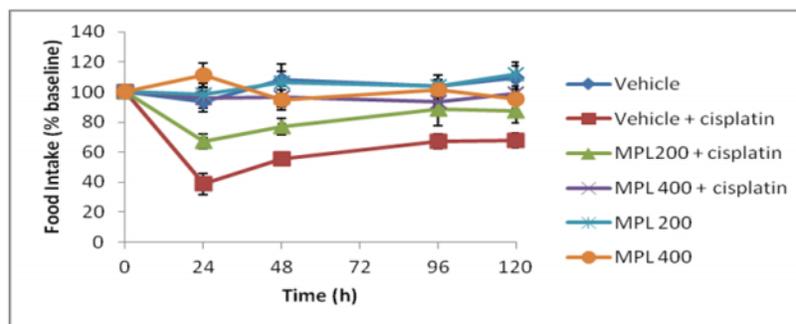


Fig 2. Effect of cisplatin (3 mg/kg) and cisplatin plus *MPL* (200 and 400 mg/kg) on reduced food intake (% baseline) induced by cisplatin in rats. Values are expressed as mean \pm SD. ^a $p < 0.05$ with respect to normal, ^b $p < 0.05$ with respect to control.

72, 96 and 120 h compared to normal animals of group I (97 baseline) compared to the group II ($P < 0.05$). 168
169 ($p < 0.05$). The *MPL* (200 mg/kg) pretreatment 198 Additionally, after 48 h, reduction of food intake was
170 significantly decreases the kaolin intake compared to 199 insignificant as compared to baseline, suggesting that
171 the group II at 24, 48, 72 and 96 h ($p < 0.05$). Kaolin 200 *MPL* significantly improved the reduction in food intake
172 intake at 24 h (4.1 ± 0.27 g) was significantly lower in 201 induced by cisplatin at 24 and 48 h. Further, no
173 *MPL* (200 mg/kg) pretreated animals than the animals 202 significant variation was found in food intake by the
174 of group II (6.9 ± 0.43 g). However, kaolin intake was 203 animals of group I, V and VI, compared to its baseline
175 still higher than normal baseline intake at 0 h (0.3 ± 0.02 204 (0 h).

176 g). Pretreatment with *MPL* (400 mg/kg) significantly 177
177 reduced kaolin intake compared to group II at 24, 48, 205
178 and 72, 96 and 120 h ($p < 0.05$). Moreover, the kaolin 206

179 consumption was near to the baseline intake at 0 h. This 207
180 suggests that *MPL* at 400 mg/kg reduced the pica for 208 from *Pluchea lanceolata* attenuated kaolin intake (pica)
181 longer and to a greater magnitude compared to *MPL* at 209 in cisplatin-treated rats. Additionally, the antioxidant
182 200 mg/kg. The group I, V and VI did not show any 210 activity of *MPL* may be one of the mechanisms by
183 significant variation in kaolin intake during the 211 which *MPL* attenuates cisplatin-induced nausea/emesis.
184 experiment when compared to its baseline (0 h). Fig 2 212
185 shows the effect of pretreatment with *MPL* on food 213 cisplatin (3 mg/kg; i.p.) induced an alteration in food
186 intake following cisplatin administration. 214 habit, indicated by increased kaolin consumption and

187 Treatment with cisplatin in the group II resulted in a 215 reduced food intake in rats. The increase in pica
188 significant reduction in food intake at 24 h (38.6% of 216 corresponds to a nausea/emesis induced by cisplatin in
189 baseline) and 48 h (55.7% of baseline) compared to the 217 humans [22]. The study also showed that methanolic
190 control group I ($p < 0.05$). When pretreated with *MPL* 218 extract of *Pluchea lanceolata*, effectively attenuated
191 200 mg/kg, food intake was significantly improved at 219 cisplatin-induced pica.

192 24 h as reduction in intake remained to 67.2% of 220
193 baseline. However, the food intake was still less, 221 nausea/vomiting is possibly mediated via cytotoxic
194 compared to the control group ($p < 0.05$). The treatment 222 damage to the enterochromaffin cells in the small
195 with *MPL* 400 mg/kg, food intake improved 223 intestine by ROS release [23-25] and treatment with an
196 significantly at 24 h (reduction in intake; 95.9% of 224 antioxidant should reduce these side effects. Based on

DISCUSSION

The present study inferred that methanolic extract

from *Pluchea lanceolata* attenuated kaolin intake (pica)

in cisplatin-treated rats. Additionally, the antioxidant

activity of *MPL* may be one of the mechanisms by

which *MPL* attenuates cisplatin-induced nausea/emesis.

The present study demonstrated that a single dose of

cisplatin (3 mg/kg; i.p.) induced an alteration in food

intake following cisplatin administration.

habit, indicated by increased kaolin consumption and

reduced food intake in rats. The increase in pica

corresponds to a nausea/emesis induced by cisplatin in

humans [22]. The study also showed that methanolic

extract of *Pluchea lanceolata*, effectively attenuated

cisplatin-induced pica.

The mechanism of cisplatin-induced

nausea/vomiting is possibly mediated via cytotoxic

damage to the enterochromaffin cells in the small

intestine by ROS release [23-25] and treatment with an

antioxidant should reduce these side effects. Based on

- 224 these facts, the present investigation was done to 281 9.
 225 evaluate the efficacy of *Pluchea lanceolata*, in cisplatin- 282
 226 induced pica. *In vitro* antioxidant activity of methanolic 283
 227 root extract of *Pluchea lanceolata* was already 284 10.
 228 determined by DPPH free radical scavenging assay and 285
 229 hydrogen peroxide scavenging activity [26,27]. The 286
 230 results showed that *MPL* at dose of 200 mg/kg and 400 287 11.
 231 mg/kg reduced cisplatin-induced pica. This suggests 288
 232 that cisplatin-induced pica (nausea) could be treated 289 12.
 233 with *MPL*. Although low doses of *MPL* caused reduced 290
 234 pica in cisplatin-treated rats, the improvement was still 291
 235 less as compared to normal kaolin intake. 292
 236 These findings support the notion that herbal 293 13.
 237 medications, such as *MPL*, could be an effective and 294
 238 inexpensive alternative for preventing chemotherapy- 295
 239 induced emesis without troublesome side effects. 296 14.
 240 Further, earlier studies also showed that herbal 297
 241 antioxidants may have a role in attenuating cisplatin- 298
 242 induced nausea and vomiting [28]. However, it is 299
 243 important to examine the interaction between the herbal 300
 244 extract and cisplatin, which could either hamper or 301 15.
 245 augment the anticancer actions of cisplatin. As cisplatin 302
 246 act by oxidative stress in tumor cells and treatment with 303 16.
 247 antioxidants could detoxify ROS, the herb may prevent 304
 248 oxidant injury to tumor cells and sensitize the tumor 305
 249 cells to the anticancer effects of chemotherapy [29]. 306 17.
 250 We conclude that herbal antioxidants potentially 307
 251 represent a new class of low-cost antiemetic agents for 308
 252 the treatment of chemotherapy-induced 309 18.
 253 nausea/vomiting. Additional studies are required to 310
 254 further investigate the antiemetic actions of such herbal 311
 255 medications and the effects of interaction with the 312
 256 chemotherapeutic agents. 313
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