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Ethanolic leaf extract of *Holoptelea integrifolia*, Planch. decreases cisplatin-induced pica in rats

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ABSTRACT

Treatment of nausea/vomiting caused by cisplatin, a potent chemotherapeutic agent and one of the most emetogenic stimuli, requires a combination of different antiemetic drugs. In this study, we investigated the effects of ethanolic extract of leaves of *Holoptelea integrifolia*, Planch., an antioxidant herbal medicine, on cisplatin-induced nausea using a rat model. Rats react to emetic/nausea-producing stimuli, such as cisplatin, with altered feeding habits, manifested by pica or increased consumption of kaolin (a type of clay). We measured pica in rats to quantify cisplatin-induced nausea, and to evaluate the antinausea effect of pretreatment with ethanolic extract of *Holoptelea integrifolia*, Planch (HIE) given orally. Cisplatin at 3 mg/kg (i.p) induced significant pica accompanied by reduced food intake, suggesting the presence of nausea. Hence, this cisplatin dose was selected for testing the antinausea activity of HIE. Cisplatin-induced pica decreased significantly when animals were pretreated with HIE at doses of 250 mg/kg p.o and 500 mg/kg p.o (P<0.01). HIE pretreatment decreased cisplatin-induced kaolin intake in the rat model of simulated nausea, suggesting that HIE and its active constituent(s) may play a therapeutic role in chemotherapy-induced emesis.

KEYWORDS: Cisplatin, pica, *Holoptelea integrifolia*, Planch, ROS.

INTRODUCTION

Nausea, vomiting and abdominal discomfort are common side effects associated with the use of chemotherapy in cancer patients, and adversely affect patients quality of life (1,2). More importantly, these side effects may lead to dehydration, compromised patient compliance or refusal of potentially curative cycles of chemotherapy (1) and therefore need to be treated.

Cisplatin, a potent chemotherapeutic agent, is known to cause significant nausea/vomiting (1,3). A number of studies have shown that cisplatin, like other chemotherapeutic agents, generates free radicals and releases reactive oxygen species (ROS) (4,5,6,7). Such enhanced oxidant activity in the gastrointestinal tract could cause injury to enterochromaffin cells, as well as other cells, and result in serotonin (5-hydroxytyptamine, 5-HT) release. Ensuing stimulation of vagal afferent sensory nerves and the chemoreceptor trigger zone in the brain stem caused by the released 5-HT could ultimately result in emesis (8,9). Therefore it is postulated that antioxidants could attenuate cisplatin-induced oxidant gut injury and reduce nausea/vomiting.

Since nausea/vomiting induced by cisplatin may be mediated by oxidants, we proposed to investigate the effectiveness of an antioxidant herb, *Holoptelea integrifolia,* Planch. (10), in a cisplatin-treated rat model.

Rats react to emetic stimuli by altering their feeding habit, manifested as increased consumption of non-nutritive substances such as kaolin (a type of clay), a phenomenon known as pica (11,12,13,14). This rat model has been used previously to simulate nausea and vomiting and shows reduced pica in response to antiemetic drugs (12,13). In the current study, we measured pica to quantify nausea in cisplatin-treated rats, and evaluated the effects of ethanolic extract of *Holoptelea integrifolia*, Planch (HIE) on cisplatin-induced pica.

MATERIALS AND METHODS

Preparation of ethanolic extract of H. integrifolia, Planch (HIE)

Plant leaves of *H. integrifolia,* Planch were obtained from the foothills of Yercaud (Tamil Nadu, India) in the month of August 2007 and were authenticated at Dept. of Botany, Amravati University, Amravati, Maharashtra. The dried and coarsely powdered leaves (400 g) extracted successively with 1.5 L each of petroleum ether (60–80^0 C), ethanol and in a Soxhlet extractor for 72 h. The extracts were concentrated to

dryness under reduced pressure and controlled temperature (40–50^0 C). The petroleum ether extract yielded a yellowish green sticky semisolid, weighing 3 g (3%) The ethanol extracts yielded brown and semi-solid residues, weighing 7.0 g (7.0%).

Phytochemical screening

The presence of phytochemicals alkaloids (Draggendorff's), flavonoids (Shibata's reaction), saponins (Frothing test), tannins (5% ferric chloride), terpenoids (2,4 dinitrophenylhydrazine), glycosides (fehling's solution), steroids (Liebermann's Burchard test) were evaluated.

Animals

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Amravati University, Amravati, Maharashtra, India. Swiss Albino mice (weighing 20–25 g) of either sex were used to perform acute toxicity studies & Male Wistar strain rats, weighing between 150 and 200 g, were used to evaluate antiemetic activity. Animals were housed in standard isolation cages (45×35×25 cm) under environmentally controlled conditions with 12-h light/12-h dark cycle. Rats were allowed free access to water, standard laboratory rat chow (Hindustan Liver Pvt. Ltd, Mumbai) and kaolin (see below), which were placed in separate containers continuously available throughout the experiment.

Acute toxicity test

Test animals were divided into groups (n = 6 per group) which were administered different doses of the crude extract (62.5, 125, 250, 500, 1000, 2000 and 4000 mg/kg p.o.), while the control group received only the vehicle (1% Tween 80 in water, p.o.). The general signs and symptoms of toxicity were observed for 24 h and mortality was recorded for each group at the end of this period (15).

Kaolin preparation

Kaolin was prepared based on a method described previously (11,12). Briefly, pharmacological grade kaolin (or hydrated aluminum silicate; Fisher, Fair Lawn, N.J.) and acacia (or Gum Arabic; Fisher) were mixed at a ratio of 99:1. Distilled water was used to form a thick paste of this mixture. The paste was rolled and cut into pieces that resembled regular rat chow pellets. The pellets were dried at room temperature for 72 h.

Experimental protocol

There was a 3-day adaptation period prior to study period (day 0). During this period, animals were placed in individual cages to allow access to both regular food and kaolin. Prior to testing the effects of HIE, three doses of cisplatin (Cipla Pharmaceuticals, Goa, India; available in aqueous form at 1 mg/ml), i.e. 3 mg/kg (n=6), 5 mg/kg (n=3) and 10 mg/kg (n=3), were given intraperitoneally (i.p.) to the animals. Based on the observation, cisplatin 3 mg/kg was selected for the evaluation of the effects of HIE pretreatment. We observed that one rat had mild diarrhea following administration of cisplatin at 10 mg/kg.

On Day 0, all four groups of rats received two i.p. injections at 2 P.M. and 2:30 P.M. Group 1 animals (n=6) received normal saline (vehicle). Group 2 animals (n=6) received normal saline and cisplatin 3 mg/kg (i.p). Group 3 animals (n=6) received HIE 250 mg/kg p.o and cisplatin 3 mg/kg. Group 4 animals (n=6) received HIE 500 mg/kg p.o and cisplatin 3 mg/kg.

At 3 p.m. on each experimental day, the kaolin intake was measured for five consecutive days. To measure kaolin intake, the remaining kaolin were collected including that spilled outside the containers. The collected kaolin was dried for 24 h to obtain dry weight values to the nearest 0.1 g.

No irritation, restlessness or other adverse effects (e.g., respiratory distress, abnormal locomotion, or catalepsy) were detected in rats following i.p. injection.

Statistical analysis

Data were analyzed using one-way ANOVA followed by Dunnett's test. The statistical analysis was performed with Prism 4.0 (GraphPad software Inc., San Diego, CA). $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION:

Results of the preliminary phytochemical analysis carried out on the crude ethanol extract indicated the presence of alkaloids, glycosides, sterols, flavonoids, tannins and saponins. No lethal effects were observed within 24 h after the administration of the extract at any of the doses used, even at the highest dose tested (4000 mg/kg). Therefore, the lethal dose (LD_{50}) of the extract in mice could not be determined. Dose levels of 250 and 500 mg/kg body weight were chosen for the pharmacological screening.

We demonstrated that a single dose of cisplatin induced an alteration in food habit, characterized by prolonged increased kaolin consumption (up to and at 120 h) in rats as shown in fig 1. The prolonged increase in pica corresponds to a prolonged and delayed emetic response to cisplatin in humans (2). We also demonstrated that pretreatment with HIE, an antioxidant herb, effectively attenuated cisplatin-induced kaolin intake.

Cisplatin-induced nausea/vomiting is possibly mediated

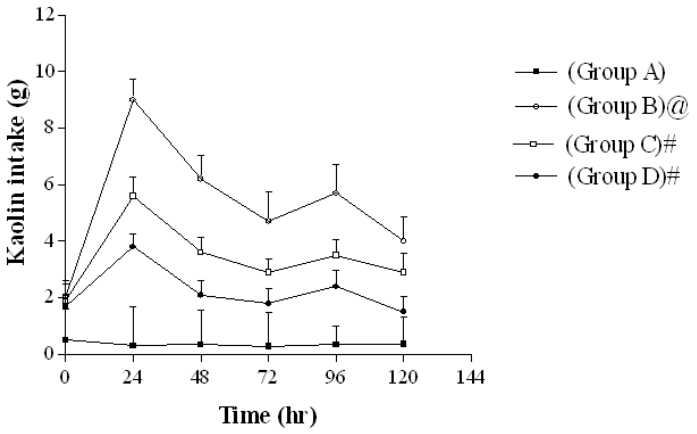

Figure 1. Effect of cisplatin and HIE on kaolin intake. The increase in kaolin intake induced by cisplatin at 3 mg/kg i.p was decreased by administration of HIE at 250 mg/kg p.o and 500 mg/kg p.o. Values are mean ± SD (n=6). P < 0.01 vs. control; one-way ANOVA followed by Dunnett's test. Statistical comparison is as follows: @ - vs A; # - vs B.

via cytotoxic damage to the enterochromaffin cells in the small intestine (13,14,16). The resultant 5-HT release could cause the emesis and nausea associated with cisplatin treatment (17-20). Since chemotherapeutic agents are known to release ROS (Reactive Oxygen Species) (4), it is possible that cisplatin could induce oxidant injury to enterochromaffin cells that results in 5-HT release, stimulation of 5-HT_3 receptors located on the vagal afferents, and initiation of the emetic reflex in the brain stem (21-24). ROS release by cisplatin has been confirmed in other studies (6,8). If ROS cause cisplatin-induced nausea/vomiting, then treatment with an antioxidant should reduce these side effects. This supposition has been confirmed in a study in which cisplatin-induced emesis was effectively prevented by preadministration of the antioxidant *N*-(2-mercaptopropionyl)-glycine (7). Additionally, cisplatin-induced emesis is exaggerated by ferric chloride, which is known to catalyze the production of cytotoxic oxygen radicals, and is ameliorated by deferoxamine, an iron chelator (22). Based on these facts, we proposed to investigate the efficacy of an antioxidant herb, HIE, in treating cisplatin-induced pica or simulated nausea.

Holoptelea integrifolia, Planch. is a widely used herb in traditional medical systems of India (10). The major constituents of *Holoptelea integrifolia,* Planch. are flavonoids, glycosides and terpenoids. The effects of HIE may result in part from its constituent flavones, which can attenuate oxidant stress and protect cells from lethal oxidant damage. We therefore sought to determine whether the antioxidant, HIE, could be used to treat cisplatin-induced symptoms mediated through putative oxidant mechanisms.

Data from the present study showed that HIE at both 250 mg/kg and 500 mg/kg reduced cisplatin-induced pica as shown in fig 1. This suggests that cisplatin-induced pica (nausea) could be treated with HIE. The mechanism of the antinausea effect could be mediated by the antioxidant properties of HIE (10).

Clinically, cisplatin-induced nausea/vomiting has been described as a biphasic phenomenon, with each phase responding to distinct antiemetic drugs (16). Overall, conventional antiemetics such as dopamine antagonists, antihistamines, anticholinergics, and glucocorticoids have a modest efficacy against chemotherapy-induced emesis, either when administered alone or in combination. A newer class of antiemetics, that is the 5-HT_3 receptor antagonists such as granisetron and ondansetron, have improved the therapy of chemotherapy-induced emesis. However, these drugs, in addition to their high cost, appear not to be effective against the delayed emetic response to cisplatin (16,25). Neurokinin receptor antagonists have demonstrated better efficacy in treating the delayed emetic phase (26,27). Thus patients who are treated with cisplatin chemotherapy have to consume additional multiple drugs to treat severe side effects, which in turn leads to additional side effects such as extrapyramidal effects, constipation etc (28-31). A herbal medicine, such as HIE and its flavonoids, could be an effective and inexpensive alternative to preventing chemotherapy-induced emesis without troublesome side effects. Our

results suggest that HIE at a dose of 500 mg/kg attenuated kaolin consumption in both phases. The mechanism involved in the attenuation of acute pica could by the antioxidant effects of HIE, but the mechanism involved in the reduction in pica in the later phase is not clear.

It is important to examine the pharmacokinetic and pharmacodynamic interaction between the antioxidant herb and cisplatin, which could either hamper or augment the anticancer actions of cisplatin. Antineoplastic agents result in oxidative stress in cells that may interfere with their antineoplastic activity. Treatment with antioxidants could detoxify ROS, prevent oxidant injury to tumor cells and sensitize the tumor cells to the anticancer effects of chemotherapy (4). Thus concomitant antioxidant use may potentiate cisplatin activity. A recent study by Cipak et al. has indicated that flavonoid antioxidants either potentiate or inhibit cisplatin-induced apoptosis depending on the specific flavonoid (32). The effect of flavonoids in HIE on cisplatin activity has not been studied previously and further studies are needed to confirm that HIE administration does not adversely alter the pharmacological parameters of cisplatin.

We conclude that herbal antioxidants potentially represent a new class of low-cost antiemetic agents for the treatment of chemotherapy-induced nausea/vomiting. Additional studies are required to further investigate the antiemetic actions of such herbal medications and the effects of interaction with the chemotherapeutic agents.

REFERENCES

1. Pendergrass K.B. Options in the treatment of chemotherapy-induced emesis. *Cancer Practice* **6:**276–281 (1998).
2. Grunberg S.M., Hesketh P.J. Control of chemotherapy-induced emesis. *N Engl J Med* **329:**1790–1796 (1993).
3. Bartlett N., Koczwara B. Control of nausea and vomiting after chemotherapy: what is the evidence? *Intern Med J* **32:**401–407 (2002).
4. Conklin K.A. Dietary antioxidants during cancer chemotherapy: impact on chemotherapy effectiveness and development of side effects. *Nutr Cancer* **37:**1–18 (2000).
5. Sodhi A., Gupta P. Increased release of hydrogen peroxide (H_2O_2) and superoxide anion (O^{-2}) by murine macrophages in vitro after cisplatin treatment. *Int J Immunopharmacol* **8:**709–714 (1986).
6. Torii Y., Mutoh M., Saito H., Matsuki N. Involvement of free radicals in cisplatin-induced emesis in *Suncus murinus. Eur J Pharmacol* **248:**131–135 (1993).
7. Torii Y., Saito H., Matsuki N. Induction of emesis in *Suncus murinus* by pyrogallol. A generator of free radicals. *Br J Pharmacol* **111:**431–434 (1994).
8. Hannemann J., Baumann K. Cisplatin-induced lipid peroxidation and decrease of gluconeogenesis in rat kidney cortex: different effects of antioxidants and radical scavengers. *Toxicology* **51:**119–132 (1988).
9. Schworer H., Racke K., Kilbinger H. Cisplatin increases the release of 5-hydroxytryptamine from the isolated vascularly perfused small intestine of the guinea-pig: involvement of 5-HT3 receptors. *Naunyn Schmiedebergs Arch Pharmacol* **344:**143–149 (1991).
10. Chandrasekar, D., Madhusudhana, K., Ramakrishna, S., & Diwan P.V. Evaluation of antimicrobial, antioxidant and wound-healing potentials of *Holoptelea integrifolia. J Ethnopharmacol* **115:** 249-256 (2008).
11. Mitchell D., Wells C., Hoch N., Lind K., Woods S. C., Mitchell L. K. Poison induced pica in rats. *Physiol Behav* **17:**691–697 (1976).
12. Takeda N., Hasegawa S., Morita M., Matsunaga T. Pica in rats is analogous to emesis: an animal model in emesis research. *Pharmacol Biochem Behav* **45:**817–821 (1993).
13. Takeda N., Hasegawa S., Morita M., Horii A., Urio A., Yamatodani A., Matsunaga T. Neuropharmacological mechanisms of emesis ll. Effects of antiemetic drugs on cisplatin-induced pica in rats. *Methods Find Exp Clin Pharmacol* **17:**647–652 (1995).
14. Rudd J. A., Yamamoto K., Yamatodani A., Takeda N. Differential action of ondansetron and dexamethasone to modify cisplatin induced acute and delayed kaolin consumption ("pica") in rats. *Eur J Pharmacol* **454:**47–52 (2002).
15. Lorke, D. A new approach to acute toxicity testing. *Arch Toxicol* 54:275–287 (1983).
16. Cubeddu L. X., Serotonin mechanisms in chemotherapy-induced emesis in cancer patients. *Oncology* **53** (Suppl 1):18–25 (1996).
17. Cubeddu L. X., O'Connor D. T., Parmer R. J. Plasma chromogranin A: a marker of serotonin release and of emesis associated with cisplatin chemotherapy. *J Clin Oncol* **13:**681–687 (1995).
18. Cubeddu L. X. Mechanisms by which cancer chemotherapeutic drugs induce emesis. *Semin Oncol* **19:**2–13 (1992).
19. Fukui H., Yamamoto M., Ando T., Sasaki S., Sato S. Increase in serotonin levels in the dog ileum and blood by cisplatin as measured by microdialysis. *Neuropharmacology* **32:**959–968 (1993).
20. Lindley C., Blower P. Oral serotonin type 3-receptor antagonists for prevention of chemotherapy-induced emesis. *Am J Health Syst Pharm* **57:**1685–1697 (2000).
21. Lindley C., Blower P. Oral serotonin type 3-receptor antagonists for prevention of chemotherapy-induced emesis. *Am J Health Syst Pharm* **57:**1685–1697 (2000).
22. Matsuki N., Torii Y., Saito H. Effects of iron and deferoxamine on cisplatin-induced emesis: further evidence for the role of free radicals. *Eur J Pharmacol* **248:**329–331 (1993).
23. Andrews P. L., Davis C. J., Bingham S., Davidson H. I. M., Hawthorn J., Maskell L. The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity. *Can J Physiol Pharmacol* **68:**325–345 (1990).
24. Yuan C. S., Barber W. D. Area postrema: gastric vagal input from proximal stomach and interactions with nucleus tractus solitarius in cat. *Brain Res Bull* **30:**119–125 (1993).
25. Hill R. P., Lubarsky D. A., Phillips-Bute B., Fortney J. T.,

Creed M. R., Glass P. S., Gan T. J. Cost-effectiveness of prophylactic antiemetic therapy with ondansetron, droperidol, or placebo. *Gen Pharmacol* **21:**1–10 (1990).

26. Campos D., Pereira J. R., Reinhardt R. R., Carracedo C., Poli S., Vogel C., Martinez-Cedillo J., Erazo A., Wittreich J., Eriksson L. O., Carides A. D., Gertz B. J. Prevention of cisplatin-induced emesis by the oral neurokinin-1 antagonist, MK-869, in combination with granisetron and dexamethasone or with dexamethasone alone. *J Clin Oncol* **19:**1759–1767 (2001).
27. Singh L., Field M. J., Hughes J., Kuo B. S., Chauhan N. S., Tuladhar B. R., Wright D. S., Naylor R. J. The tachykinin NK1 receptor antagonist PD 154075 blocks cisplatin-induced delayed emesis in the ferret. *Eur J Pharmacol* **321:**209–216 (1997).
28. Sanger G. J., Wardle K. A. Constipation evoked by 5-HT3-receptor antagonism: evidence for heterogeneous efficacy among different antagonists in guinea pigs. *J Pharm Pharmacol* **46:**666–670 (1994).
29. Wolfe S. G., Chey W. Y., Washington M. K., Harding J., Heath A. T., McSorley D. J., Dukes G. E., Hunt C. M. Tolerability and safety of alosetron during long-term administration in female and male irritable bowel syndrome patients. *Am J Gastroenterol* **96:**803–811 (2001).
30. Ballard H. S., Bottino G., Bottino J. Ondansetron and chest pain. *Lancet* **340:**1107 (1992).
31. Marin J., Ibanez M. C., Arribas S. Therapeutic management of nausea and vomiting. *Gen Pharmacol* **21:**1–10 (1990).
32. Cipak L., Rauko P., Miadokova E., Cipakova I., Novotny L. Effects of flavonoids on cisplatin-induced apoptosis of HL-60 and L1210 leukemia cells. *Leuk Res* **27:**65–72 (2003).

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