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CISPLATIN INDUCED REPRODUCTIVE TOXICITY AND ITS MODULATION BY *ANDROGRAPHIS PENICULATA* IN SWISS ALBINO MICE

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ABSTRACT

Cisplatin is an anticancer drug used against various types of epithelial cancers which produces reproductive toxicity when used during pregnancy. Andrographis paniculata is a popular herb in Asia and South East Asia which is traditionally used for treatment of diabetes, hypertension and sore throat. The present study was done to observe that Andrographis paniculata may reduce Cisplatin induced reproductive toxicity in Swiss albino mice. Pregnant mice were randomly divided into four groups. Group I mice received distilled water from gestation day (GD) 10 to 17, mice in group II was intraperitoneally administered with Cisplatin on GD 10, Group III mice received Andrographis paniculata (from GD 10 to 17) and Group IV was treated with both drugs. Maternal weight of all pregnant dam was taken from GD 0 to GD18. On GD 18, experimental animals were sacrificed, fetuses were removed, weighed, CRL was measured, observed for external anomalies and preserved in 10% formalin for further studies. Cisplatin treatment significantly reduced the maternal weight gain, fetal weight and CRL whereas increased the frequency of congenital malformations in the experimental animals. These effects were reduced by treatment of Andrographis paniculata extract along with Cisplatin. Thus Andrographis paniculata proved to be beneficial in Cisplatin induced reproductive toxicity.

Key words: Kalmegh, Anticancer, Congenital malformations, Fetal weight, Maternal weight

Cisplatin (*cis-diaminedichloroplatinum*) is one of the most effective chemotherapeutic agents extensively used for the treatment of head and neck, lung, testicular, ovarian and bladder cancer.[1,2] It acts as alkylating agents[3] which stops growth by formation of covalent adduct and intra- and enter- DNA cross-link between DNA bases and platinum compound.[4,5] These DNA- adducts interferes and inhibit the replication and transcription as well as mechanism of DNA repair in mammalian cells.[6]

According to the United States Food and Drug Administration (USFDA), Cisplatin is classified as 'D' drug which may be acceptable to pregnant woman due to its suitable benefits although the positive evidence of existence of fetal risk. Moreover several studies in pregnant mice, rats and monkeys demonstrated that it crosses the placental barriers and induces embryo-lethality and toxicity.[7,8] As it does not distinguish between a malignant and normal fast growing cell, it eliminates both types of cells producing anomalies in fetal liver, kidney and nervous system[15] along with restriction in growth and development of fetuses.[9,10]

The plant of family Acanthaceae, *Andrographis peniculata* (Kalmegh) has been used for many years in herbal medicine. It contains therapeutically important active principle diterpene lactones such as andrographolide, neoandrographolide, deoxyan drographolide and didehydroandrographolide in its aerial parts.[11,12,13] Extensive research in the past decade indicates that because of the presence of active principles, it possesses a wide range of

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pharmacological activities such as antioxidative,[14,15,16] anti-inflammatory[17,18,19] and anti-toxic activities.

Application of chemotherapy during pregnancy is a demanding issue by reason of to treat the mother with reducing the effect of chemotherapeutic agent simultaneously in favor of survival of fetuses. So for reduction of toxicity of Cisplatin and survival of fetuses some natural agents could be essential. Therefore in the light of antioxidant and antitoxic properties of *Andrographis peniculata*, present study was performed to demonstrate that it may reduce reproductive toxicity induced by Cisplatin in terms of maternal weight gain during the treatment, fetal weight and crown rump length with possible fetal malformations.

MATERIALS AND METHODS

Animals

In the present experimental study male and female Swiss albino mice 12-15 weeks of age were obtained from the animal house of the department of Anatomy, Institute of Medical Sciences, Banaras Hindu University and used after at least one week of acclimatization. The animals were allocated in animal house in polypropylene cages. During the experiment, mice were maintained at temperature of 23 ± 2 ºC, altering lighting with 12 h light/ dark cycle under maintained relative humidity (50 ± 10 %) conditions feeding with standard lab chaw and water *ad libitum*. All experimental protocol was used accordance with Ethical principles of animal research and was approved by the Ethics committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Drug preparation

Andrographis peniculata (aerial parts- leaves and stems) was collected from the Ayurvedic garden of Institute of Medical Sciences, Banaras Hindu University, Varanasi. Collected aerial parts were air dried, powdered with the help of grinder and then processed for extraction in a soxhlet apparatus in the presence of solvent methanol (250 gm powder/2.5 liter of methanol). After completion of extraction process, the extract was kept in incubator (for 3-6 days) to evaporate the methanol in order to obtain solidify extract. This extract was used for oral administration after making the desired dilutions in distilled water. Cisplatin was purchased from Cipla under the trade name of "Cytoplatin-10" dissolved in normal saline (0.9%).

Experimental design

Female mice during their proestrous phase of estrous cycle and male mice of the same stock were caged overnight for the matting in the ratio of 2:1. In the next morning (between 9.00- 10.00 am) vagina were examine to confirm the pregnancy. Presence of vaginal plug was considered as gestational day (GD) 0 of pregnancy.

These GD 0 pregnant mice were randomly divided into four groups with five animals per group. Group I mice were control, received single dose of normal saline on GD 10 via intraperitoneal injection, group II mice were administered with Cisplatin at the dose of 6 mg/ kg body weight on GD 10 whereas group III mice were treated with *Andrographis peniculata* (50 mg/ kg body weight) from GD 10- 17 via oral route and group IV mice received Cisplatin (6 mg/ kg body weight) on GD 10 intraperitoneally along with *Andrographis peniculata* (50 mg/kg body weight) from GD 10 to 17 via oral route. The maternal weight was recorded throughout pregnancy for all groups of animals. On the GD 18 the pregnant dams were sacrificed by cervical dislocation. Uterine horns were exposed, the fetuses and placentas were removed and weighed. All fetuses were rinsed in tap water, observed for external malformations and preserved in 10% formalin for further study.

Statistical analysis

All experimental data were calculated and presented in form of Mean ± SD. Data of different groups were analyzed and compared to check

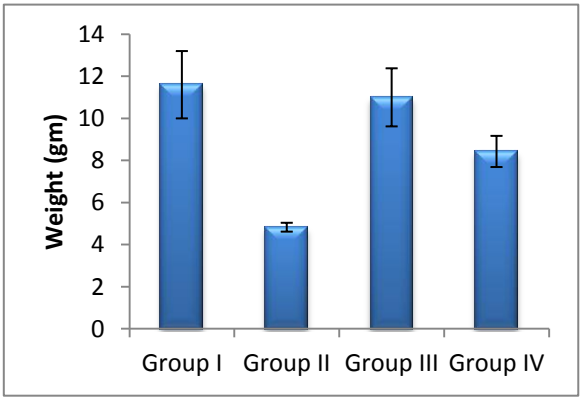

Fig. 1: Effect of treatments on maternal weight gain

statistical significance by using one way analysis of variance (ANOVA) test with help of software SPSS (Version 16). $p \leq 0.05$ was considered as significant.

RESULTS

Weight gained by pregnant mice

Figure 1 summarizes the finding of maternal weight gain of the pregnant mice of different groups at the 18th day of gestation. There was significant difference ($p<0.001$) of the treatment in the maternal weight gain. Group wise comparison showed that Cisplatin (CP) treatment significantly decreased ($p<0.001$) maternal weight gain in group II

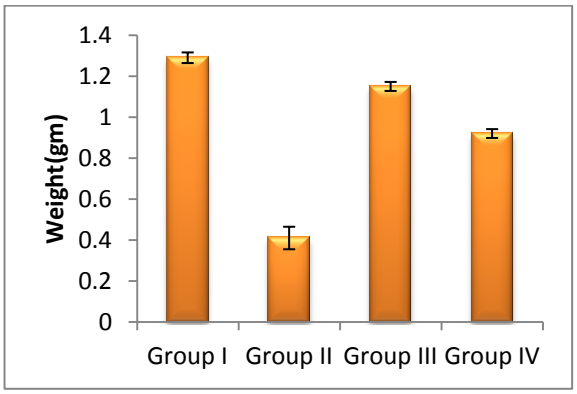

Fig. 2: Effect of treatments on fetal weight

as compared to that of control in group I whereas the *Andrographis peniculata* treatment along with Cispaltin significantly increased ($p<0.003$) the maternal weight gain in group IV as compared to

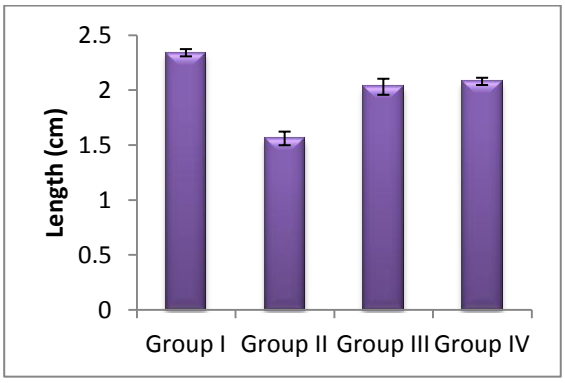

Fig. 3: Effect of treatments on fetal crown rump length

that in Cisplatin treated dams in group II. No significant difference ($p>0.05$) in maternal weight gain was observed between groups I, III and IV ($p>0.05$).

Fetal weight and crown rump length (CRL)

The data of fetal weight and CRL of different groups were presented in figure 2 and 3 respectively. Significant effect of treatments in fetal weight ($p<0.001$) and CRL ($p<0.001$) was observed among the groups. Groupwise comparison showed that Cisplatin treatment severely decreased ($p<0.001$) the fetal weight and CRL in group II as compared to control (group I). When *Andrographis peniculata* was given along with Cispaltin in group IV, the fetal weight and CRL was dramatically increased ($p<0.001$) as compared to those in Cispaltin treated fetuses of group II. The fetal weight was significantly decreased ($p<0.01$) in group IV as compared to that in group I but no statistical difference ($p>0.05$) in CRL was observed.(Fig. 4)

Congenital malformations

Congenital malformations were observed in fetuses of group II and IV. In group IV, there was a single fetus with hemorrhagic patches. In group II, congenital malformations were observed in 28% of the fetuses. There were 2 fetuses with anophthalmia, 2 fetuses with intracranial hemorrhage, 2 fetuses with shortening of limbs and 1 fetus hemorrhagic patches (Fig. 5).

DISCUSSION

According to the evaluation of American Cancer Society, cancer is second most common cause of death during pregnancy. Westernization of society could lean women to delay their child bearing. Generally they planned for family in third or fourth decade of life which may leads to incidence of gestational cancer. Although diagnosis of cancer during pregnancy is rare but one incidence has been occurred in every 1000 pregnancies.[22] As for the treatment of cancer with complication of pregnancy, chemotherapeutic agents are used though positive evidence of existence of fetal risk is reported. To overcome these problems some drugs are needed which benefit maximum to mother with minimal harm to fetus at the same time. The present experiment was done to study the protective effect of *Andrographis peniculata* against Cisplatin induced reproductive toxicity in mice.

In this study, single dose Cisplatin administration on GD 10 severely affects the dam's weight gain during gestation. Similarly the fetal weight and crown rump length were also severely affected. Cisplatin is a lower molecular weight (300 kD)

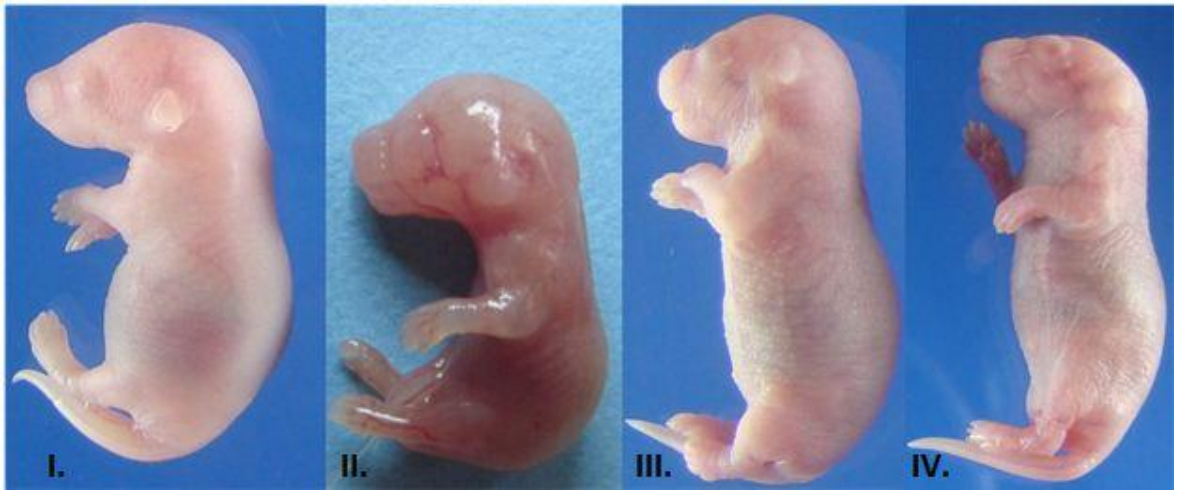

Fig. 4: Comparison of fetuses of different groups

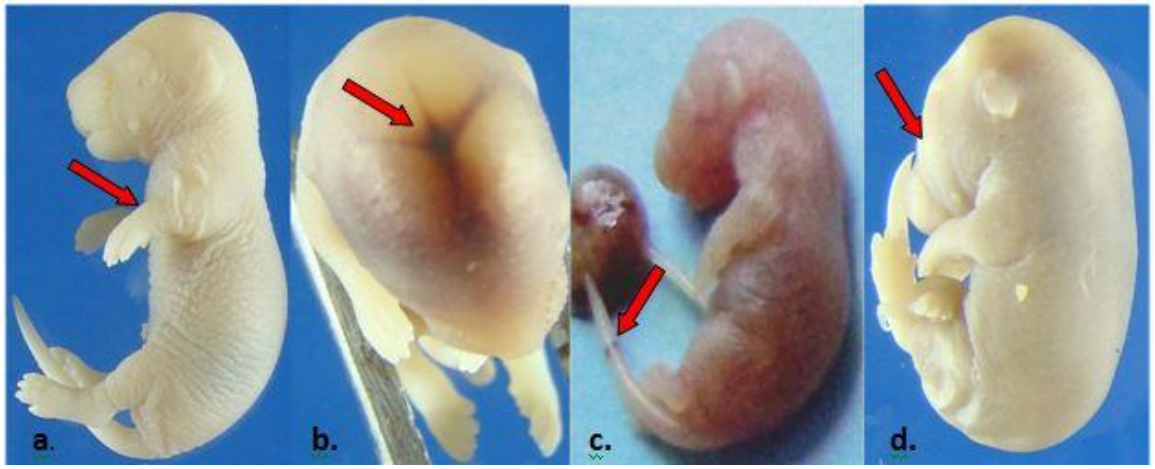

Fig. 5: Fetuses with congenital malformations; (a) A fetus with shortening of left upper limb, (b) A fetus with intracranial hemorrhage, (c) A fetus with hemorrhagic patch on tail and (d) A fetus with anophthalmia

platinum compound which crosses the placental barrier easily[23]. In function, it acts as alkylating agents[3]. The heavy metal platinum binds with nitrogenous bases of DNA results in a covalently linked DNA- adducts. These DNA adducts may be monofunctional, bifunctional or act as topoisomerase inhibitor along with free radical generators. By forming DNA- adducts CP ultimately brings about chromosomal changes. Further, modified DNA product (DNA adducts) hampered cell division along with RNA and protein synthesis. Besides Cisplatin also increases stress level which enhances the production of reactive oxygen species (ROS)[24,25] and nitrogen reactive species (NRS)[26] as well as diminishes the antioxidant systems[24-26] which also might resulted in feto-toxicity and congenital malformations as seen in the present study.

At the same time when *Andrographis peniculata* extract was administered along with Cisplatin, the dam's weight was significantly increased. The fetal weight and crown rump length were also increased and the rate of congenital malformations was decreased. Active components of *Andrographis peniculata* are diterpenoids such as 14-de-oxy-11-oxoandrographolide, 12-didehydroandrographolide and neo-andrographolide, collectively termed as andrographolides have shown several pharmacological properties including antioxidant,

vasorelaxant, antiplatelet, hypotensive and anti-inflammatory activities. The methanolic extract of the plant has been reported to inhibit the formation of oxygen derived free radicals such as superoxide, hydroxyl radicals, lipid peroxidation and nitric oxide[18]. So the protective effect of *Andrographis peniculata* against Cisplatin induced reproductive toxicity in the present study might be due to antitoxic and anti-oxidative properties of *Andrographis peniculata* which neutralized the toxic effect of Cisplatin.

According to previous studies though Andrographolide possesses anticancer activity which inhibit the effect of DNA topoisomerase II and effective against many tumor cell lines such as leukemia, myeloma, Hela, colon (HT-29), human peripheral blood lymphocytes (HPBLs) and human breast cancer MCF-7.[27,28] So the use of *Andrographis peniculata* along with Ciplatin might be beneficial to the mother in treating the disease. It is also advantageous to the developing fetus in increasing the feral weight and crown rump length and reducing the congenital malformation when the drugs were exposed during 2nd trimester of pregnancy as shown by the present study.

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