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ROLE OF *CYAMOPSIS TETRAGONOLOBA* AGAINST CISPLATIN INDUCED GENOTOXICITY: ANALYSIS OF MICRONUCLEUS AND CHROMOSOME ABERRATIONS *IN VIVO*

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ABSTRACT

The present study aim to examine the cytoprotective effect of herbal drug *Cyamopsis tetragonoloba* against cisplatin induced Genotoxicity. In the study mice were exposed to cisplatin. MeOH extract of herbal drug *C .tetragonoloba* (1000-500mg/kg body wt) was administered orally. Bone marrow protection was evaluated by scoring the different type of indusual aberrant, aberrant metaphase and micronuclei formation. Significant reduction in number of aberrant cell and different type of aberration was observed in treated group as compared to cisplatin treated group of animal. The administered of MeOH leaf extract of *Cyamopsis tetragonoloba* to the animal showed significant reduction in micronucleus induction also. Our findings support the use of *Cyamopsis tetragonoloba* as protective chemotherapeutic agent, promoting food and its potential for clinical use.

Keywords*: Cyamopsis tetragonoloba; cisplatin; Bone marrow; micronuclei; chemotherapeutic agent.*

Number of Tables : 5 **Number of Figures** : 3 **Number of References** : 17

INTRODUCTION

Cisplatin is a platinum co-ordination complex that is hydrolysed intracellularly to produce a high reactive moiety which cause cross linking of DNA. Cisplatin damage the human DNA, this agent also cause (myelotoxicity) bone marrow suppression. It is important to protect biological system from cisplatin genotoxicity.

Evaluation of herb as chemoprotective agent, the natural product and their active component used for prevention and treatment of chronic deasease. The main chemoprotective agent is thiol synthetic compound such as Amifostine, Dextrazoxane and Mesna. Amifostine is a powerful chemotherapeutic agent, it approved by FDA in1995 help reduced the level of renal injury in some cancer patient treated with chemotherapy. Dextrazoxane is approved by the FDA 1995; it has resulted in a decrease in cardiac event in cancer patient undergoing certain chemotherapy treatment. Mesna is approved by the 1988, it used to decrease bladder irritation (haemorrhage cystitis) coused by certain high dose chemotherapeutic procedure.

Many leguminous micronutrients, anthrocy-anins, trypsine inhibitor & lecithin have protective activity against cancer. The mutagenicity, genotoxicity, antimutagenicity and antigenotoxicity of cooked and dehydrated black bean have been investigated in mouse bone marrow and peripheral blood cells by micronucleus and comet assay test. *Cyamopsis tetragonoloba* is a rich source of soluble fiber for hypolipideamic influences in anthrogenic situation along with a known hypocholestremic, The benificial influences of dietory *Cyamopsis tetragonoloba* on serum, liver & billiary lipid in high cholesterol fed situation. The *Cymopsis tetragonaloba* range genotypic & phenotypic coefficient of variation, heritability genetic advanced and correlation among seven qualitative characters were estimated in 40 genotypic variations of cluster bean. The genotypic effect showed considerable amount of variability for all the traits, irrespective their place of correlation. The genotypic were grouped in to seven different clusters.

Several research studies have demonstrated that herbal plant contain medicinal effect eg. taxol, paclitaxal, vincristine , vinblastine, tiecotecon, irinotecon, itoposide, teniposide etc. Herbal medicine is the oldest form of health care known to mankind. There are many natural compound which plant origin have protective effect against genotoxicity and carcinogenicity of drug and chemical, some dietary natural compound minimize effect of genotoxins and carcinogens like fruit, cereals, nut, spices, vegetables, beverages, such as tea and coffee. Many conventional drug originated from plant source; A century ago, most effective drug were plant based. Example include- aspirin (Willow bark), digoxin (foxglove), quinine (Cinchona bark), and morphine (Opium poppy). World, wide herbal medicine receive a boost when the WHO encouraged developing countries to the traditional plant medicine to fulfill need unmet by modern system. With this background and presence

of abundant source of unique active component in *Cyamopsis tetragonoloba* , the presence study was taken up on this plant namely *Cyamopsis tetragonoloba* belongs to the family Leguminosae. *Cyamopsis tetragonoloba* is used traditional medicine for its Anti-inflammatory activity of alcoholic and aqueous extract, decreasing the level in blood glucose level and total lipid level reduction the risk of cardiovascular disease.

In the present study, we reported that *Cyamopsis tetragonoloba* extract possesses the *invitro* antioxidant potential. In continuation of this study, the *in vivo* cytoprotective activity was also assessed by using cisplatin as an oxidative DNA damaging agent and in evaluation of reduced chromosomal aberration and micronucleus formation in mouse bone marrow cells.

MATERIAL AND METHOD

Collection, Authentication and Extraction of plant:

The *Cyamopsis tetragonoloba* leaves are commercially available, purchased in the month of July from local market in Bhopal (M.P) authenticated by taxonomist (Zia Ul Hasan), Department of Botany, Sofia College of Science, Bhopal (M.P.), India. The plant material was collected and dried under shade and then powderd with mechanical grinder. The powder (300 gm) of *Cyamopsis tetragonoloba* is extracted using in petroleum ether for defatting according to the polarity. *C. tetragonoloba* was extracted by using maceration procedure at 37ºC with methanol : distilled water (70:30, v/v) .The macerated mixure was then filtered through muslin cloth and the solvent was removed under reduced pressure (120 mm Hg). The total yield found was 10 gm. This extract was stored at a temperature of 4ºC for further investigations.

Determination of total Phenolic content:

The amount of total Phenolic content was determined with the Folin-Ciocalteau reagent[11]. Galic acid was used as a standard and total Phenolic were expressed as mg/mg Galic acid equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg /ml galic acid were prepared in methanol. Concentration of 0.1 and 1 mg/ml of plant extract prepared in methanol and 0.5 ml of each sample were introduced in to test and mixed 2.5 ml of a 10 fold dilute Foluin reagent and 2 ml of 7.5%. sodium corbonate. The tube were covered with parafilm and allowed 30 minute at room temprature before the absorbance read at 760 nm spectrometrically.The Folin Ciocalteau reagent is sensitive to reducing compound including polyphenol. The absorbance of the blue colour that devoloped was measured at 760 nm.The concentration of total phenolic content was expressed as mg/g of dry extract.

Determination of total flavonoids content:

Total Flavonoids were measured by a colorimetric assay according to (Dewanto *et al*.,) An aliquat a dilute sample or standerd sample rutin was added to a 75 ul of NaNo2 solution and mixed for 6 minute before adding 0.15 ml AlCl3 (100 g/L), after 5

minute, 0.5 ml NaoH was added. Final volume was make up, up to 25 ml with distilled water and thoroughly mixed. Absorbance was determined at 510nm, against the same mixure, withought sample, as a blank. Total Flavonoids content was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of rutin.

Animals:

Swiss albino mice weighing 25 ±2 gm were maintain in ventilated animal house of Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (India), (Reg No.1283/c/09/CPCSEA). All mice were kept at controlled environmental condition (22±2ºC, 60±5 % humidity) with 12 hours light and dark cycle. Animal were fed with standard pellets (Golden Feed, New Delhi) and *ad labitum*. Animal were transferred to experimental room for acclimatization. It approval references No. is PBRI/12/IAEC/PN-271.

Acute oral toxicity study (OECD 423, 2001)

Acute oral toxicity was performed as per OECD 423 guidelines. Four dose levels were selected for acute oral toxicity 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg were used as dose range.

Chemotherapeutic agent:

Cisplatin (Trade name- cisplatin, Brand name-Platin, marketed by Cadila Healthcare according to FDA , book) is a chemotherapeutic drug. It was a first member of class, platinum containing anticancer drug, which now also include carboplatin and oxaliplatin.The platinum complex react *in vivo* ,binding to and causing cross linking of DNA, which ultimately triggers apoptosis (Programmed cell death). Bone marrow depression due to damage to stem cell couse reduction in blood white cells, platelets and red cell count.

Experimental design:

Mice were randomized into four groups of six animal each (n=6):

Group1. Vehicle (Normal saline only)

Group2. Vehicle + Cisplatin (5mg/kg i.p.bw)

Group3. Extract *C. tetragonoloba* (1000mg/kg bw)+ Cisplatin (5mg/kg i. p.)

Group4. Extract *C. tetragonoloba* (500mg/kg bw)+ Cisplatin (5mg/kg i. p.)

Bone marrow study:

Chromosomal aberration assay:

After 24 hour of treatment schedule, mice was injected with colchicines (5mg/kg i.p.) and after 90 minute animal was sacrificed by cervical dislocation and femurs were quickly removed, muscle was cleaned away from the bone and both femurs were placed on the edge of a renumbered centrifuge tube which correspond to animal number. The tube contain 5 ml normal physiological saline pre

warmed to 37ºC. Bone marrow was flushed with a hypodermic syringe fitted with a 22-g needle, and dispersed with spinal needle, and tube were centrifuge for 5 min. at 1000rpm. The supernant was removed and collect the remaining pellet then add 0.075 M pottasium chloride (prewarmed at 37ºC) drop wise with agitation to get the required quantity (5 ml) and incubate it for 20 minute at 37ºC in water bath then centrifuge if properly. Finally to fix the cells add Carnoy's fixative and allowed it to stand for 20 minute at room temprature. Slides was prepared by air drop method.[16] Slides was observed using microscope (). Total 100 metaphase were analyzed per animal and number of aberration, namely chromosome and chromatid break, fragment and ring were scored.

Microneuclease assay:

The *micronucleus assay* is a biological dosimeter of *in vivo* cisplatin induced. The bone marrow flushed out in centrifuge tube by using fetal calf serum. Then, centrifuge it at 1000rpm for 5 minute then supernatant was discarded. Slide were prepared and air dried then stain with May-Grunewald followed by Giemsa stain and observed under a light microscope for presence of micronuclei in polychromatic erythrocytes (PCEs) and Narmochromatic erythrocytes (NCEs).

Statistical analysis:

All experimental data are given as Mean ± SD. Statistical analysis was carried out using the one way analysis of variances (ANOVA), (Graph Pad Prism software). P< 0.05 is found significant.

RESULT AND DISCUSSION

Standerd curve of Gallic acid (10ug/ml - 60ug/ml) have a regression coefficient value 0.931 with line regression y = 0.033x+1.719 and the total amount phenol present in the *C. tetragonoloba* is shown in figure 1.In 100gm. of *C. tetragonoloba* extract, 26.6 + 0.05 mg Galic acid equivalent of phenol was found.

Table1: *Total Phenolic content:*

Line of regression	R^2	Absorbance	TPC (GAE) µg/mg extract
Y=0.033X+1.719	**0.931**	**2.6**	**26.6**

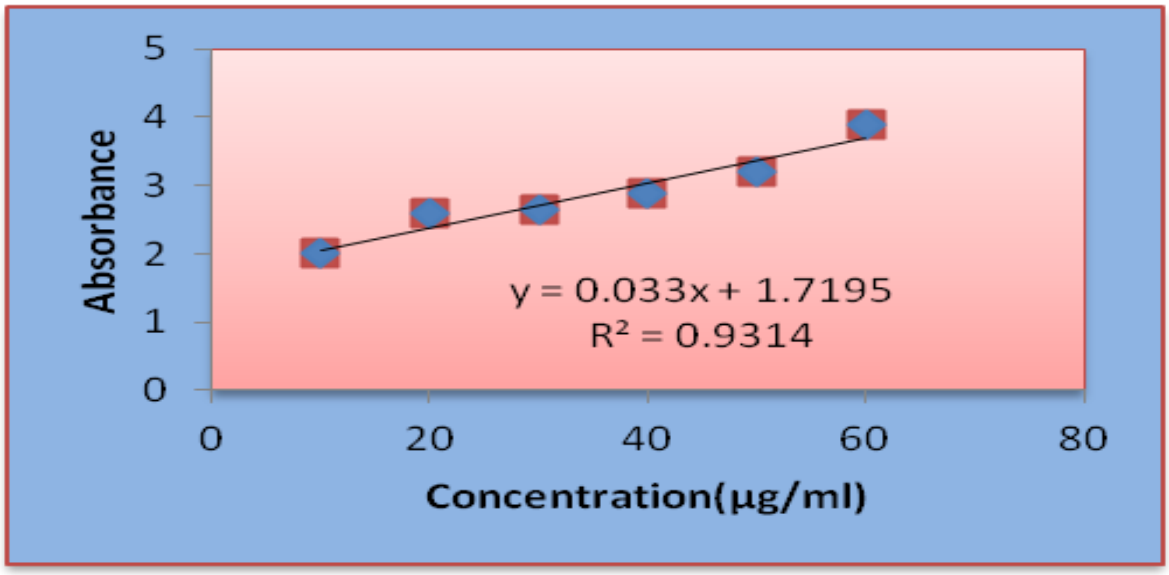


Fig 1: Total phenolic content

Determination of total Flavonoids content:

Rutin is Flavonoids glycosides reduce the fragility of blood vessel found in hemorrhagic disease and hypertension in human. In this study, rutin was used as standard. The *C. tetragonoloba* extract was found to contain 238 ± 0.09 mg of rutin equivalent. Flavonoids content of *C. tetragonoloba* extract was expressed as rutin equivalent in mg/g of extract**.**

Table 2: *Total Flavonoids content*

Line of regression	R^2	Absorbance	TFC (RE) µg/mg extract
Y=0.001X+0.103	**0.987**	**0.341**	**238**

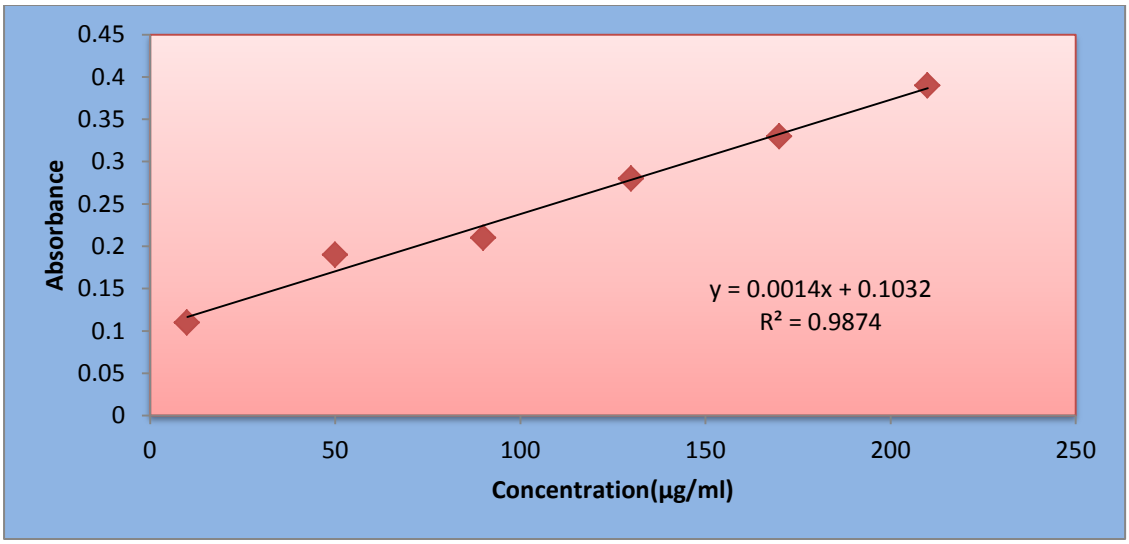


Fig: 2. Total Flavonoids content and Line of regression

Effect of *C. tetragonoloba* on cisplatin induced chromosomal aberrations: Chromosomal aberration has been used as a sensitive monitor of DNA damage in studies of several cytoprotectors. In the study, control group show less than 4 % aberrant cells, cisplatin produced a significant increase in the percent of aberrant cells.Corresponding, increase aberration in all indusual animal. The treatment with MeOH extract of *C. tetragonoloba* , it decrease the chromosomal aberration like chromatid break, centric ring, acentric fragment, delition, dicentric and total abnormal metaphases in bone marrow cells compared to narmal control group.

Effect of *C. tetragonoloba* on cisplatin induced micronuclei formation:

The control group of mice had (0.516±0.476 %) micronucleus polychromatic erythrocytes and cisplatin induced micronucleus formation results in (2.83±0.983%) micronucleus polychromatic erythrocytes . The treatment with *C. tetragonoloba* extract results in decreased micronucleus formation (0.666±0.816%)and (1.5+0.783 %) at 1000mg/kg bw and 500mg/kg bw respectively.

Table 3: *Effect of extract on aberrations (Break, Fragment, Deletion, Ring and Dicentric)*

Treatment	% Chromosomal aberration				
	Break	**Fragment**	**Deletion**	**Ring**	**Dicentric**
Vehicle	1.833±0.752	2.166±0.752	0.666±0.816	0	0.333±0.516
Vehicle+cisplatin	5.1661±.722	4.5±2.167	3.833±0.752	2.83±30.752	2±0.632
Extractd1+cisplatin	3.16±61.669	1.5±1.048	1.166±0.752	0.5±0.836	0.166±0.408
Extract+d2+cisplatin	3.833±1.169	2. 5±1.870	2.166±0.983	1.666±1.032	0.5±0.836

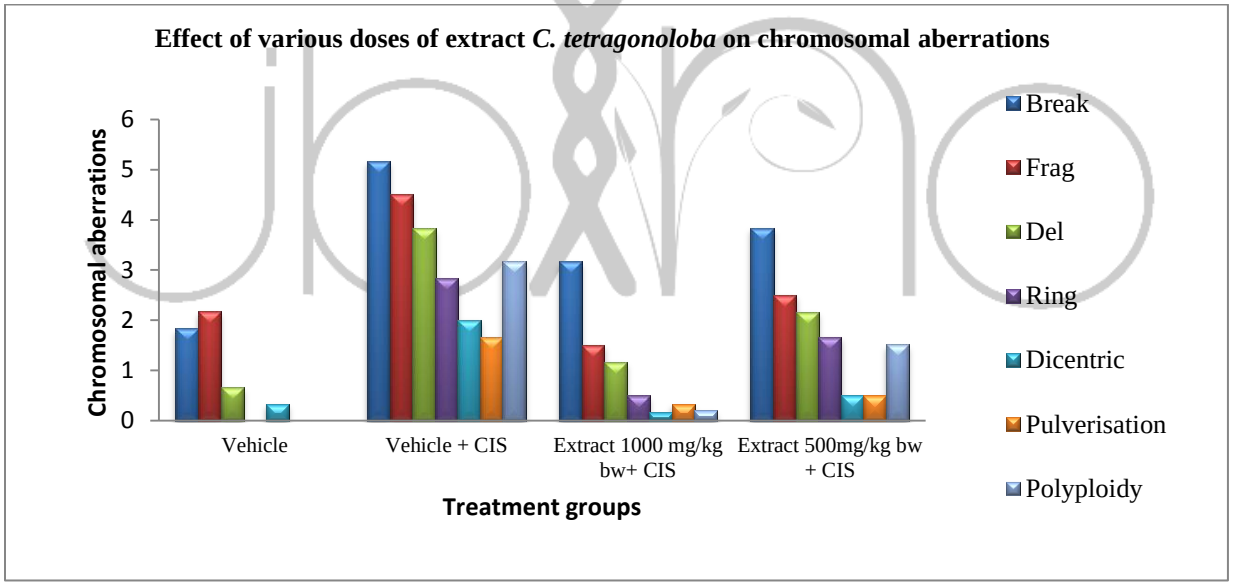
The values are Mean ± SD, n=6

P<0.05, + cisplatin is compared with vehicle + cisplatin and Extract C. tetragonoloba (1000mg/kg bw)+ Cisplatin (5mg/kg i. p.) Extract C. tetragonoloba (500mg/kg bw)+ Cisplatin (5mg/kg i. p.)

Table 4: *Effect of extract on aberrations (pulverized, polyploidy)*

Treatment	% Chromosomal aberration	
	Pulverized	Polyploidy
Vehicle	0	0
Vehicle+cisplatin	1.6661±.211	3.166±1.471
Extract d1+cisplatin	0.333±0.516	0.2±0.516
Extract d2+cisplatin	0.5±0.836	1.51±.048

The values are Mean ± SD, n=6 P<0.05, + cisplatin is compared with vehicle + cisplatin and Extract C. tetragonoloba (1000mg/kg bw) + Cisplatin (5mg/kg i. p.)and Extract C. tetragonoloba (500mg/kg bw)+ Cisplatin (5mg/kg i. p.)



The values are Mean ± SD, n=6

P<0.05, + cisplatin is compared with vehicle + cisplatin and Extract C. tetragonoloba (1000mg/kg bw)+ Cisplatin (5mg/kg i. p.) Extract C. tetragonoloba (500mg/kg bw)+ Cisplatin (5mg/kg i. p.)

Figure: 3. Effect of various doses of extract C. tetragonoloba on chromosomal aberrations

Effect of extract *C. tetragonoloba* on Micronucleus assay:

Table 5: *Effect of extract C. tetragonoloba on Micronucleus assay*

Treatment	% MN- PCE
Vehicle	0.516±0.476
Vehicle + cisplatin	2.830±0.983
Extract d1+cisplatin	0.666±0.816
Extract d2+cisplatin	1.500±0.763

CONCLUSION

The cytoprotective effect of *C. tetragonoloba* extract was seen against the genotoxicity induced by cisplatin. Extract was found to be rich in carbohydrates, phytosterol, tannins and phenol.

The protective potential of *C. tetragonoloba* extract was ascertained on the basis of total number of aberrations such as chromatid break, ring, acentric fragment, delition, dicentric, pulverizations, polyploidy and total percentage aberrant metaphases. All the results obtained were statistically significant. Our study concluded that Methanolic fraction of *C. tetragonoloba* possesses cytoprotective potential.

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