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# Protective Potential of *Bauhinia Variegata*Methanolic Leaves Extract against CisplatinGenotoxicity and *in Vitro* Antioxidant and Quantitative Estimation

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**Abstract**: Cancer is the worldwide diseases in developed or non developed countries. Cancer serves many types of toxicity even small exposure of treatment. It may cause serious side effect like ototoxicity, hepatotoxicity, and nephrotoxicity etc. Chemotherapy drugs targeted cancerous cell as well as non cancerous cell. It effect on the DNA structure of normal cell and may causegenotoxicity. It can be estimated through micronucleus assay etc.*B. varigata* is folk remedies in different cases and it contains huge chemical constituents that may help to reduce the genotoxicty induced by cisplatin. Pretreatment with 200mg/kg or 400 mg/kg methanolic extract of *B.varigata*orally for consecutively for five days and treated with cisplatin I.P. We observed that our extract were able to reduced the micronucleus frequency in extract treated group.

**Keyword: Micronucleus, *B. Variagata*, Chemotherapy.**

## Introduction:

Chemotherapy is a form of cancer treatment that involves taking one or more of type of drug which interferes with the DNA (genes) of fast-growing cells.Cisplatin or Cis-Diamminedichloroplatinum(II) or CIS-DDP has had a central role in cancer chemotherapy for the last 40 years and continues to be among the most widely-used antineoplastic drugs in clinical use (Ta et. al., 2009). CisDiamminedichloroplatinum(II) and its derivative, carboplatin, are widely used agents in various tumour types including lung and ovarian cancers (Germain et. al., 2010). They are used alone or in combination regimens to treat many types of human malignancies, including testicular and ovarian cancers, tumours of the bladder, head and neck, esophagus, and some lung cancers (Zlatanova, 1998). Cisplatin produced serious toxicity like peripheral sensory neuropathy or haematological suppression (Foltinova et. al., 2008),Nephrotoxicity (Hutchinson et. al., 1988), Neurologic toxicity (Abou-Elghait et. 2010) Genotoxicity (Vilar, J. B 2008)

## Material and method

### Animals

Albino Wistar Rats (200-220 gm) were separately group housed in ambient room temperature (25 ± 2°*C*) and relative humidity (50 ± 5%), maintained at 12:12 h dark–light cycle. Food and water were available *ad libitum*. All protocols were approved by Institutional Animal Ethics Committee of Pinnacle Biomedical Laboratories Pvt. Ltd. Bhopal (M.P.) India and carried out under strict compliance with Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests, Government of India.(Ref: 1824/PO/Ere/S/15/CPCSEA) and protocol approval references

### Plant material and extraction

Plant material (*B.varigata*) was collected locally in the month of march. Herbarium was prepared, and was identified at the Department of Botany, Safiacollege of science, Bhopal, M.P (voucher specimen-427/Bot/Safia/17). Locally collected shaded dried and grinded followed by extraction with pet ether for two days and then methanol at room temperature for 5 days by maceration method. The methanol extract was then rotary evaporated to dryness.

**Preliminary Phytochemical Screening**(Kokate et.al 2006)

Preliminary phytochemical screening for the detection of various constituents was carried out by using standard procedures.

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**Quantitative estimation**

- Total Phenolic Content Estimation (Ainsworth EA et.al 2007)
- Total Flavanoid Content estimation (Zhishen, J et.al 1999)

***In-vitro* antioxidant activity**

- DPPH assay(Gulçin I et.al 2006)
- Super oxide assay(Nishikimi, M. 1972
- Reducing power assay(R Jain and SK Jain,2011)

**Acute Oral Toxicity Studies**

Acute oral toxicity study was carried out in rat as per OECD-423 guidelines. The four fixed dose levels were selected as 5, 50, 300, 2000 mg/kg body weight. The rats were continuously observed for their mortality and behavioral response for 24 hr and thereafter once in a day for 14 days.

**Experimental Groups**

Different groups (n = 6) of rats were treated with either vehicle (normal saline 10 mg/kg p.o) or *B. variegata*extract in the doses of 200 and 400 mg/kg per oral for five consecutive days alone, or along with a single dose of cisplatin (5mg/kg i.p). Cisplatin was administered on 5th day 1 h after *B. variegata* extract treatment. All animals were sacrificed 24 h after the treatment ofcisplatin. And estimate micronucleus assay.

**Micronucleus Assay**(Schmid, 1975)

A rat's bone marrow micronucleus test was carried out according to earlier standard method [6] with some modification. The animals were sacrificed by cervical dislocation, 24 h after DOX treatment. The bone marrow from both the femurs was flushed in the form of a fine suspension into a centrifuge tube containing bovine serum albumin (BSA) about 2ml The cells were dispersed by gentle pipetting and collected by centrifuge at 1000 rpm for 5min. Cell pellets were re-suspended in drops of BSA, and bone marrow smears were prepared. After 24 h of air-drying, the smears were stained with May- Grunwald/Giemsa stain. In this method, polychromatic erythrocytes were stained reddish-blue, while nuclear materials are stained as dark purple. Six rats were used for each experimental point. The 2000 polychromatid erythrocytes were scored per animal. Cytotoxicity of DOX was determined by deceased in PCEs to total erythrocytes ratio (references as PCE/(PCE+NCE)), as well as we also determine the total micro nucleus formation (MNPCE%)

**RESULTS**

**Table 1 Phytochemical screeningof petroleum extract and methanolic extract**

| S. No. | Sec. Metabolites | Test Name | Pet Ether | Methanol |
|---|---|---|---|---|
| 1. | **Saponins** | Froth test | Negative | Negative |
| 2. | **Carbohydrates** | Molish<br>Benedict | Positive<br>Positive | Positive<br>Positive |
| 3. | **Protein / Amino acid** | Biuret`s<br>Ninhydrin | Negative<br>Negative | Negative<br>Negative |
| 4. | **Glycosides** | Borntrager<br>Legal`s<br>Keller-killani | Negative<br>Negative<br>Negative | Negative<br>Negative<br>Negative |
| 5. | **Alkaloids** | Mayers<br>Wagner's<br>Hangers | Negative<br>Negative<br>Negative | Negative<br>Negative<br>Negative |
| 6. | **Flavonoid** | Alkaline reagent<br>Lead acetate | Negative<br>Negative | Positive<br>Positive |
| 7. | **Terpinoids or steroids** | Salkowski<br>LibermannBurchard`s | Negative<br>Negative | Positive<br>Positive |
| 8. | **Tannins and phenolic** | Ferric chloride<br>Dilute iodine | Positive<br>Positive | Positive<br>Positive |

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**Table 2Acute oral toxicity study**

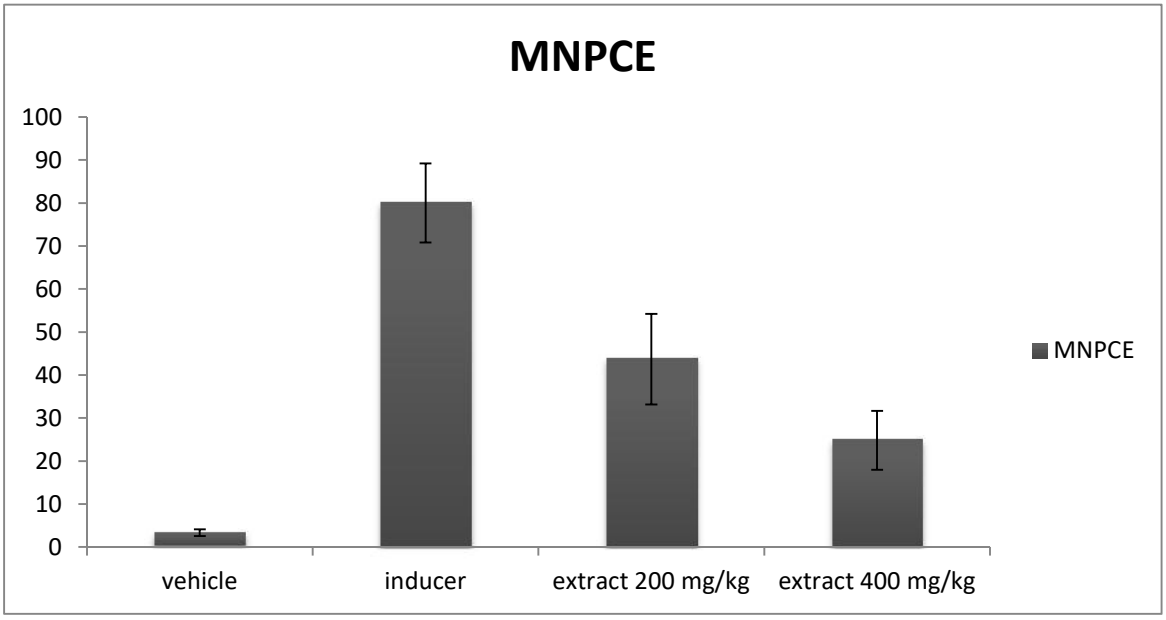
| Treatment Groups | *Fragariaananassa* Extract dose (mg/kg, p.o) | Observation after 2 hours | Mortality (dead/live) |
|---|---|---|---|
| **Group 1** | 5 | Normal | 0/3 |
| **Group 2** | 50 | Normal | 0/3 |
| **Group 3** | 300 | Normal | 0/3 |
| **Group 4** | 2000 | Normal | 0/3 |

Extract was found to be non-toxic up to 2000 mg/kg body weight. Finally, the dose of 200 mg/kg and 400 mg/kg were chosen for further studies

**Table 3 Effect of extract of *B.variagata*on cisplatin induced micronucleus polychromatid erythrocytes (MN-PCE) and ratio of polychromatid to nonchromatide (PCE/NCE) in bone marrow cells**

| | | |
|---|---|---|
| **Vehicle** | 3.33±0.816 | 3.13±0.545 |
| **Total MN-PCE (%)±SD** | 80.00±9.187** | 1.55±0.333** |
| **Vehicle+cisplatin** | | |
| **Extract 200 mg/kg** | 43.67±10.52** | 2.42±0.11(NS) |
| **Extract 400 mg/kg** | 24.83±6.853** | 2.77±0.727(NS) |

All data are represented in Mean ± SD format. Data were analyzed by a software stat-32 followed by One Way ANOVA comparison with many groups and then post doc by Bonferroni Test. When data compared with vehicle group ** is represented by P<0.001, * is represented by P>0.005 and non significant data is represented by (NS).




**Graph1. Total % Micronucleus polychromated erythrocytes**

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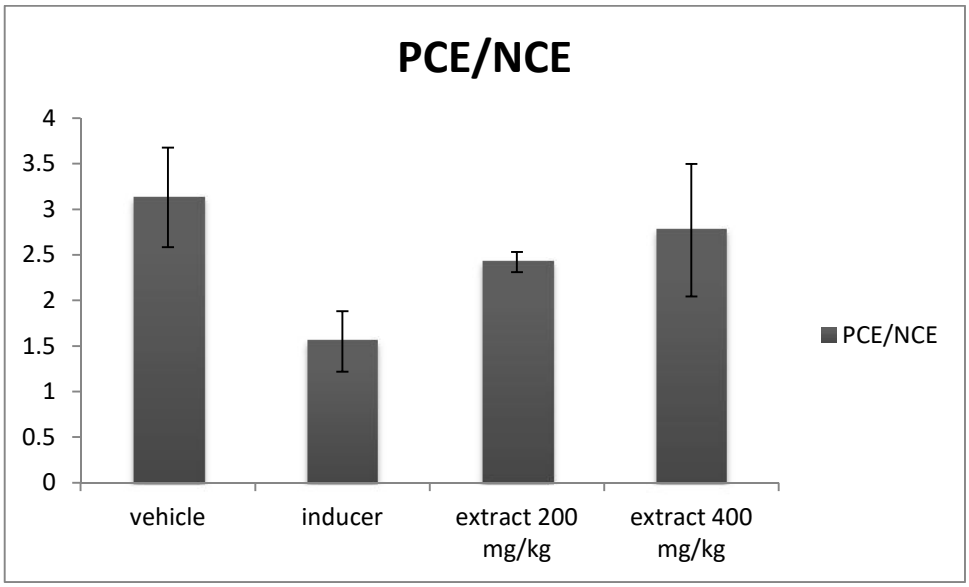
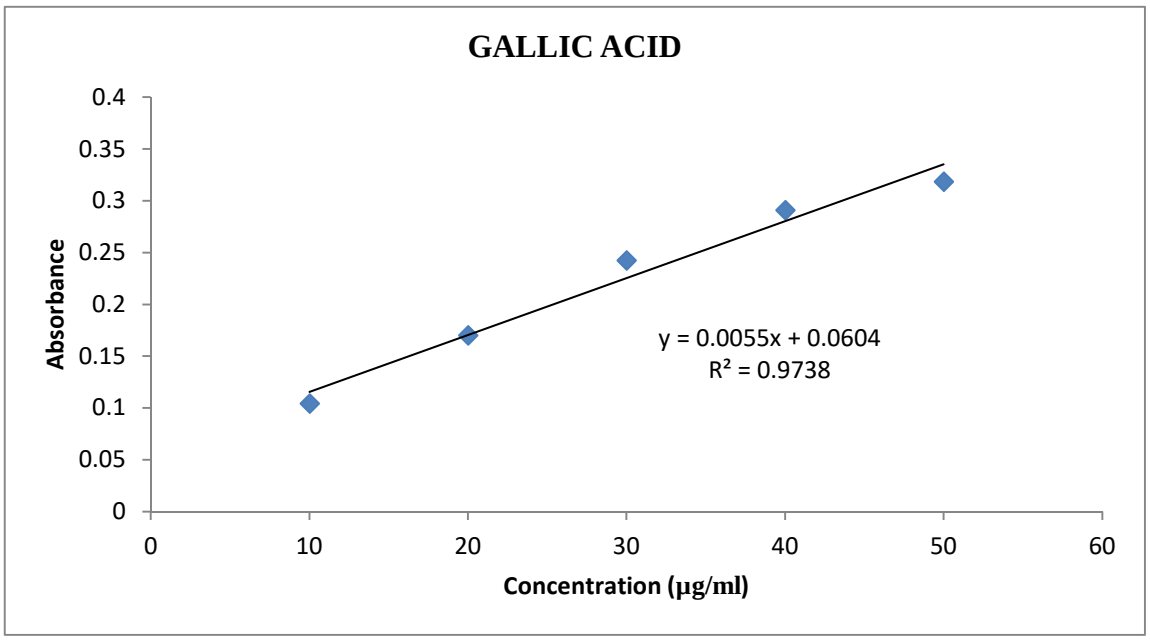



**Graph2. Ratio of polychromated erythrocytes and non chromate erythrocytes**

**Table 4 Standard different conc. 10 µg/ml to 50 µg/ml**

| S No | Conc (µg/ml) | Absorbance |
|---|---|---|
| **1** | 10 | 0.1042 |
| **2** | 20 | 0.1703 |
| **3** | 30 | 0.2426 |
| **4** | 40 | 0.2911 |
| **5** | 50 | 0.3187 |




**Graph 3: Graph of standard Gallic acid graph.**

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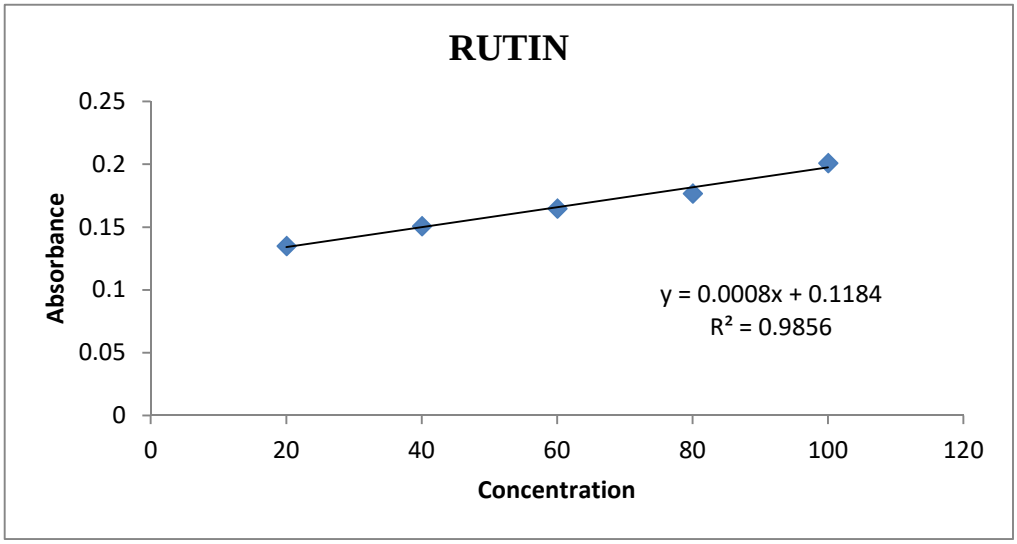
**Table 5 Total phenolic content of *Bauhinia variagata*methanolic.**

| S.No. | Conc. | TPC (mg/g) |
|---|---|---|
| **1.** | **1mg/ml** | 25.73±0.115 |

**Table 6 Total flavanoid content of Bauhinia*variegata*methanolic extract using Gallic acid as standard different conc. 10 µg/ml to 50 µg/ml**

| S.No. | concentration | Absorbance |
|---|---|---|
| **1.** | 20 | 0.135 |
| **2.** | 40 | 0.151 |
| **3.** | 60 | 0.165 |
| **4.** | 80 | 0.177 |
| **5.** | 100 | 0.201 |




**Graph 4: Graph Of Standard Rutin Graph**

**Table 7 Total flavonoid content *Bauhinia variegata*methanolic extract**

| s.no. | Conc. | TFC µg/ml |
|---|---|---|
| **1.** | 1µg/ml | 25.73±0.11547 |

**Table 8 DPPH of Ascorbic acid as standard**

| S.No. | Conc. | Absorbance of sample | Absorbance of control |
|---|---|---|---|
| **1.** | 20 | 0.397 | 0.699 |
| **2.** | 40 | 0.34 | 0.699 |
| **3.** | 60 | 0.269 | 0.699 |
| **4.** | 80 | 0.211 | 0.699 |
| **5.** | 100 | 0.142 | 0.699 |

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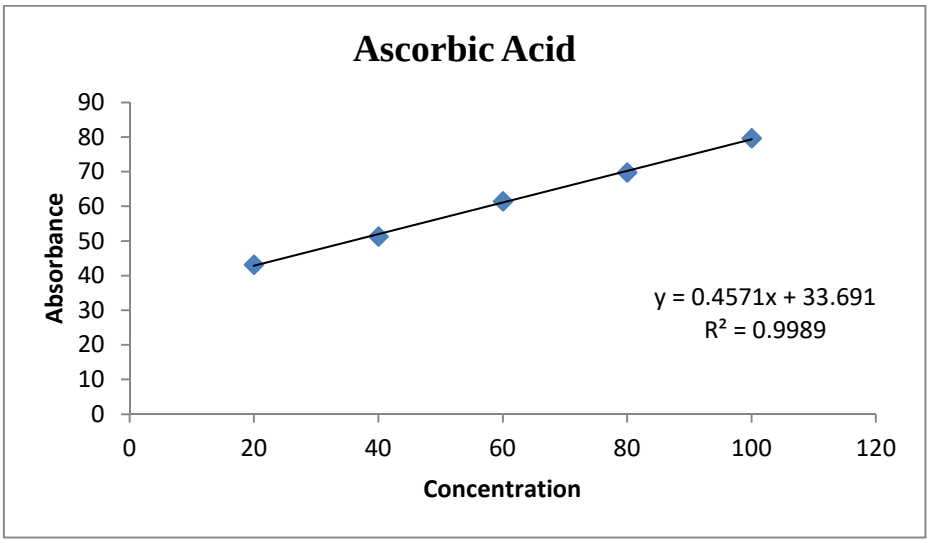
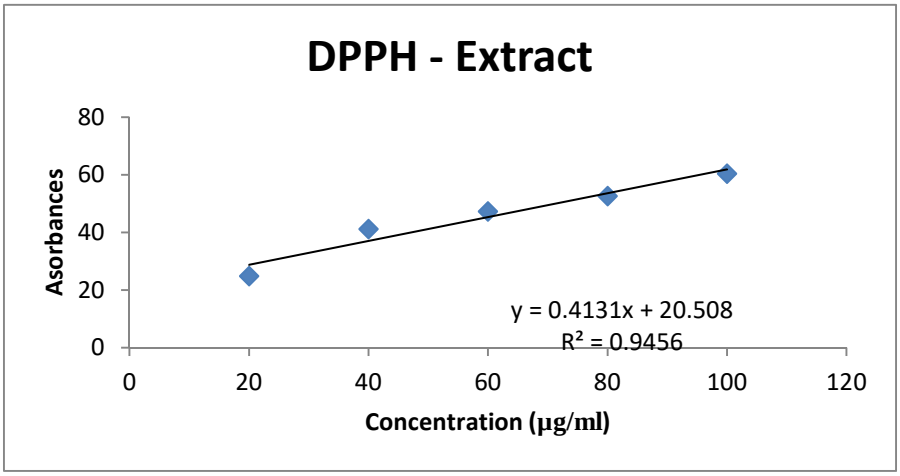



**Graph 5: Graph of DPPH of Ascorbic acid as standard**

**Table 9DPPH of *Bauhinia variegata*methanolic extract.**

| S. NO | Conc µg/ml | Absorbances test |
|---|---|---|
| **1.** | 20 | 0.355 |
| **2.** | 40 | 0.278 |
| **3.** | 60 | 0.213 |
| **4.** | 80 | 0.197 |
| **5.** | 100 | 0.122 |




**Graph 6: DPPH assay of *Bauhiniavariegata*methanolic extract**

**Table 10 Super oxide scavenging Test Bauhinia*variegata*methanolic extract**

| s.no. | Conc µg/ml | ABS of sample |
|---|---|---|
| **1.** | 10 | 0.581 |
| **2.** | 20 | 0.552 |
| **3.** | 30 | 0.521 |
| **4.** | 40 | 0.47 |
| **5.** | 50 | 0.413 |

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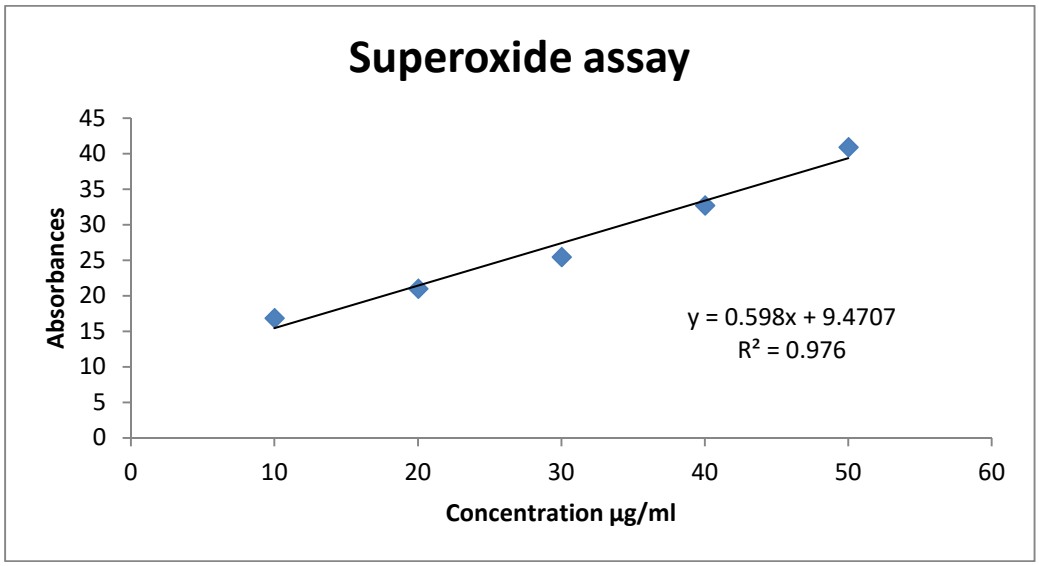
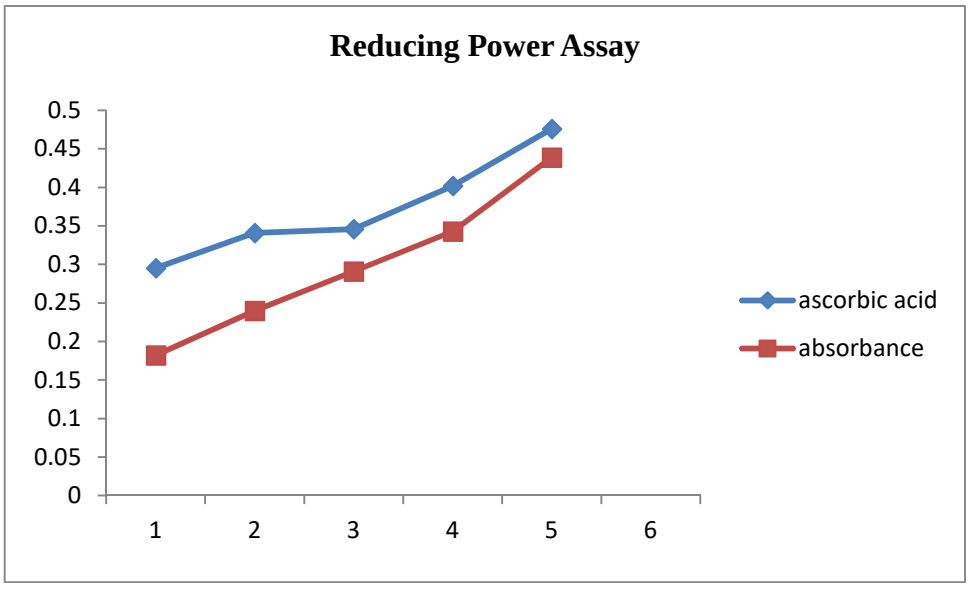



**Graph 7: Super Oxide Scavenging Test of*Bauhinia Variegata*of methanolic extract**

**Table 11 Reducing Power Assay Bauhinia*variegata*methanolic extract**

| s.no. | Conc µg/ml | Absorbance of ascorbic acid(Standard) | Absorbance of B.variagata |
|---|---|---|---|
| **1.** | 20 | 0.295 | 0.182 |
| **2.** | 40 | 0.341 | 0.24 |
| **3.** | 60 | 0.346 | 0.291 |
| **4.** | 80 | 0.402 | 0.343 |
| **5.** | 100 | 0.476 | 0.439 |




**Graph 8: Reducing Power Assay *Bauhinia Variegata*Methanolic Extract.**

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**Histology of micronucleus assay**

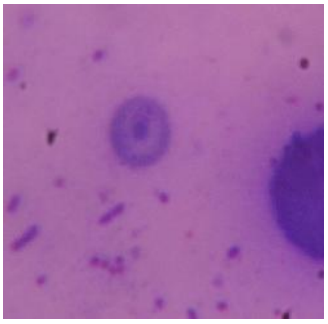

**Fig 1: Micronucleus**

**Discussion:**

Present investigation is dedicated to all those suffering patients that face the toxicity during chemotherapy. Many investigation and researches were regularly done on the chemotherapy toxicity to reduced its effect. many herbal medicaments , many isolated chemical constituents were reported likeEmblicamyrobalan(Celik, T. A. (2012) , Ficusbenghalensis(Sunny.S et.al 2011), Solanumlycopersicum(Raja, W et.al 2010), Crocus sativus(Sakr, S. A et.al 2014) etc.phytochemical investigation shows that our methanolic extract were contain flavanoids, phenolic,terpinoids are present and from quantitative estimation proved the exact quantity of flavanoid and phenolic when compared to the standard. Our methanolic plant extract content good antioxidant activity that were shown by DPPH,reducing power assay, superoxide scavenging assay. From in vivo micronucleus assay we observed that cisplatin were capable to gradually and significantly increased the frequency of micronucleus and that gradually and dose dependly reduced the micronucleus assay.so we concluded that our plant extract were having capability to reduce the Genotoxicity caused by cisplatin single I.P.

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