Evaluation of Chemoprotective and Anticarcinogenic Activity of *Cuscuta reflexa*

Shweta Mishra¹², Jignyasha Amit Rawal², Ram Kumar Sahu³

¹GRKIST Pharmacy College, Barela, Kukrikheda, Jabalpur (M.P.)-483001, India  
²Department of Pharmacy, Pacific University, Udaipur (R.J.)-313001, India  
³University College of Pharmacy, Pt. Deendayal Upadhyay Memorial Health Sciences and Ayush University of Chhattisgarh, Raipur (C.G.)-493111, India

**Article Information**  
Received 17 June 2019  
Received in revised form 23 Oct 2019  
Accepted 24 October 2019

**Keywords:**  
*Cuscuta reflexa*, Chemoprotective, Anticarcinogenic

**Corresponding Author:**  
E-mail: mishrasetu87@gmail.com  
Mob.: +919630014433

**DOI:** 10.20510/ukjpb/7/5/1580547431

**Abstract**  
The plant products are best option for the treatment of cancer due to its lower side effects. In the present study, it was planned to evaluate the chemopreventive and anticarcinogenic activity of *Cuscuta reflexa* extract in experimental rats. The hydroalcoholic extract was prepared, and acute toxicity, anticarcinogenicity activity and Chemopreventive effect were evaluated. The hydroalcoholic extract was free from toxicity up to dose of 5000 mg/kg body weight. The present investigation showed significant effect of the hydroalcoholic extract (250 and 500 mg/kg) in preventing melanoma tumor by B16F10 cell line in experimental animals. The result of micronucleus assay indicate that hydroalcoholic extract (250 and 500 mg/kg) have preventive potential against cyclophosphamide induced micronucleus formation in experimental animals. The findings suggested that the anticarcinogenicity and chemopreventive activity of *Cuscuta reflexa* may be due to antioxidant activity.

**1 Introduction**  
Malignant growth is in charge of around 25% of passings in created nations and 15% of all passings around the world. In malignant growth chemoprevention, synthetic operators are utilized to invert, smother, or anticipate cancer-causing movement. Cyclophosphamide tranquilize is for the most part recommended for treatment of different disease and dangerous tumors. The incessant utilization of Cyclophosphamide may actuate lethal impact to various fundamental organ of body in particular renal and liver disappointment. The overall utilization of normal items including therapeutic plants has turned out to be progressively vital in essential medicinal services. Common items are amazing choices for therapeutics, especially in creating nations, as a result of their moderately entrenched wellbeing profile. Likewise, concentrate of plants show low danger; in this manner they assume a vital role in pharmaceutical research.

Manufactured anticancer medications utilized for the treatment of different sorts of strong and hematogenous tumors including malignancy of blood, bone, ovaries, thyroid, head and neck, bladder, balls, stomach and delicate tissues and so forth. Nonetheless, utilization of manufactured anticancer medication is regularly confined due to its broad unfavorable symptoms that incorporate queasiness, mutagenicity, hemopoetic nephrotoxicity, urotoxicity, cardiotoxicity, hepatotoxicity, immunotoxicity, cancer-causing nature, and teratogenicity.

Herbal drugs have been appeared to be an exceptional and reliable sources for the improvement of new medications. Flavonoids and phenolic mixes are found to show different natural properties, including hepatoprotective, hostile to bacterial, and anticancer activity. The upsides of phenolic mixes are for the most part thought to be because of their antioxidant agent and free radical rummaging properties.

The plant chose for complete investigation depended on its simple accessibility, level of research work which isn't finished.
There are numerous restorative properties recorded on the extract of *Cuscuta reflexa*, yet none of the researchers have focused on chemoprotective and anticarcinogenic activity of the *Cuscuta reflexa*. In perspective on its assorted range of pharmacological properties, it was advantageous to explore and set up the chemopreventive and anticancer capability of extract of *Cuscuta reflexa* in exploratory rodents.

### 2 Material and Method

#### 2.1 Collection and identification of plant material

For the experimental work, *Cuscuta reflexa* was gathered from the nearby local forest of district Jabalpur (M.P.) in the month of Nov. 2014, and gathered species was validated by Botanist from Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur. The plant materials were shade dried, reduced to coarse powder and stored in airtight container till further use.

#### 2.2 Preparation of extract

The dried and powdered of whole plant of *Cuscuta reflexa* (500 gm) were successively extracted on a Soxhlet apparatus, employing chloroform and hydroalcoholic respectively. The extracts were further concentrated under reduced pressure with a rotary evaporator.

#### 2.3 Acute Toxicity

According to OECD 423 guidelines the acute oral toxicity was accomplished. Four dose levels (5, 50, 500, 1000, 2000 and 4000 mg/kg) were selected for acute oral toxicity. As per annexure 2a of OECD 423 guidelines, following methodologies were used⁶.

#### 2.4 Skin carcinogenesis bio assay (Papilloma model)

This assay has done as per the method of two stage protocol reported by Berenbeum (1975) in skin carcinogenesis assay and also standardized by us (Agrawal et al 2009).

The animals were divided randomly in to different groups each group comprises of 6 animals. Hair was removed from the dorsal region in an area of 2 cm² in all the groups. Treatments have given topically on the shaved areas⁷⁻⁸.

1. Group 1 (Vehicle control): 100μl acetone 2 times a week up to 16 weeks.
2. Group 2 (DMBA alone): 100μg DMBA was dissolved in 100μl acetone and single application was given.
3. Group 3 (Croton oil): 1% croton oil was applied on the skin 2 times a week up to 16 weeks.
4. Group 4 (Hydroalcoholic extract): 250 mg/kg extract was given 2 times a week up to 16 weeks.
5. Group 5 (Hydroalcoholic extract): 500 mg/kg extract was given 2 times a week up to 16 weeks.
6. Group 6 (DMBA + Croton Oil): Single dose of 100μg DMBA was dissolved in 100μl acetone given afterwards 1% croton oil was applied on skin 2 times a week up to 16 weeks.
7. Group 7 (DMBA + Croton Oil + Hydroalcoholic extract): Single dose of 100μg DMBA was dissolved in 100μl acetone was given afterwards the 100μl *C. reflexa* in Hydroalcoholic extract at the dose of 250 mg/kg was given after 1 hr. 1% croton oil was applied on skin 2 times a week up to 16 weeks.
8. Group 8 (DMBA + Croton Oil + Hydroalcoholic extract): Single dose of 100μg DMBA was dissolved in 100μl acetone given afterwards the 100μl *C. reflexa* Hydroalcoholic extract at the dose of 500 mg/kg was given after 1 hr. 1% croton oil was applied on skin 2 times a week up to 16 weeks.

The cumulative number of papillomas, tumor incidence, tumor yields and tumor burden were estimated.

#### 2.5 Melanoma tumor model

The original melanoma cell line (B16F10) was received from National centre for cell science Pune. The melanoma cell line (B16F10) maintained in-vivo in department of research was obtained and injected s.c. in animals of each of group.

- The C57BL hybrid mice of both sexes of the mean weight of 25 gm and 6-7 weeks old were obtained from the animal colony of our institute.
- Cell suspension having total 5 lacks cell / animal were injected subcutaneously.
- Animal were kept under observation for implantation of the melanoma cell line, and experiment was started after 10 days when the 1 cm tumours were seen.
- The treatment was given orally for 30 days and tumour volume and survival time of each animal was recorded.

Group 1: melanoma cell line (B6F10) were injected subcutaneously (S.C.) in all six mice

Group 2 (Hydroalcoholic extract): Melanoma cell line was injected by s.c. route. The tumor bearing mice were orally given dose of 250 mg/kg extract

Group 3 (Hydroalcoholic extract): Melanoma cell line was injected by s.c. route. The tumor bearing mice were orally given dose of 500 mg/kg extract

The treatment of extract was given orally for 30 days and tumour volume and survival time of each animal was recorded⁶.

#### 2.6 Hematological parameters

At the end of the experimental period, all mice were sacrificed by cervical dislocation. Blood was collected from freely flowing tail vein and used for the estimation of Hemoglobin (Hb) content, red blood cell count (RBC) and white blood cell count (WBC). WBC

Pharm & Biosci J. 2019; 7(5); 34
differential count was carried out from Leishman stained blood smears.

2.7 Micronucleus test

The animals were divided into following groups consisting of 6 animals each group.

Group 1: Cyclophosphamide 50mg/kg
Group 2: 250 mg/kg hydroalcoholic extract
Group 3: 500 mg/kg hydroalcoholic extract
Group 4: 250 mg/kg hydroalcoholic extract + Cyclophosphamide 50 mg/kg
Group 5: 500 mg/kg hydroalcoholic extract + Cyclophosphamide 50 mg/kg

After completion of study, the MNPC and PCE/NCE Ratio were estimated.

2.8 Data analysis

Results were analyzed using one way analysis of variance (ANOVA) followed by the tukey’s test by using statistical software package, Graph Pad Prism; version 5.03. Values were expressed as mean ± SEM and the p <0.05 were considered as statistically significant.

3 Results and Discussions

3.1 Acute oral toxicity (OECD 423)

The HAE was administered orally (5 - 2000 mg/kg) in albino mice and rodents. After administration of extract no any manifestations of lethality was watched. The extracts at the dose of 5000 mg/kg body weight were safe to use. Hence one tenth of dose of extracts (250 mg/kg and 500 mg/kg) were considered for treatment of the animals.

3.2 Skin carcinogenesis bio assay (Papilloma model)

Table 1 shows the 1st appearance of papilloma, cumulative number of papilloma, mean number of papilloma tumour yield in vehicle alone, DMBA alone, croton oil alone, hydroalcoholic extract alone (250mg/kg), hydroalcoholic extract alone (500mg/kg), DMBA + Croton oil (Control) group, DMBA + Croton oil + HAE (250mg/kg) and DMBA + Croton oil + HAE (500mg/kg) treated groups.

It suggests that papillomas were induced only in one control and two treated groups. 1st papilloma appeared early in DMBA + Croton oil (control) group i.e. on 56th day which was delayed in both the test group and was 84th and 98th day in HAE (250mg/kg) and HAE (500mg/kg) treated group, respectively. Cumulative number and mean number of papilloma was also higher in DMBA + Croton oil (control) group which were reduced in both the treated groups. DMBA + Croton oil + HAE (500mg/kg) have the lower number of papillomas as compared to DMBA + Croton oil + hydroalcoholic extract (250mg/kg) treated group. Tumour yield was also highly reduced in DMBA + Croton oil + HAE (500mg/kg) group as compared to DMBA + Croton oil (Control) & DMBA + Croton oil + hydroalcoholic extract (250mg/kg) treated group.

Table 1: Cumulative number of papilloma in different groups

| Groups                        | Dose          | 1st appearance of Papilloma | Cumulative No. of Papilloma | Papilloma Yield | Tumour Appears |
|-------------------------------|---------------|-----------------------------|-----------------------------|-----------------|----------------|
| Vehicle alone                 | 100μl         | _                           | _                           | _               | _              |
| DMBA alone                    | 104μg         | _                           | _                           | _               | _              |
| Croton Oil alone              | 1%            | _                           | _                           | _               | _              |
| Hydroalcoholic extract        | 250mg/kg      | _                           | _                           | _               | _              |
| Hydroalcoholic extract        | 500mg/kg      | _                           | _                           | _               | _              |
| DMBA + CO                     | 104μg + 1%    | 56th Day                    | 28.1±2.32                   | 4.66            | 100%           |
| DMBA + CO + Hydroalcoholic    | 104μg + 1% +  | 84th Day                    | 8.4±3.47*                   | 1.33            | 66.66%         |
| extract series                | 250mg/kg      |                             |                             |                 |                |
| DMBA + CO + Hydroalcoholic    | 104μg + 1%    | 98th Day                    | 3.2±1.89*                   | 0.5             | 33.33%         |
| extract series                | 500mg/kg      |                             |                             |                 |                |

Table 2 present the week wise appearance of papilloma in different control and test groups. It was found that papilloma was appeared in DMBA + Croton oil (Control) group DMBA + Croton oil + HAE (500mg/kg) and DMBA + Croton oil + HAE (250mg/kg) treated groups. Papilloma was started appearing from 5th week and reached to cumulative number 28 by the end of 16th week of experiment in DMBA + Croton Oil group. The papillomas was delayed and reduced in both the treated group. The maximum...
delay and reduction was found in DMBA + Croton oil + HAE (500mg/kg) test group as compared to DMBA + Croton oil (Control) and DMBA + Croton oil + HAE (250mg/kg) treated group.

3.3 Melanoma tumor model

The study showed the effect of HAE of *C. reflexa* on B16F10 melanoma tumour bearing mice. The preventive effect of extract was calculated using the parameter of inhibition rate (IR), Increase the life span (ILS), and Volume of tumour.

Table 3 showed the significant increment in tumour volume in control and test groups. When tumour volume of test group have compared to control group it was found that tumour volume increased slowly in extract treated group as compared to untreated group. The results suggest that *C. reflexa* extract have a preventive or reducing effect on tumour volume.

Table 4 shows the mean survival time and tumour doubling time and increased in life span of animals in different groups in transplanted tumour model. It is clear from below table that mean of survival, tumour doubling time and increased in life span have increased in test group where animal were treated with *C. reflexa* extract. These results suggest that *C. reflexa* extract have a antitumor effect on transplant tumour too.

| No. of Papillomas | 4th Week | 8th Week | 12th Week | 16th Week |
|-------------------|----------|----------|-----------|-----------|
| Vehicle alone     | –        | –        | –         | –         |
| DMBA alone        | –        | –        | –         | –         |
| Croton Oil alone  | –        | –        | –         | –         |
| Hydroalcoholic extract (250mg/kg) | –        | –        | –         | –         |
| Hydroalcoholic extract (500mg/kg) | –        | –        | –         | –         |
| DMBA + CO         | _        | 5.3±1.56 | 26.1±2.43 | 28.4±1.39 |
| DMBA + CO + Hydroalcoholic extract (250mg/kg) | _        | –        | 2.3±1.34* | 8.1±2.14* |
| DMBA + CO + Hydroalcoholic extract (500mg/kg) | _        | _        | 1.5±2.12* | 3.2±1.47* |

Data are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with DMBA + CO group

| Tumour volume in mice of different groups |
|------------------------------------------|
| Group | 1st Week | 2nd Week | 3rd week | 4th Week |
|-------|----------|----------|----------|----------|
| Control | 906.78±7.08 | 2121.37±4.62 | 3584.92±8.90 | –        |
| Hydroalcoholic extract (250mg/kg) | 455.84±6.90* | 935.12±4.87* | 1891.12±5.97* | 2104.49±6.82 |
| Hydroalcoholic extract (500mg/kg) | 340.19±5.24* | 724.56±8.46* | 1465.37±6.17* | 1734.65±8.46 |

Data are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with control group

| Various parameter in melanoma model of different groups |
|-------------------------------------------------------|
| Group | Mean of Survival (Day) | Tumour Doubling Time (Days) | Increase Life Span (%) |
|-------|------------------------|-----------------------------|------------------------|
| Control | 16.2±5.59 | 6.5±1.0 | – |
| HAE (250mg/kg) | 26.8±4.89* | 9.5±0.57* | 162 |
| HAE (500mg/kg) | 29.3±4.89* | 10.3±0.57* | 164 |

Data are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with control group
3.4 Hematological parameters

Hematological parameters of tumor bearing mice on day 16 were observed to be fundamentally modified contrasted with the ordinary gathering. The complete WBC tally was observed to be expanded with a decrease of Hb substance of RBC. The all out number of RBC demonstrated an unassuming change. In differential check of WBC, the percent of neutrophils expanded while the lymphocyte and monocytes tally diminished. At the same time interval on HAE extracts (250 and 500 mg/kg) treatment restored all the altered hematological parameters (Table 5).

| Treatment           | Hb content | Total RBC cells/ml×10⁶ | Total WBC cells/ml×10⁶ | Differential count |
|---------------------|------------|------------------------|------------------------|--------------------|
| Control (Group I)   | 11.29±0.3  | 1.19±0.01              | 20.37±0.28             | Lymphocyte (%)     |
| HAE (250mg/kg)      | 14.21±0.2* | 1.42±0.04              | 9.43±0.27*             | 55.73±5.1*         |
| (Group II)          |            |                        |                        | Neutrophils (%)    |
| HAE (500mg/kg)      | 16.36±0.3* | 1.56±0.05*             | 6.27±0.24*             | 70.12±4.6*         |
| (Group III)         |            |                        |                        | Monocytes (%)      |

Data are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with control group

3.5 Micronucleus assay

The micronucleus study showed that the single application of the Cuscuta reflexa hydroalcoholic extract at the dose of 250mg/kg and 500mg/kg body weight, prior to the administration of cyclophosphamide (50mg/kg) have preventive appearance on micronucleus formation in the dose dependent manner (Table 6; Fig 1). The PCE/NCE ratio of drug alone not suppressed as compared to control group.

| Group   | Treatment                                      | MNPCE±SEM | PCE/NCE Ratio |
|---------|------------------------------------------------|-----------|---------------|
| 1       | Cyclophosphamide alone (50mg/kg)               | 2.83 ± 0.75 | 1.035 ± 0.015 |
| 2       | HAE (250mg/kg) alone                           | 0.66 ± 0.57* | 1.05 ± 0.03   |
| 3       | HAE (500mg/kg) alone                           | 0.45± 0.31*  | 1.05 ± 0.01   |
| 4       | HAE (250mg/kg) + Cyclophosphamide (50mg/kg)   | 1.33 ± 0.51* | 1.05 ± 0.03   |
| 5       | HAE (500mg/kg) + Cyclophosphamide (50mg/kg)   | 0.98 ± 0.63* | 1.036 ± 0.028 |

Data are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with control group

Fig 1: Micronucleus cells

Chemoprevention is at present an essential procedure for controlling the procedure of malignant growth acceptance. In this way, there is a need to investigate therapeutic plants or other common operators that can fill in as chemopreventive agents. The present examination shows the chemopreventive capability of HAE of Cuscuta reflexa on DMBA-initiated skin tumorigensis in male Swiss pale skinned person and C57 BL mice. The skin show in exploratory creatures has been observed to be a helpful framework for researching the impact of dietary chemopreventors both robotically and operationally. The present examination exhibited that topical utilization of the HAE (250 and 500 mg/kg body weight) at the pre advancement stage demonstrated a critical decrease in tumor frequency, tumor trouble, tumor weight, tumor estimate, combined number of papillomas, in HAE regarded bunches when contrasted with the cancer-causing agent treated control. The antitumour movement of ethanolic concentrate of B.variegata was accounted for in Dalton’s austere lymphoma (DAL) in Swiss pale skinned person mice and in liver tumor in rodents. The definite components of anticarcinogenic and antimutagenic impacts of Cuscuta reflexa are not yet known. In any case, huge impacts were accomplished; suggesting that the plant concentrate may have either hindered the digestion of...
DMBA to its dynamic structure, deferred the advancement period of carcinogenesis, or down controlled responsive oxygen species arrangement. Such exhaustion of tumorigenesis inferable from comparative components and in different plants has been accounted for by others14-19. Proof has amassed to recommend this is maybe because of responsive oxygen species, which assume an imperative job in tumor inception by improving or encouraging the metabolic actuation or potentially starting impacts of cancer-causing agents. The methanolic concentrate of Bauhinia racemosa likewise delivered a defensive impact by diminishing the dimension of serum compounds, bilirubin and expanded the protein and uric acid dimensions17-19. The flavonoids which are available in the concentrate and have been accounted for to group’s antimutagenic and anticarcinogenic activity might be conceivable the action of Cuscuta reflexa extract. The present work recommends further assessment of the adequacy of this outstanding plant is justified.

4 Conclusion

The entire plants of Cuscuta reflexa is wealthy in flavonoids and polyphenol mixes, and observed to be dynamic against different pharmacological activity. Consequently from present examination it tends to be presumed that HAE of Cuscuta reflexa showed critical anticarcinogenic action. The flavonoids which are available in the extract and have been accounted for to group's anticarcinogenic activity might be possible the action of Cuscuta reflexa extract.

5 Conflict of Interest

Nil

6 Author’s contributions

SM and JAR participated in experimental work, RKS contributed interpretation of data. All authors read and approved the final manuscript.

7 References

1. Murthy P, Manjunatha N, Subodh BN, Chand PK, Benegal V. Indian J Psychiatry. 2010; 52(1): 89-99.
2. Hogle WP. Cytoprotective agents used in the treatment of patients with cancer. Semin.ONcol.Nurs. 2007; 23: 213-224.
3. Bhatt S, Upadhyay U, Upadhyay S, Soni H, Patel P. Chemotherapy facts and figures. Res. J. pharm. 2013; 4(5): 213-215.
4. Ravi Shankar P, Subish P, Mishra P, Dubey AK. Teaching pharmacovigilance to medical students and doctors. Indian J Pharmacol. 2006; 38(5):316-9.
5. Parthasarathi G, Karin NH, Milap C, Nahata. Adverse drug reactions. In: G Parthasarathi and Sten Olsson. A textbook of clinical pharmacy practice essentials concepts and skills. 2007; 84-102.
6. Organization for Economic Co-operation and Development (OECD) Guidance Document on Acute Oral Toxicity Testing 420. Organization for Economic Co-operation and Development; Paris, France: 2008.
7. Agrawal RC, Jain R, Wasim R, Oves M. Anticarcinogenic effect of Solanum lycopersicum fruit extract on Swiss albino mice. Asian Passific Journal of Cancer prevention. 2009: 10: 379-382.
8. Berenblum I. Suquential aspect of carcinogenesis is skin cancer, A comprehensive treatise", Plenum Press New York. 1975; 1: 323-344.
9. Aron CS, Sorg S, Zimmer D. The mouse bone marrow micronucleus test: Evaluation of two drug candiclate, Mutation. Research. 1989; 223: 129-140.
10. Schimid S. The Micronucleus test. Mutation Research. 1975; 31: 9-15.
11. Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E. Human Micronucleus Project. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutant Res. 2003: 534(1-2): 65-75.
12. Montbriand MJ. Senior and health-professionals mismatched perceptions and communication about prescription and non-prescription medication. Can J Aging. 2000; 19: 35-56.
13. Rajkapoor B, Jayakar B, Murgeshand ND, Akthisekaran. Chemoprevention and cytotoxic effect of Bauhinia variegata against N-nitrosodiethylamine induced liver tumors and human cancer cell lines. J Ethnopharmacol. 2006; 104: 407-9.
14. Kausar H, Bhasin G, Zargar MA, Ather M. Palm oil alleviates 12-O tetradecanoyl–phorbol-13 acetate-induced tumor promotion response in murine skin. Cancer Lett. 2003; 192: 151-60.
15. Sancheti G, Jandal A, Kumari R, Goyal PK. Chemopreventive action of Emblica officinalis on skin carcinogenesis in mice. Asian Pac J Cancer Prev. 2005; 6: 197-201.
16. Kumar M, Soni KA, Shukla S, Kumar A. Chemopreventive potential of Tribulus terrestis against 7,12 dimethylbenz (a)anthracene induced skin papillomagenesis in mice. Asian Pac J Cancer Prev. 2006; 7: 289-94.

Pharm & Biosci J. 2019: 7(5): 38
Mishra et al., Evaluation of Chemoprotective and Anticarcinogenic Activity of Cuscuta reflexa

17. Ather M. Oxidative stress and experimental carcinogenesis. Indian J Exp Biol. 2002; 40: 656-67.

18. Kumar RS, Sunderam RS, Sivakumar T. Effect of Bauhinia racemosa stem bark on N-nitrosodiethylamine induced hepatocarcinogenesis in rats. Am J Chin Med. 2007; 35: 103-14.

19. Brown JP. A review of the genetic effect of occurring flavonoids, Anthraquinones and related compounds. Mutat Res. 1980; 75: 243-77.

20. Hirano T, Oka K, Akiba M. Antiproliferative effect of synthetic and naturally occurring flavonoids on tumour cells of human carcinoma cells lines. Res Comm Chem, Pathol Pharmacol. 1989; 64: 69-78.