Journal of Pharmaceutical Research International

33(59A): 216-228, 2021; Article no.JPRI.76966
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Antimutagenic Activity of Cassia Auriculata Linn Fractions along with Anticancer Activity in Male Albino Mice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i59A34267

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/76966

Original Research Article

Received 10 October 2021
Accepted 15 December 2021
Published 16 December 2021

ABSTRACT

Background: In recent years, there has been a surge in interest in studying plant-derived materials and their impact on DNA. Herbal products include a number of natural substances that may help protect cells against mutagen-induced cell damage.
Aim: The purpose of this research was to assess the genotoxic effects of Cassia Auriculata Linn flavonoids (CAF) and Cassia Auriculata Linn saponin (CAS) rich fractions on mouse bone marrow cells utilizing chromosomal aberration test and micronucleus assay.
Methodology: The suppressive impact of CAF and CAS on 7, 12-dimethylbenz (α) anthracene (DMBA) and Croton oil induced skin tumor promotion in mice with topical administration twice

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weekly for 18 weeks is also investigated in this work. Three dosages of 100 and 200 mg/kg body weight were used. Single oral dosages of CAF and CAS Fraction at the three levels did not enhance the number of micronucleate polychromatic erythrocytes in the micronucleus experiment.
Result: In mice bone marrow cells, a single oral treatment of CAF and CAS fraction revealed no significant alterations in mitotic indices or chromosomal aberration induction. The clastogenicity of CYP was considerably decreased by pretreatment with CAF and CAS fraction. As a result, it can be stated that CAF and CAS fraction had no genotoxic impact on mouse bone marrow cells.
Conclusions: The portions of *Cassia Auriculata* have been shown to be non-genotoxic and non-clastogenic at the quantities utilized in this investigation. CAF and CAS Fraction might possibly be a promising skin tumor promotion reducing agent, according to this research.

Keywords: Cassia Auriculata; cyclophosphamide; Genotoxic effects; anticancer activity.

1. INTRODUCTION

Natural products, in different forms such as extracts, fractions, or as a chemical platform, continue to play an important role in the treatment of numerous illnesses [1]. Plants have historically been the most common source of medicines throughout human history, owing to their secondary metabolites, which have a wide range of pharmacological activities. The knowledge of the many medicinal powers of plants has mostly been passed down via folk traditions and subsequently proven through scientific data [1,2]. Natural bioactive compounds in the prevention and/or treatment of chronic illnesses, which have been dubbed the public health challenge of the twenty-first century, are now one of the trendiest topics in medicine [2]. Indeed, as Lunenfeld and Stratton explain, the rise in healthcare systems and life expectancy in developed countries, as well as the decrease in fertility rate due to chromosomal abnormalities, has resulted in a rapid increase in global population ageing, with an increase in chronic degenerative disease [2,3]. According to the World Health Organization (WHO, 2018), cancer is the second leading cause of death (9.6 million in 2018), with malignancies of the liver (782 000 deaths) and breast (627 000 deaths being the most prevalent. DNA damage has long been known as a cause of cancer formation, as stated [3]. In reality, oncogenes and tumor suppressor genes are affected by mutations or chromosomal abnormalities, leading to malignant transformation of cells [4]. As a result, antimutagenic, antigenotoxic, and anticarcinogenic chemicals play a critical role in cancer prevention [4,5]. A wide range of medicinal plants and their metabolites have been studied in recent years for their potential to reduce the mutagenic and carcinogenic effects of potentially harmful chemicals [6,7]; in fact, these natural compounds can inhibit free radicals and oxidative stress-induced DNA and cellular damage [8,9].

Many mutagens and carcinogens work by creating reactive oxygen species (ROS), which are well known for causing oxidative damage to cell structures and biomolecules such lipids, nucleic acids, and proteins in living systems [10]. As a result, the evaluation of the possible genotoxicity of conventional medications is a crucial problem, as damage towards the genetic material can result in significant changes and, as a result, an increased cancer risk or other disorders. Genetic toxicology research has spawned a slew of testing methodologies, including both vitro and in vivo, as a result of their findings. In order to determine the effects of different test chemicals on genetic material and, as a result, the danger to living creatures, including humans, several processes have been devised and tested [11,12].

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are preferred, but according to recent research by Lobo et al. [13], they may have major negative consequences on human health. Plants and medicinal plants have been advised to counteract the effects of free radicals/mutagens because they may stimulate phase II enzymes, lowering the activity of cancer and other degenerative illnesses at the initiation, promotion, and progression phases [14–17].

Tanners Senna, *Cassia auriculata* Linn (Family: *Caesalpiniaceae*), is found across India's hot deciduous woods and maintains a renowned place in Ayurveda and Siddha systems of treatment. It is said to include alkaloids, terpenoids, phenols and tannins, sugar saponins, flavonoids, quinines, steroids, and proteins, among other phytoconstituents. The *C. auriculata* was found to be used to cure diabetes, joint pain and inflammation, muscular discomfort, sickness,

cold, venereal disease, hair cleaner, decrease body heat, stomach pain, vomiting, diarrhea, and toothache in an ethnobotanical survey. Anti-diabetic, anti-oxidant, hepatoprotective, anti-cancer, anti-inflammatory, anti-hyperlipidemic, and other pharmacological effects of *C. auriculata* have been reported [18-19].

2. MATERIAL AND METHOD

2.1 Collection of Plant Material

Cassia auriculata Linn. roots were obtained from farms around Walgaon Road in Amravati, Maharashtra. Safia College of Science, Peer Gate, Bhopal, Madhya Pradesh, validated this plant and assigned it the voucher specimen number 159/Bot/Safia/2010 (*Cassia auriculata* Linn.).

2.2 Material and Reagent

Sigma-Aldrich Co. provided 7,12-dimethylbenz(α)anthracene (DMBA) and acetone (United States). TCI chemicals provided the croton oil (Japan). All of the other reagents were readily accessible. The tumor initiator DMBA was dissolved in acetone at a concentration of 100 g/100lμl. Croton oil was dissolved in acetone to make a % croton oil solution, which was used as a tumour promoter. Curcumin was dissolved in acetone at a dosage of 10 mg/kg as a positive control. All other chemicals were bought from Hi-media Laboratories Pvt. Ltd in Mumbai, India, and were of analytical quality.

2.3 Extraction of the Plant Material

Cassia auriculata Linn. roots (2 kg) were dried in the shade before being ground into a coarse powdered substance. The powdered roots of the plant were extracted with the aid of water using the decoction technique [2020] of extraction at 40°C 5°C. The aqueous extract was then filtered through funnel, and alcohol (Ethanol) was gently added to the aqueous liquid extract to precipitate out polysaccharides found in individual plant roots. The solution was then filtered, and the filtrate was evaporated to a quarter of its original volume. After evaporating 1/4 of the total volume of the solution, it was extracted with an equivalent quantity of ethyl acetate using a separating funnel to get a fraction of root components in ethyl acetate. To improve the yield of extract, the ethyl acetate extract was acidified with 0.1 N HCl. The ethyl acetate portion of the plant's root was then evaporated to produce a precipitate, which was then dissolved in methanol and slowly evaporated to produce crystalline powder (CAF) [20].

Similarly, saponin-rich fractions were extracted from the roots of the plants. After defatting with petroleum ether, pulverized plant material was treated with a 70:30 mixture of ethanol and water for maceration for seven days (40:60). During this time, the mixture was agitated at regular intervals. After filtering through muslin cloth and filter paper, the obtained extract was concentrated using a rotary vacuum evaporator (40°C), with care taken to ensure that the extract did not become powdered. To get the n-butanol soluble fraction, the concentrated extract was treated with n-butanol. The soluble fraction of n-butanol was then treated with cold diethyl ether. Precipitate was generated after treatment with cold diethyl ether. This precipitate-containing mixture was stored at -20°C for 24 hours. Centrifugation was used to further separate the precipitates. To get crystalline powder (CAS), these precipitates were further dissolved in methanol and then evaporated [20-21].

2.4 Animals

This study was conducted in accordance with ethical procedures and policies approved by the Institutional animal ethical committee (IAEC) of PBRI (1283/c/09/CPCSEA). *Swiss albino* mice (Male; 4-5 months; 20-30 gms) were obtained from animal house of Pinnacle Biomedical Research Institute. The animals were randomly assigned to different control and treatment groups (Six animals in each group). All experimental and housing conditions for animals were maintained as per CPCSEA guidelines. Naive animals were selected at random from animal house of PBRI. Animals were kept in group of six in propylene plastic cages with sterilized husk as bedding material. Animals were provided standard feeding pellets (Golden feeds, New Delhi) and water *ad libitum*. Temperature was maintained at 22±2°C, with light and dark cycle of 12:12 hrs. The animals were transferred to the laboratory at least 1h before experiment for proper acclimatization. The experiments were performed during day (08:00-16:00 h).

2.5 Acute Toxicity Study

Toxicology experiments were conducted on young male Swiss albino mice. Each mouse's weight was recorded. The animals were separated into two groups, each with three

animals, and then labelled. Plant extract solution was produced and delivered orally in dosages of 5, 50, 100, 200, and 500 mg/kg. Up to 36 hours, no detrimental effects or mouse death were found. For any additional pharmacological action, treatment doses of 1/10th and 1/20th of the highest tolerable safe dosage were used [22-23].

2.6 Chromosomal Aberration Assay

2.6.1 Dose and treatment

The Chromosomal aberration assay of the tested substance was performed on 36 mice, age 4 - 5 months, weight - 20-30g. Before and during the experiments, all groups of animals were kept under natural light conditions with free access to food and water (Dose- 100 mg/kg; 200 mg/kg).

2.7 Procedure

In the aforesaid group of animals, CYP was utilized as a mutagenic agent and was administered as an intraperitoneal (i.p.) injection at a single dosage of 40 mg/kg b.w 2 hours after the final administration of extract on the seventh day. The positive control group got just a single CYP i.p. injection. The negative control got merely a single 0.4 mL distilled water (d.w.) i.p. injection. For 7 days, the control groups were given p.o. extract at a rate of 200 mg/kg b.w per day for 7 days.

2.8 Chromosomal Analysis

The animals in all groups were slaughtered by cervical dislocation at the sampling time of 24 hours after treatment (colchicine was administered at a dosage of 4 mg/kg of body weight 2 hours before to killing to halt the metaphase stage). Preston et alprocedure's for cytogenetic analysis was followed. Hank's balanced salt solution was used to drain the bone marrow from both femurs (pH 7.2). To allow osmotic swelling of cells, the cells were centrifuged at 1000 rpm for 5 minutes and the pellet was re-dispersed in a hypotonic solution of 0.56 % (w/v) KCl for 30 minutes at 37°C. Swollen cells were fixed in ice-cold Carnoy's fluid, transferred to slides, and stained with phosphate-buffered 5% Giemsa solution. The mitotic index was estimated from a scan of 2000 cells per animal and 75 well-spread metaphase plates per animal in each group were tested for chromosomal abnormalities at a magnification of 100 X. Breaks, fragments, exchanges, and numerous chromosomal abnormalities were classed as chromosomal aberrations (cells with 10 or more aberrations were classified as multiple). The mitotic index, percentage of occurrence of aberrant cells, and percentage of suppression of chromosomal abnormalities were among the criteria studied. The mitotic index was determined as a proportion of dividing cells out of total bone marrow cells measured for cytotoxicity assessment. The number of abnormal cells was calculated as a proportion of the total number of damaged cells (aberrant metaphases).

The suppression percentage of chromosomal aberrations was calculated as:

100− [percent incidence of aberrant cells in extract pre-treated and CYP post treated groups/ percent incidence of aberrant cells in CYP alone treated group) × 100] [24-26].

Table 1. Dose and treatment selection for Chromosomal Aberration Assay

Group No	Group name	Treatment	Dose	No of animal/group
1	Vehicle	No treatment	Nil	06
2	Control	CYP Only	Nil	06
3	CYP+CAF	Extract + CYP	100 mg/kg	06
4	CYP+CAF	Extract + CYP	200 mg/kg	06
5	CYP+CAS	Extract + CYP	100 mg/kg	06
6	CYP+CAS	Extract + CYP	200 mg/kg	06

Table 2. Dose and treatment selection for Micronucleus assay

Group No	Group name	Treatment	Dose	No of animal/group
1	Vehicle	No treatment	Nil	06
2	Control	CYP Only	Nil	06
3	CYP+CAF	Extract + CYP	200 mg/kg	06
4	CYP+ CAS	Extract + CYP	200 mg/kg	06

2.9 Micronucleus Assay

2.9.1 Dose and treatment

The Micronucleus assay of the tested substance was performed on 36 mice, age 4 - 5 months, weight- 20-30 g. Before and during the experiments, all groups of animals were kept under natural light conditions with free access to food and water (Dose- 100 mg/kg; 200 mg/kg).

2.10 Bone Marrow MN Test and Scoring

The bone marrow MN tests were performed on the same experimental animals. Cervical dislocation was used to kill the animals. The femur and tibia were surgically removed. The modified Schmid technique was used to create bone marrow MN slides. The pellet was centrifuged at 1000 rpm and resuspended in BSA solution after marrow suspension from femur and tibia bones was produced in % bovine serum albumin (BSA). Smears were created and the slides were air-dried after a drop of this suspension was applied on clean glass slides. MN were found in the forms of RBCs, i.e., polychromatic erythrocytes as PCEs, when the slides were fixed in methanol and stained with May-Grunwald-Giemsa. MN was detected in around 2000 PCEs per animal [27-29].

2.10.1 Anticancer activity

2.10.1.1 Dose and treatment

The Anticancer activity of the tested substance was performed on 24 mice, age 4 - 5 months, weight - 20-30g. Before and during the experiments, all groups of animals were kept under natural light conditions with free access to food and water (Dose- 100 mg/kg; 200 mg/kg).

2.11 Procedure

2.11.1 Tumor induction

Different groups of 06 animals were treated with a single dose of DMBA (7,12-dimethylbenz[a]anthracene) (100 µg/100 µl of acetone) over the shaven area of the skin of the mice. Two weeks later, croton oil (1% in 100 µl of acetone) was applied as a promoter 3 times per week until the end of the experiment (i.e., 16 weeks).

2.11.2 Parameters to be noticed

Tumor incidence: The number of mice carrying at least 1 tumor, expressed as a percentage incidence.

Tumor yield: The average number of papillomas per mouse.

Tumor Burden: The average number of tumors per tumor bearing mouse.

Body weight: The weight of the mice was measured weekly.

Average Latent Period: The lag between the application of the promoting agent and the appearance of 50% of tumors was determined. The average latent period was calculated by multiplying the number of tumors appearing each week by the time in weeks after the application of the promoting agent and dividing the sum by total number of tumors.

$$\textbf{Average latent period} = \sum fx / n$$

Where f is the number of tumors appearing each week, x is the numbers of weeks, and n is the total number of tumors.

2.12 Histopathological Study

Skin was fixed with buffered formalin for 24 hours. Afterward skin was embedded in paraffin wax by standard protocol. Serial section of 4 µm was cut by microtome with rotary microtome. Sections were stained with Hemtoxyline and eosin staining. Sections were observed at 40x and 100x for histological variations. Important areas were photographed using microscope with digital camera [30-32].

Table 3. Dose and treatment selection for anticancer activity

Group No	Group name	Treatment	Dose	No of animal/group
1	Control	DMBA + Croton oil	Nil	06
2	CAF + CAS	DMBA + Croton oil + Extract of CAF + CAS	200 mg/kg	06
3	CAF	DMBA + Croton oil + Extract of CAF	200 mg/kg	06
4	CAS	DMBA + Croton oil + Extract of CAS	200 mg/kg	06

3. RESULT AND DISCUSSION

3.1 Acute Toxicity Study

Acute oral toxicity is a key criterion for determining a component's safety. The acute oral toxicity of test samples was determined using the OECD 423 criteria in this study. Four dosage levels were employed, according to the guidelines: 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg. No mortality was reported in Cassia auriculata flavonoid rich extracts (CAF) up to a level of 2000 mg/kg. No fatality was seen in the case of Cassia auriculata saponin rich extract (CAS) up to a dosage of 300 mg/kg, but one death was recorded at a dose of 2000 mg/kg. As a result, it was determined that the LD50 of both extracts was more than 2000 mg/kg. This upper limit was also utilized to determine dosages for the current study. As a result, for future research, dosages of 1/10th and 1/20th of the maximum allowed dose, i.e., 200 mg/kg and 100 mg/kg, were used [22-23].

3.2 Chromosomal Aberration Assay

In present study effect of test samples was assessed against cyclophosphamide (CYP) induced mutagenicity. Break, Fragment, Deletion, polyploidy, pulverized and ring type of aberrations were quantified in various treatment groups. Generally, chromatid breaks can be induced in the S and G2 phases of the cell cycle, when the chromosome has split into 2 chromatids. Many chemical agents, especially alkylating agents cause predominantly chromatid-type aberrations. In vehicle treated animals total break was found to be 3.83±1.72 % which was significantly elevated (P<0.05) in CYP only treated animals with 31.33±3.01 %. Flavonoid rich extract of Cassia auriculata significantly lowered percentage break up to 8.16±1.47 % and 5.16±1.72 % at 100 and 200 mg/kg respectively (P<0.05). In saponins rich extract increased mutagenicity was observed at lower dose as well as higher doses. Fragments are single chromatid without centromeres. In CYP treated animals 24.16±2.56 % fragments were observed which were significantly higher (P<0.05) as compared to vehicle treated animals in which 2.83±1.47% fragments were present [24-26].

3.3 Effect of CAF & CAS on Cyclophosphamide induced Genotoxicity

3.3.1 Study of effect of CAF& CAS in micronucleus assay

During anaphase, Mn products from entire chromosomes or central chromosomal segments were not involved in cell division. Mn formation might be used as a biomarker for exposure to both clastogenic and eugenic hazards. Mn is a useful biomarker for biological dosimetry in the event of acute radiation exposure in humans, according to studies. Radiation dosage has an effect on Mn frequency. After being exposed to ionizing radiation in the 50-500 Msv range, Fenech discovered a rise in Mn frequency in human cells. The Mn test was used to assess biological damage in populations living in locations with high levels of radioactivity, as well as in workers who are exposed to ionizing radiation on the job.Counting the amount of PCE among 1000 cells was used to assess the cytotoxic capability of CAF and CAS. Table 6 shows the number of MNPCEs among 2000 PCE, which indicates genotoxicity. At a dosage of 200 mg/kg, the number of MNPCEs among 2000 PCE generated by CAF was significant (p<0.001). CAF reduced the yields of MN induced by CYP in pre-, simultaneous, and post-treatments by a statistically significant amount. Except for the two highest dosages, all of the CAF doses tested were shown to be beneficial in lowering the incidence of MN caused by CYP. There was also a significant decline in overall MN yield (MN in PCE) [27-29]. CAS were shown to be ineffective in lowering the frequency of MN.

Table 4. Acute toxicity study

Groups	No. of animals	Dose (mg/kg)	Result
***C. auriculata* root, crude flavonoids extract**			
1.	3	5	Not observed (0/3)
2.	3	50	No death
3.	3	300	No death
4.	3	2000	No death
***C. auriculata* root, crude saponin extract**			
5.	3	5	No death

Groups	No. of animals	Dose (mg/kg)	Result
6.	3	50	No death
7.	3	300	No death
8.	3	2000	01 Dead

Table 5. Effect of CAF & CAS on Cyclophosphamide induced Genotoxicity

S. No.	Treatment	Break	Fragment	Deletion	Polyploidy	Pulverized	Ring
1	Vehicle	3.83 ± 1.72[d]	2.83 ± 1.47	3.66 ± 1.63	0	0	0
2	CYP	31.33 ± 3.01[a]	24.16 ± 2.56[a]	17.33 ± 3.26[a]	6.66 ± 1.96[a]	6.16 ± 1.47[a]	5.83 ± 1.60[a]
3	CYP+CAF (100 mg/kg)	8.16 ± 1.47 [abc]	7.17 ± 2.22 [abc]	7.83 ± 2.63 [abc]	2.33 ± 1.21 [abc]	1.83 ± 1.169 [abc]	2.16 ± 0.98 [abc]
4	CYP+CAF (200 mg/kg)	5.16 ± 1.72 [abc]	4.33 ± 1.86 [abc]	3.67 ± 1.63 [abc]	1.16 ± 0.75 [abc]	1.16 ± 1.60 [abc]	0.83 ± 1.16 [abc]
5	CYP+CAS (100 mg/kg)	37.66 ± 2.58	29.83 ± 2.71	23.16 ± 3.31	10.33 ± 2.25	10.66 ± 2.50	9.17 ± 1.47
6	CYP+CAS (200 mg/kg)	30.83 ± 2.31 [a]	23.67 ± 3.32 [a]	16.83 ± 3.31 [a]	5.83 ± 1.94 [a]	5.66 ± 1.96 [a]	5.33 ± 2.16 [a]

All Data presented in mean ± SD, [a] P<0.05 as compared to CYP+CAS treated group, [b] P<0.05 as compared to CYP treated group, [c] P<0.05 as compared to CYP+CAS treated group, [d] P<0.05 as compared to CYP+CAF treated group

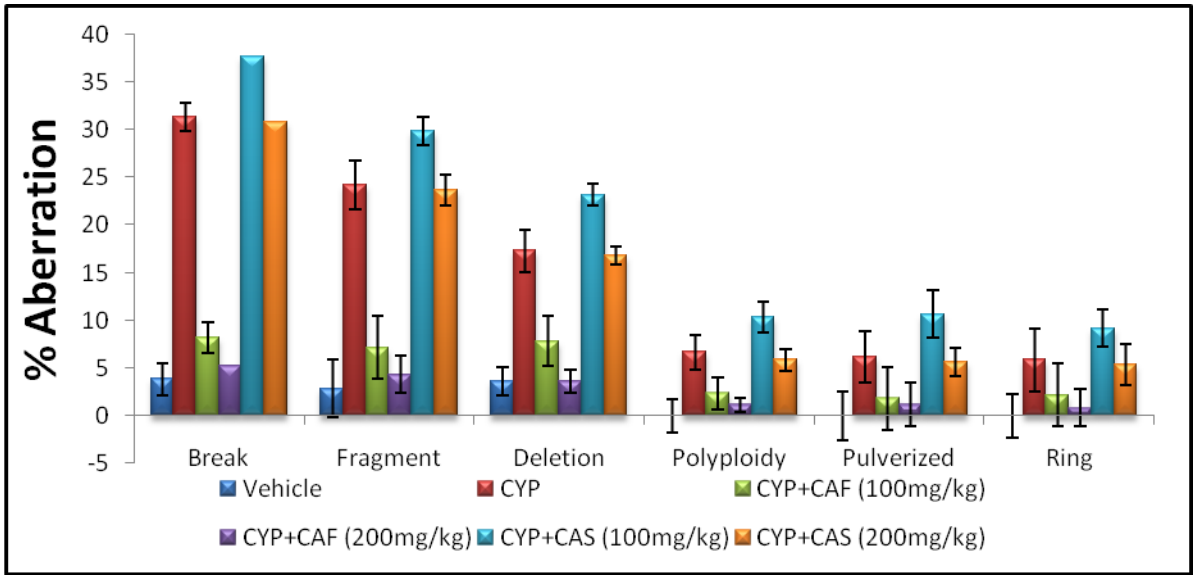

Fig. 1. Effect of CAF & CAS on Cyclophosphamide induced Genotoxicity

Table 6. Effect of CAF & CAS in Micronucleus Assay

S. No.	Treatment	MN-PCE
1.	Vehicle	0.24±0.11
2.	CYP	6.12±0.42
3.	CYP+CAF	2.48±0.63 [abcd]
4.	CYP+ CAS	5.92±0.54

All Data presented in mean ± SD, [a] P<0.05 as compared to CYP treated group, [b] P<0.05 as compared to CYP+ Caf treated group, [c] P<0.05 as compared to CYP+ CAS treated group

3.4 Anticancer Activity

At weekly intervals, body weight, tumor yield, percentage of tumors incidence, and tumors burden were examined and assessed. Only tumors with a diameter bigger than 1 mm that had been present for more than one week were included for data analysis. When the first tumors developed, the latency time of tumor development was established. The number of tumor-bearing mice was divided by the total number of mice in a group and multiplied by 100 percent to get the percentage of tumor incidence. The total number of tumors was divided by the number of tumor-bearing mice in a group to get the tumor burden. The volume of the tumor was calculated by multiplying the length, breadth, and height of the tumor by Π/6.

At the conclusion of the trial, all groups' average body weights had increased significantly (Table 7). During the promotion stage, tumors begin to form on the skin between weeks 6 and 9. Tumor development began one week sooner in groups I and IV than in groups II and III, in the sixth week.

The incidence of tumors differed considerably between the treatment Control group and the remainder group.Statistical analysis also revealed that there is a significant variation in tumor burden across groups. Tumor burden is greatest in group I, with a value of 32.5, followed by 18.83,22.83, and 29.83 in groups II, III, and IV, respectively.In terms of tumor volume, there was no significant difference between groups.

However, there was a statistically significant difference in tumor volume between groups I (32.5) and II (18.83) when compared to group III, while group IV (22.83 and 29.83) was lower than group I, albeit there was no statistically significant difference between these two groups.

In terms of tumor incidence, tumor yield, and tumor burden, groups II and III performed better than the carcinogen control group (group I). The suppressing impact of group II was equivalent to that of the positive control group (group III), but the suppressing effect of group IV was even larger than that of group I, notably in terms of tumor incidence and tumor burden. Nonetheless, as compared to the carcinogen control group, Group II had a higher tumor incidence and tumor volume.

Morphological investigation (Fig. 2) revealed substantial epidermal hyperplasia (as shown by the thicker epidermal layer) as well as many keratin pearls and rete ridges in the carcinogen control-treated group in all protocols. In the skin sections of the DMBA plus croton oil-treated group, irregular distribution with finger-like projections (papilloma) suggestive of malignant development was identified.

Table 7. Result of anticancer parameters

Group	Parameters	Week 0	Week 8	Week 16
Control	Body weight (gms)	22.82±1.79	32.28±3.84	31.82±2.23
	Tumor yield	0	3.67	32.5
	Tumor incidence	0	83.33	100
	Tumor burden	0	4.4	32.5
CAF+CAS	Body weight (gms)	23.07±0.90	30.35±2.27	31.02±2.58
	Tumor yield	0	2	18.83
	Tumor incidence	0	50	100
	Tumor burden	0	4	18.83
CAF	Body weight (gms)	23.63±0.53	31.14±1.31	31.38±1.89
	Tumor yield	0	2	22.83
	Tumor incidence	0	50	100
	Tumor burden	0	4	22.83
CAS	Body weight (gms)	22.83±0.61	30.69±0.83	29.7±0.85
	Tumor yield	0	3.33	29.83
	Tumor incidence	0	50	100
	Tumor burden	0	6.67	29.83

Furthermore, pieces of the basement membrane were disturbed in several tissue slices, suggesting that the tumors had advanced to a premalignant stage. In all methods, skin tissue samples from the different extracts treated groups showed a decreased degree of epidermal hyperplasia, keratinpearls, and rete ridges. Because the basement membrane remained intact and the dermis had not been infiltrated, the tumors developed were deemed benign.As a consequence of the histology examinations, it was discovered that the level of cellular diversity in various treatment groups correlated with tumor outcomes.

In all treated groups, further histological investigation revealed varied degrees of hyperplasia and keratin pearls. In comparison to the carcinogen control (group I), the epidermis in groups II and III is less hyperplastic, but group IV has more keratin pearls and a hyperplastic epidermis that is equivalent to the carcinogen control. The tumors that develop are benign papillomas that remain contained inside an intact basement membrane with no evidence of invasion into the dermis. Premalignant lesions, on the other hand, were seen in the carcinogen control group [30-32].

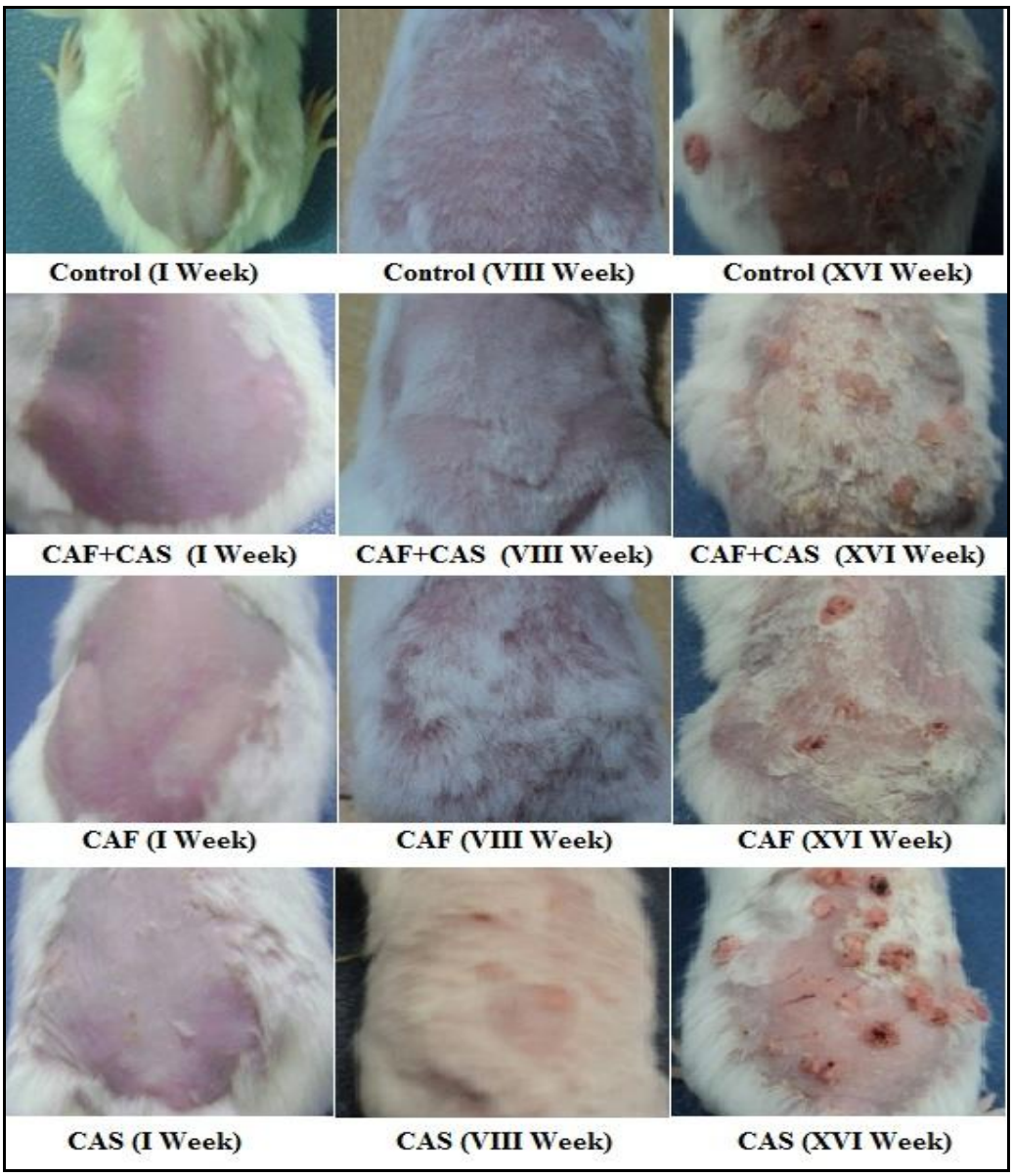

Fig. 2. Morphological examination of anticancer activity on mice

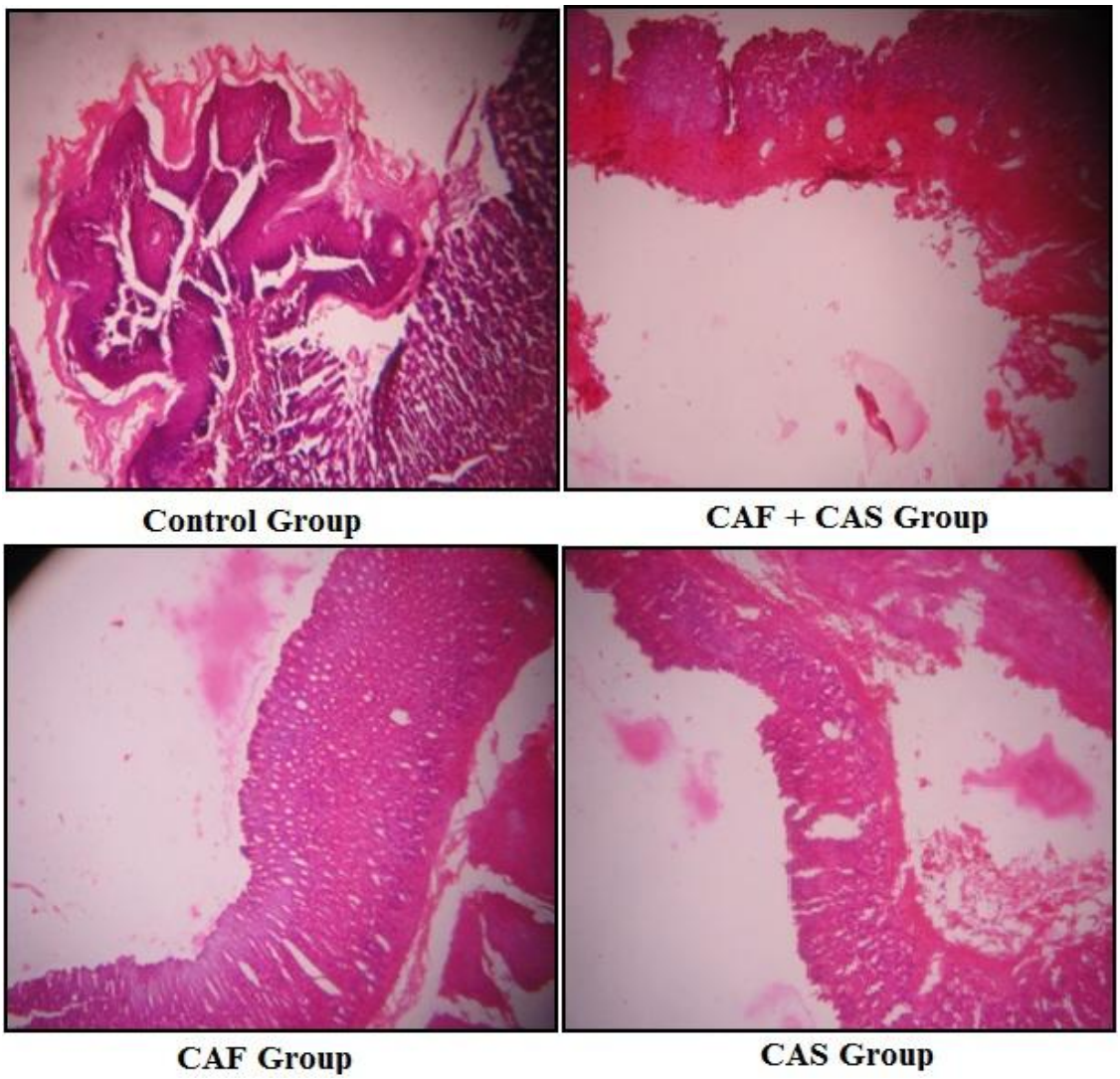

Fig. 3. Histopathological analysis of anticancer activity on mice

4. CONCLUSION

Traditional medical systems have grown in importance over the last decade as a result of their safety. According to current estimates, a significant section of the population in many developing nations depends significantly on traditional practitioners and medicinal herbs to cover their basic health care requirements. Despite the pharmaceutical industry's breakthroughs in the production of unique and highly effective medications for the treatment of a broad variety of disorders, the use of herbal remedies has increased significantly in the world's most wealthy nations. Every medicine has side effects, but a useful pharmacologically active substance should have a good balance of therapeutic and harmful or undesirable side effects. A battery of genotoxic and/or mutagenicity tests must be done to screen the toxicity mechanism to assure the safety and effectiveness of natural compounds. There is no one test that can collect enough data to predict the chemical risks to human health. The findings of this investigation show that the Cassia Auriculata Linn CAF and CAS Fractions are neither genotoxic or clastogenic at the quantities employed. Our findings further suggest that pretreatment with Cassia Auriculata Linn CAF and CAS Fraction reduces CYP-induced clastogenicity while maintaining its cytotoxic capability. Because it prolongs the development of tumors in the skin and reduces the hyperproliferative response evoked by CYP, it can be inferred that CAF and CAS Fraction of Cassia Auriculata functions as a modulator of two-stage skin carcinogenesis in Swiss albino mice. Furthermore, it reduces oxidative cell

damage, which is inextricably linked to the development of cancer. Further research into the exact mode of action of the genetic toxicity of isolated compounds from this plant species is needed to gain a better understanding of the genotoxic mechanisms described herein, as well as to investigate the protective role of Cassia Articulata's CAF and CAS fractions against genotoxic agents in the environment. It is necessary to understand their processes as cancer treatments.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was conducted in accordance with ethical procedures and policies approved by the Institutional animal ethical committee (IAEC) of PBRI (1283/c/09/CPCSEA). Swiss albino mice (Male; 4-5 months; 20-30 gms) were obtained from animal house of Pinnacle Biomedical Research Institute.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/76966