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Therapeutic potential of *Carica papaya* leaf extract and Zamzam water in a rat model of brain tumor

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ABSTRACT

Background: Plant products are increasingly seen as promising alternatives to anticancer therapy due to their intrinsic safety and non-toxicity profiles. **Objective:** The main area of our research is searching for pharmacologically active compounds from a natural source. The present study aimed to screen the role of *Carica papaya* (*C. papaya)* leaf extract and Zamzam water on cadmium-induced brain tumours and oxidative stress in rat models. **Material and Methods:** Oxidative stress and cancer were induced by oral Cadmium for 60 days, followed by treatment with *C. papaya* leaf ethanolic extract at 200 and 400 mg/kg and Zamzam water 1 mL/Kg for 30 days. At the end of the study, the animals were sacrificed, and the antioxidant activity of extracts was assayed by *in vivo* antioxidant tests (CAT, MDA, GSH). To assess anti-tumour activity, expression of Bmi-1 and p53 was evaluated in brain tissue. **Results:** Ethanolic extract of leaves of *C. papaya* in the dose of 200 mg/kg and 400 mg/kg significantly decreased Cadmium-induced oxidative stress. At the same time, Zamzam water reduced the stress, but it was not significant. *C. papaya* in high dose (400 mg/kg) and Zamzam water reduced the expression of Bmi-1 while increasing the expression of p53 in both low and high doses (200 mg/kg and 400 mg/kg). **Conclusion:** Our findings suggest that *C. papaya* leaf extract and Zamzam water have anti-tumour potential.

Keywords: *C. papaya,* Zamzam Water, Anti-Cancer, Anti-Oxidant, Cadmium, Bmi-1, P53 Gene Expression.

INTRODUCTION

Brain tumours encompass a wide range of extremely severe cancers that develop from various brain cells as well as systemic tumours that have spread to the brain. The 10th most common cause of mortality for both men and women is cancer of the brain and other neurological systems. It was predicted that the primary brain and CNS tumours might cause 18,020 adult deaths (10,190 men and 7,830 women) [1]. Temozolomide: An oral chemotherapeutic drug most often prescribed to patients with high-grade gliomas.

Brain tumours are among the deadliest of all tumour types, despite decades of research. Due in part to the special cell-intrinsic and microenvironmental characteristics of brain tissues, these tumours can withstand practically all traditional and experimental therapies. Currently, available plant-based anticancer medications fall into four categories: vinca alkaloids (Vinblastine, Vincristine, and Vindesine), epipodophyllotoxins (Etoposide and Teniposide), taxanes (Paclitaxel and Docetaxel), and camptothecin derivatives (Camptothecin and Irinotecan). Plants are a rich source of natural compounds that may have chemoprotective properties against cancer and still hold immense promise for developing novel medications.

Cadmium is a natural element found in tiny amounts in air, water, soil, and food. All soils and rocks contain some cadmium, including coal and mineral fertilizers. Exposure to cadmium occurs mainly in workplaces where cadmium products are made. The major routes of occupational exposure are inhalation of dust and fumes and incidental ingestion of dust from contaminated hands, cigarettes, or food. Many plants and their extracts have been shown to significantly reduce the tumour volume and increase the overall survival in an orthotopic GBM animal model [2].

C. papaya is mainly cultivated in tropical and subtropical countries. It is used as food, and many parts are used as traditional medicine to treat many diseases [3,4]. Several studies have asserted the benefits of papaya comprising protection against cancer attributed to its antioxidant properties [5-8]. Otsuki N *et al.* evaluated the effects of *C. papaya* leaf extract on cancer cell lines and recognized it to possess significant growth inhibitory activity [9]. Almost. Five thousand compounds from the papaya plant have been identified to be associated with anticancer properties, among which three bioactive compounds have led to an interest in anticancer studies: phenolics, carotenoids, and glucosinolates compounds [10].

Zamzam water is naturally alkaline and is obtained from an ancient well in Makkah, Saudi Arabia. Zamzam water exhibits cytotoxic activity against many cancer cell lines [11-15]. An animal study on rats showed that intake of Zamzam water for ten weeks helped in decreasing fasting blood sugar, serum insulin, and insulin resistance [16]. Riaz B *et al* have reported Zamzam water's anticataleptic and neuroprotective effects [17]. It also possesses antimicrobial activity against salmonella [18]. Therefore, this study explores the anti-cancer and antioxidant potential of *C. papaya* leaves and Zamzam water *in vivo* in albino rats.

MATERIAL AND METHODS

Chemicals and Reagents

Cadmium, RNA later (Qiagen, Germany), TRI reagent (Sigma - Aldrich, USA), Chloroform (Qualigens chemicals, India), Isopropanol (Thermofisher scientific, India), Alcohol (Chanshu Hongsheng Fine Chemicals, China), Nuclease-free distilled water (Thermofisher scientific, India), High-capacity cDNA extraction kit (Thermofisher scientific, India), PCR Master mix (Thermofisher scientific, India), Agarose (SRL chemicals, India), DNA ladder (Thermofisher scientific, India). All the reagents were of reagent grade with 99.0% purity.

Plant extract

At the Jawaharlal Nehru Medical College's Department of Pharmacology (Aligarh Muslim University, Aligarh, Uttar Pradesh, India), *C. papaya* extract was made using dried, finely powdered leaves (100 gm), which was then extracted for 72 h in 400 mL of absolute alcohol using the Soxhlet apparatus. In Petri dishes, the extract was gathered and allowed to air dry for a week. The resulting dried mass was weighed, and the yield was determined, it was then packed with aluminium foils and placed in a refrigerator for further experimentation [19]. Zamzam water was obtained from online procurement site (zamazemah.com.sa) and was used in its original form.

Animals

Adult albino Charles foster rats of either sex weighing 100-200 g were obtained from Central Animal House, JNMC, Aligarh Muslim University, Aligarh. The animals were housed in polypropylene cages bedded with paper strips in the Pharmacology section of Central Animal House. The animal room was well-ventilated under standard conditions (Temperature 27±3 °C and 12-hour light/dark cycle) throughout the experimental period. All animals were allowed free access to food and water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) on 01.10.2018 (Reg. no. 1495/Pharma). All animal experiments were carried out as per the rules and regulations of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) under the "Guidelines for Care and Use of Animals in Scientific Research."

Experimental design

The rats of either sex were acclimatized to the laboratory conditions for one week before the experiments, weighed and randomly divided into six groups of eight rats each. Oxidative stress was induced by oral administration of cadmium for a period of 60 days [20-21]. Treated as follows.

Group 1- normal control group was given orally distilled water 1 mL/kg orally for ninety days.

Group 2- negative control group treated orally with 1:1 (w/v) a mixture of Cadmium and distilled water 1 mg/kg orally for sixty days followed by distilled water for thirty days,

Group 3- positive control group was treated orally with a 1:1 (w/v) mixture of Cadmium and distilled water 1 mg/kg orally for sixty days, followed by Temozolomide 50 mg/ kg orally for thirty days.

Group 4- treated orally with 1:1 (w/v) mixture of Cadmium for sixty days followed by *C papaya extract* 200 mg/kg for thirty days.

Group 5- treated orally with 1:1 (w/v) mixture of Cadmium for sixty days followed by *C papaya extract* 400 mg/ kg for thirty days.

Group 6- treated orally with 1:1 (w/v) mixture of Cadmium for sixty days followed by Zamzam water 1 mL/ kg) for thirty days. On the 91st day, blood samples were collected by cardiac puncture for enzymatic analysis, and rats were sacrificed using pentobarbitone in the dose of 100 mg/kg intraperitoneally, and their brain samples were preserved in RNA later.

Extraction of total RNA from tissue samples

RNA of brain tissue was isolated with TRI reagent (SIGMA). RNA quantification was done using UV Spectroscopy (UV-1800 Shimadzu) to assess its purity and concentration. RNA was stored at -80 °C for further analysis. A high-capacity cDNA Reverse Transcriptase kit purchased from Thermo Scientific was used for the synthesis of cDNA from extracted RNA. cDNA was stored at -20 °C for further analysis [22-23].

Polymerase chain reaction (PCR)

PCR master mix (2X) was purchased from Thermo scientific. The annealing temperature for beta-actin, p53, was 60.5 °C and for BMI1 was 59.5 °C. Gel electrophoresis was done to separate the DNA molecules and was visualized by a gel documentation system. 2% agarose gel was made. Ethidium bromide (EtBr) was used to stain DNA in agarose gel. Freshly prepared TAE buffer was used as gel running buffer [24].

In vivo antioxidant test

The Catalase (CAT) test was done according to the procedure described by Sinha [25].

The Reduced Glutathione (GSH) test was performed according to the protocol of Ellman [26]. Absorbance was measured at 412 nm within 30 seconds. Specific gravity was calculated by the formula:

$$\frac{O.D.\ X\ 134}{0.1}$$

And they were expressed in nmol/mL.

The Malonyl dialdehyde (MDA) test was done according to the method described by Buege [27]. Optical density was measured at a wavelength of 535nm.

The MDA level was calculated by the formula

$$\frac{O.D.}{0.156}$$

And expressed in nmol/mL.

Statistical analysis

All data are expressed as mean ± Standard error of the mean (SEM). The groups were compared by one-way analysis of variance (ANOVA). A p-value of less than 0.05 was significant. ANOVA and post hoc Tukey were employed using SPSS 23.0 software (IBM, Armonk, New York, USA). The graphs were made using Microsoft Excel 2016.

RESULTS

***In vivo* antioxidant**

The ethanolic extract of leaves of *C. papaya* was prepared by Soxhlet extraction. The yield obtained was 76.3%. Ethanolic extract of *C. papaya* leaf in the dose of 400 mg/kg dose showed a significant increase in Catalase. It significantly decreased MDA activity and increased GSH activity in both low and high doses (Table 1). Zamzam water in the dose of 1 mL/kg increased Catalase and GSH activity while decreasing the activity of MDA, but the findings were not significant (Table 2).

***In vivo* Anti-cancer activity**

Expression of Bmi-1 and p53 were evaluated, and beta-actin was used as an internal control. *C. papaya* Leaf Ethanolic Extract (LEE) decreased the expression of Bmi-1, high dose (400 mg/kg) being more effective in doing so. It also increased the expression of p53 in both low and high (200 mg/kg and 400 mg/kg) doses (Figure 1). Zamzam water (1 mL/kg) decreased the expression of Bmi-1 and increased the expression of p53 suggesting its antitumor potential (Figure 2).

Table 1: *In vivo* antioxidant tests after administration of *C. papaya* leaf ethanolic extracts in Cadmium-induced oxidative stress rat model

Biochemical test (Mean ± SEM)	Group				
	Normal control	Negative control	Positive control	*C. papaya Low* dose 200 mg/kg	*C. papaya High* dose 400 mg/kg
Catalase (U/min/mg)	9.1028 ±1.086	4.2058 ± 0.840	7.2626 ± 1.804***	5.1525 ± 0.720	8.2440 ± 0.614*
MDA (nmol/mL)	0.9250 ± 0.122	2.1217 ± 0.364	0.9400 ± 0.075***	1.1700 ± 0.188***	0.7850 ± 0.094***
GSH (nmol/mL)	92.7375 ± 5.566	59.7267 ± 5.779	104.6085 ± 7.761***	87.6000 ± 6.261***	97.7466 ± 8.470***

The Negative control group was compared with a normal control group and all other groups were compared to negative control group. *p < 0.05, **p < 0.01 & ***p < 0.001

Table 2: *In vivo* antioxidant tests after administration of *Zamzam water* in Cadmium-induced oxidative stress rat model

Biochemical test (Mean ± SEM)	Group			
	Normal control	Negative control	Positive control	Zamzam Water (1 mL/kg)
Catalase (U/min/mg)	9.1028 ± 1.086	4.2058 ± 0.840	7.2626 ± 1.804***	5.2703± 0.832
MDA (nmol/mL)	0.9250 ± 0.122	2.1217 ± 0.364	0.9400 ± 0.075***	1.8867± 0.101
GSH (nmol/mL)	92.7375 ± 5.566	59.7267 ± 5.779	104.6085 ± 7.761***	69.9483± 6.058

The Negative control group was compared with a normal control group and all other groups were compared to negative control group. *p < 0.05, **p < 0.01 & ***p < 0.001

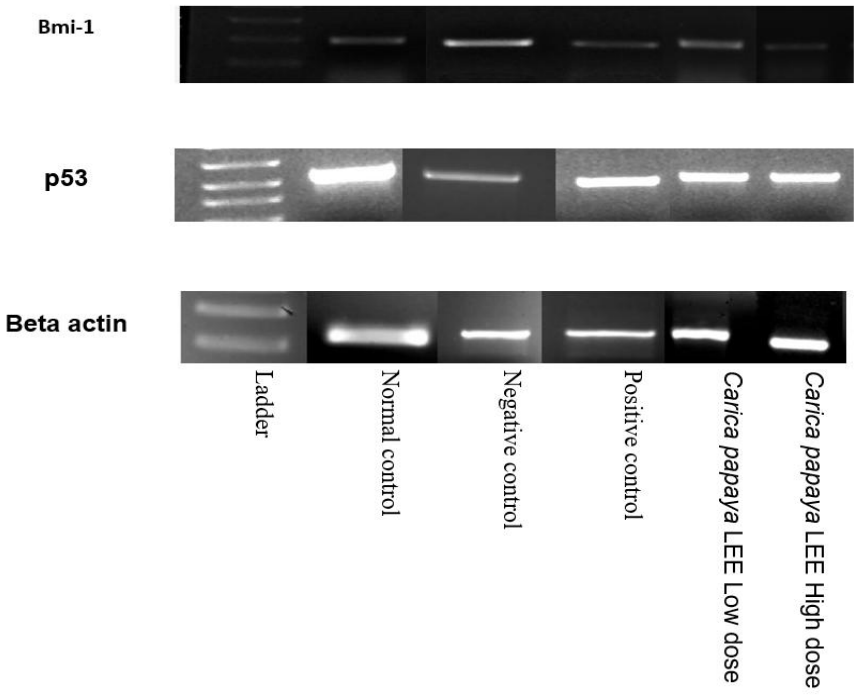

Figure 1: Expression of Bmi-1 and p53 in *C. papaya* LEE (Leaf Ethanolic Extract) treated group. Beta actin was used as internal control. Test groups were compared to negative control group. High dose (400 mg/kg) of *C. papaya* LEE decreased the expression, of Bmi-1, and both high and low doses (400 mg/kg and 200 mg/kg) of *C. papaya* LEE increased the expression of p53 when compared to the negative control group

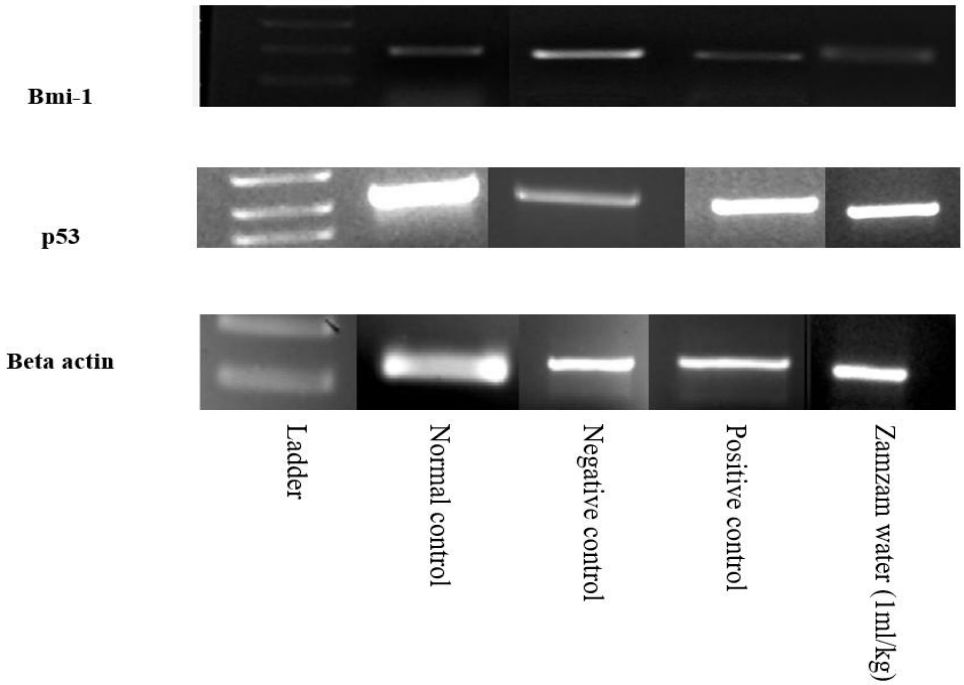

Figure 2: Expression of Bmi-1 and p53 in the Zamzam water treated group. Beta actin was used as internal control. The test group was compared to the negative control group. Zamzam water decreased the expression of Bmi-1 and increased the expression of p53 when compared to the negative control group

DISCUSSION

Ethanolic extracts of *C. papaya* leaves significantly decreased Cadmium-induced oxidative stress. The lowering of these parameters indicates this extract's antioxidant effect. These findings suggest that the extract of *C. papaya* effectively lowered the levels of reactive oxygen species. It decreased the expression of Bmi-1 in high doses and increased the expression of p53 in both low and high doses, suggesting its anti-tumour potential.

Oxidative stress is directly tied to every aspect of cancer, including tumour-bearing status, carcinogenesis, therapy, and prevention. Under long-term environmental stress, ROS are created, which causes serious harm to cell structure and function, as well as the possibility of somatic mutations and neoplastic transformation. Indeed, oxidative stress has been related to the onset and spread of cancer by promoting DNA mutations or causing DNA damage, cell proliferation, and genome instability [28-29]. We used cadmium to induce oxidative stress and cancer. It promotes the production of reactive oxygen species (ROS) and metallothionein, which results in oxidative damage to erythrocytes and other tissues and the loss of membrane functions [30]. Long-term exposure to cadmium causes Superoxide dismutase function to be inhibited and promotes lipid peroxidation, which indicates oxidative damage to the liver, kidneys, and testes [31]. Cadmium toxicity has been linked to changes in the antioxidant defence system, which normally defends against free radical toxicity by enzymes like glutathione peroxidase (GPx), glutathione-S-transferase, superoxide dismutase (SOD), and catalase (CAT), as well as non-enzymatic molecules like glutathione [32].

Cadherin disruption, oxidative stress, obstruction of DNA repair, interference with apoptosis, and gene control of proto-oncogenes are the main processes behind cadmium carcinogenesis [33]. The protocol used in our study produced tumours in all rats.

Only a few studies on the anticancer and antioxidant activity of *C. papaya* leaves are reported. Therefore, the present study was planned. To the best of our knowledge, based on the literature review, it is the first time that we are reporting the anticancer and antioxidant activity of *C. papaya* leaves. Two doses of *C. papaya* leaf extract were selected for the study (200 mg/kg and 400 mg/kg). Administration of ethanolic extracts of *C. papaya* leaf for 30 days at a dose of 400 mg/kg showed a significant increase in Catalase activity (Table 1). Both low and high doses of *C. papaya* showed a significant decrease in the activity of MDA (Table 1). It significantly increased GSH activity in both low and high doses (Table 1).

These results agree with the findings of Nandani. *et al*., 2020 and Aboobacker *et al*., 2020 [34-35]. The results suggest the antioxidant potential of *C. papaya* leaves, which may be attributed to polyphenols present in the extract.

Bmi-1 is an oncogene highly expressed in many human cancers, including medulloblastoma and adult Glioblastoma multiforme [36-37]. A high level of Bmi1 is associated with medulloblastoma invasion [38] and is a poor prognostic marker in multiple human cancers [39-41]. It is also significantly involved in tumor recurrence and chemo resistance [42].

The p53 gene, is a tumor suppressor gene, implying that its activity stops the formation of tumors. Several experimental and clinical evidence suggests that the loss of p53 function is a crucial initial event in development of gliomas along with other genetic and epigenetic alterations [43-46] and various strategies have been proposed to restore the function of p53 in cancer cell [47]. Zamzam water and *C. papaya* leaf extract decreased the expression of Bmi-1 in high dose (400 mg/kg) (Figure 1,2). Groups that received Zamzam water and ethanolic extract of *C. papaya* leaf showed an increased expression of p53 compared to the negative control. Polyphenols are the most extensive bioactive compounds with antioxidant activity among the natural antioxidants with antioxidant activity [48]. Many polyphenolic compounds have been identified, mainly flavonoids, phenolic acids, xanthones, stilbenes, coumarins, lignans, tannins, and chromones. Polyphenols are secondary plant metabolites that protect plants from microbial infection and ultraviolet rays. They are non-nutritive, hydrophilic components found in small amounts in plant-derived products. The primary dietary source of these compounds is plant-derived beverages such as fruit juices, tea, coffee, fruits, vegetables, cereals, and red wine [49-50]. In addition to preventing the onset and progression of diabetes, ageing, and neurological illnesses, which are all linked to oxidative stress, polyphenols have positive pleiotropic effects on health. These compounds exert their antioxidant effect by

different mechanisms, such as scavenging free radicals, reducing lipoperoxidation, and improving the endogenous enzymatic defences [51] Numerous *in vitro* and *in vivo* studies suggest the use of polyphenols in the treatment of drug-resistant tumour cells in combination with chemotherapeutic drugs in order to prevent human cancer [47,52].

C. papaya leaf extracts have been reported to have alkaloids, proteins, glycosides, phenol, tannin, saponin, quinine, oxalate, and anthocyanin on phytochemical analysis [4]. Additionally, prior research has suggested that the *C. papaya* leaf has the ability to scavenge free radicals [35,49]. Polyphenols' ability to prevent cancer has been linked to a number of different mechanisms of action, including changes in cellular signalling, induction of cell cycle arrest (apoptosis), induction of detoxification enzymes, prevention of oxidation, anti-inflammatory activity, and regulation of the host immune system [51,53].

Several studies have reported the anticancer and antioxidant effects of Zamzam water [11]. It is common knowledge that the microenvironment of malignant tumours is acidic, and that alkalinization of the tumour microenvironment severely hinders tumour growth and progression. and that alkaline water boosts the antioxidant mechanism [54]. It has been suggested that Zamzam's alkaline nature and trace amounts of Arsenic and Lithium may be the cause of its therapeutic benefits [55].

In contrast to previous studies, the present study did not reveal the antioxidant potential of Zamzam water which is consistent with the previous study [16]. The present study's findings are inconsistent with Bamosa *et al*, who reported a significant increase in the activity of Catalase and Glutathione.

CONCLUSION

According to our research, Zamzam water and *C. papaya* leaf extract may have anti-tumour properties. Numerous polyphenolic compounds, primarily flavonoids, phenolic acids, xanthones, stilbenes, coumarins, lignans, tannins, and chromones, have been reported from the plant that possess anticancer properties. The study's findings provide a scientific rationale for the application of this approach in conventional medicine. Additional confirmation research is required to determine its application in conventional medicine.

Conflict of interest

The authors declared no conflict of interest.

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