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Original Article

Berberine Mitigates Betel-Nut Induced Hepatocarcinogenesis, Enhances Chemosensitivity to Cisplatin and Reduces Cisplatin-Induced Nephrotoxicity in Mice Exposed to an Aqueous Extract of Betel Nut

Kasturi Bhattacharjee, Moumita Nath, Yashmin Choudhury*

ABSTRACT

Background: There is a considerable correlation between the use of betel-nut (BN) as a chewing substance and the development of various malignancies. **Objective:** The bioactive phytocompound berberine was tested as monotherapy or in combination with cisplatin to reduce BN-induced carcinogenesis in mice. We also examined how berberine affected cisplatin-induced toxicity. **Methods:** Swiss Albino mice were exposed to aqueous extract of betel-nut (AEBN) at a dose of 2 mg ml^{-1} in drinking water, for 16 weeks. Following this, the mice were given a combination of AEBN and berberine (10 mg kg^{-1}) for 8 weeks. Control mice were given drinking water without AEBN for 24 weeks. For the combination treatment, mice that had been exposed to AEBN (2 mg ml^{-1}) for 16 weeks were given AEBN+sodiumchloride+cisplatin (5 mg kg^{-1}) +berberine (10 mg kg^{-1}) for 2 weeks. Histopathology, oxidative stress, proliferation, apoptosis, oncogenic and tumor suppressor proteins, hepatotoxicity, and nephrotoxicity were assessed in tissues retrieved at treatment endpoints. **Results:** Berberine monotherapy reduced tissue dysplasia, liver nodulation, oxidative stress, proliferation (Ki-67 and Cyclin D1) markers, Akt/mTOR signaling, and pP53 (Ser-15) levels and apoptosis in AEBN-treated mice to levels comparable to cisplatin alone. Berberine with cisplatin decreased nephrotoxicity, fur shedding, and cancer phenotype more than cisplatin alone. **Conclusion:** The study results imparted a new therapeutic approach in developing more effective and less harmful cancer treatments.

Keywords: AEBN, Betel-Nut, Berberine, Cisplatin, Chemotherapy, Toxicity.

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INTRODUCTION

People from Southeast Asia and the Asian Pacific region chew areca nut, also called betel-nut (BN), which comes from the tropical palm Areca catechu. They do this to experience a sense of exhilaration and well-being.[1] Populations with a high consumption of BN are more prone to developing various types of cancer, such as laryngeal, stomach, liver, lung, and pancreatic cancers. Solid tumors can be efficiently treated with cisplatin, a platinum-based chemotherapy drug that is also used for head and neck cancer. Nevertheless, the emergence of resistance to the medication and the occurrence of major toxicities poses significant challenges to its utilization[4]. Berberine, an alkaloid derived from various parts of plants, has been found to have significant health benefits. There have been studies indicating that this herbal remedy may have potential benefits for conditions such as diabetes, Alzheimer's, and various types of cancer affecting different parts of the body.[5] Berberine suppressed PI3K/AMPK/mTOR signaling to diminish gastric cancer cell with cisplatin resistance,[6] protected against cisplatin-induced nephrotoxicity,[7] and alleviated cisplatin-induced peripheral neuropathy.[8]

While the anti-cancer efficacy of berberine is well documented, its role in mitigating BN-induced cancer is not known. This research investigates whether berberine monotherapy or berberine in conjunction with cisplatin improves a mouse model chronically exposed to aqueous betel-nut extract (AEBN).

MATERIALS AND METHODS

Drugs and Chemicals

Cisplatin(Cisdiammineplatinumdichloride-II),Polyvinyldifluoride (PVDF)(#3010040001) membrane, In situ cell detection Kit, AP(#11684809910), Creatinine AssayKit (#MAK080) and Lactate Dehydrogenase Activity Assay Kit(#MAK066) (SigmaAldrich, St.Louis, USA); Berberine chloride (Abcam, Cambridge, United Kingdom); antibodies against total and phosphorylated Akt(Ser473) (#9272 and #4060), total and phosphorylated p53(Ser15) (#2524and#12571S), total and phosphorylated mTOR (Ser-2448) (#2972S and #2971), PTEN (#9559T), Bcl2 (#3498), CyclinD1 (#2922S), Ki-67 (#12202) and β-actin(#4970S) were from Cell Signaling Technology (Massachusetts, United States). The secondary antibodies specifically designed for detecting rabbit IgG and linked to either alkaline phosphatase (AP) or horseradish peroxidase (HRP) were from Cell Signaling Technology (Massachusetts, United States).

Animals

All experiments and techniques followed Assam University, Silchar's Institutional Animal Ethics

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Committee's rules, which approved this work (AUS/IEAS/2017/PC/19dt-25/10/2017).

Aqueous Betel Nut (AEBN) extract preparation and administration

An aqueous extract of ripe, de-shelled betel-nut (AEBN) was prepared as previously explained[1] and administered at a concentration of 2 mg ml^{-1} in drinking water.[1]

Berberine and Cisplatin treatment

Over the course of eight weeks, berberine was administered orally through gavage at a daily dosage of 10 mg kg-1.[9] The intraperitoneal injection of cisplatin was administered for a duration of two weeks, with a dosage of 5 mg kg-1, after being dissolved in 0.9% sodium chloride. This is the maximal tolerated dose reported for cisplatin.[10]

Experimental design

For a single experimental study, Swiss albino female mice, aged 6-8 weeks, with a mean weight of 25 ± 0.2 g, were randomly allocated into seven groups of 10 mice each as follows:

Group I: Controls given solely drinking water for 24weeks.

Group II: Drinking water for 16 weeks and berberine (10 mg kg^{-1}) by oral gavage for the remaining 8 weeks.

GroupIII: AEBN in drinking water (2 mg ml^{-1}) for 24 weeks.

Group IV: AEBN in drinking water (2 mg ml^{-1}) for 16 weeks. Additionally, berberine was provided to the group by oral gavage at a dosage of 10 mg kg-1 for a period of 8 weeks, that is, from the 17th to 24th weeks.

Group V: AEBN for 16 weeks (2 mg ml^{-1} in drinking water) + NaCl (0.9mg kg-1) administered intraperitoneally for 2 weeks, that is, in the 17th and 18th weeks. This group served as the control for cisplatin treatment.

Group VI: AEBN in drinking water (2 mg ml^{-1}) for 16 weeks + cisplatin (5mgkg-1) suspended in NaCl (0.9mg kg-1) administered intraperitoneally for 2weeks, that is, in the 17th and 18th weeks.

Group VII: AEBN in drinking water (2 mg ml^{-1}) + cisplatin (5mg kg^{-1}) suspended in NaCl (0.9 mg kg^{-1}) intraperitoneally and berberine(10 mg kg^{-1}) through oral gavage administered for 2weeks, that is, in the 17th and 18th weeks.

The cumulative number of mice throughout three separate studies was (n=210). Weekly measurements were taken of the body weights of every treatment group, and the animals were monitored for any signs of discomfort. At the conclusion of the therapy, the mice were euthanized using cervical dislocation. The livers were then collected and cleaned of any attached tissue for further analysis. Besides the livers, the kidneys of Groups V through VII mice were also recovered for nephrotoxicity analysis.

Histopathological analysis

Liver, lung, small intestine, and kidney from various treatment groups were subjected to staining with hematoxylin and eosin (H & E), employing the previously reported approach.[1]

Immunohistochemical (IHC) Analysis and TUNEL assay

Immunohistochemical staining for Ki-67expression and TUNEL assay for study of in situ apoptosis in liver tissue sections were carried out as previously described.[11]

Hepatotoxicity and nephrotoxicity analyses

The analysis of hepatotoxicity and nephrotoxicity was conducted using the Lactate Dehydrogenase Activity Assay Kit and Creatinine Assay Kit from Sigma Aldrich, St. Louis, USA, following the instructions provided by the manufacturer.

Estimation of oxidative stress

Tissue homogenates were tested for lipid peroxidation, protein carbonylation, and catalase activity as previously described.[11]

Western Blotting

Western blotting was employed to measure total and phosphorylated protein expression levels, as previously described.[11]

Statistical Analysis

The results were reported as the mean ± standard deviation for three separate experiments. For statistical comparisons, one-way analysis of variance (ANOVA) and Tukey's multiple comparison post hoc test were implemented in Graph Pad Prism 8.0 (Inc. San Diego, CA). A $p<0.05$ signifies statistical significance.

RESULTS

Berberine improved the overall health and body weight of mice

Mice receiving only drinking water for the first 16 weeks preceding 10 mg kg-1 of berberine treatment for 8 weeks (Group II; mean body weight ±SD = 29±0.03 g) were healthy and exhibited no significant change in body weight relative to age-matched controls (Group I; mean body weight ±SD = 30±0.11 g). Mice receiving AEBN for 24 weeks also did not demonstrate a noteworthy shift in body weight (Group III; mean body weight ± SD = 29 ± 0.05 g) compared to age-matched controls (Group I) but developed poor health, hunched posture and sluggish movement. Similarly, the body weight of AEBN+NaCl treated mice did not change significantly (Group V; mean body weight ± SD = 30±0.31 g), but the mice exhibited poor health characteristics like the mice of Group III. Fur shedding, loss of appetite, irritable behaviour and significant decline in bodyweight was observed in AEBN+NaCl+cisplatin treated mice (Group VI; mean body weight ± SD = 25±0.27 g, $p<0.05$). In contrast, the AEBN+NaCl+cisplatin+berberine treated mice exhibited improved overall health, restoration of fur and reversal of irritable behavior, concomitant with a substantial rise in body weight (Group VII; mean body weight ± SD = 29± 0.04 g, $p<0.05$) with respect to the Group VI mice receiving cisplatin without berberine (Table 1 & Table 2).

Berberine restored tissue morphology and architecture of AEBN exposed mice without or with cisplatin treatment

Pronounced liver nodules (mean number ± SD=2.43±0.12; p=0.001) were observed in mice of AEBN (Group III) and AEBN+NaCl (GroupV) (mean number ± SD=1.87±.23;p=0.001) treatment groups, in comparison to controls (Group I). After the administration of cisplatin (Group VI), the liver nodules showed a noticeable decrease in number (mean value ± SD = 0.001 ±0.18; p = 0.078), but the liver nodulation persisted. Through the use of cisplatin and berberine (Group VII), the liver color and texture were successfully restored to a state of normalcy, with no visible nodulation. It is worth noting that the hepatic tissue of mice exposed to AEBN and given berberine (10 mg kg^{-1}) monotherapy for 8 weeks showed signs of liver tissue recovery without any visible nodules. This suggests that berberine monotherapy may have a restorative effect on betel-nut induced hepatic lesions similar to cisplatin alone, or cisplatin+berberine in combination (Figure 1A & 1C).

Examination of H&E-stained tissue sections revealed that AEBN induced dysplasia in the liver of group III mice characterized by fatty cell degeneration, infiltration by lymphocytes and Kupffer cells, enlarged nuclei and binucleated hepatocytes (Figure 1 A, B). While berberine monotherapy restored tissue architecture of the liver (Group IV) (Figure 1 A, B), cisplatin treatment (Group VI) (Figure 1 C, D) failed to reverse the liver tissue dysplasia induced by AEBN+NaCl treatment (Group V) (Figure 1 C, D). Similarly, distortion of structure and uniformity of cells, presence of necrotic foci and a significant increase in inflammatory cells were observed in the renal tissue of Groups V&VI, indicating AEBN+NaCl and AEBN+NaCl+cisplatin induced alterations in renal histology (Figure 1E). However,the hepatic and renal architecture of GroupVII mice were restored (Figure 1D&2E), indicating that the combination of cisplatin with berberine could induce recovery of both the liver and kidney tissues.

Berberine reduced oxidative stress, hepatotoxicity and nephrotoxicity in AEBN exposed mice without or with cisplatin treatment

AEBN (Group III) and AEBN+NaCl (Group V) exposed mice exhibited noteworthy (p=0.001) rise in the process of protein carbonylation and lipid peroxidation in the hepatocytes as well as kidneys in case of Group V mice, concomitant with significant (p=0.001) decrease in catalase

Table 1. Body weight and relative organ weight of Control, berberine, AEBN and AEBN+ berberine treated Swiss albino mice.

Group	Final body weight (g)		Relative organ weight g kg^{-1} b.w.* 100 ± SEM					
			Liver		Lungs		Small Intestine	
	16 weeks	24 weeks	16 weeks	24 weeks	16 weeks	24 weeks	16 weeks	24 weeks
Control (Group I)	27±0.11	32±0.31	1.5±0.12	1.6±0.12	0.23±0.08	0.25±0.12	1.3±0.04	1.5±0.09
Berberine (Group II)	28±0.03[a]	26±0.13	1.5±0.06	1.5±0.06	0.26±0.11	0.22±0.21	1.4±0.03[a]	1.2±0.11
AEBN (Group III)	29±0.05[a]	25±0.02[*]	1.4±0.03[*]	1.3±0.07	0.21±0.07	0.20±0.07	1.2±0.23	1.1±0.03[b]
AEBN+ Berberine (Group IV)	27±0.25	31±0.03[c]	1.5±0.01[d]	1.6±0.02[d]	0.21±0.05[c]	0.22±0.04[d]	1.3±0.05[c]	1.5±0.01[d]

Mean±SD of three independent experiments with three technical replicates each
[a]significant difference from respective controls (Group I) at p<0.05
[b] Significant difference from respective controls (Group I) at p<0.01
[c]Significant difference from respective controls (Group II) at p<0.05
[d]Significant difference from respective controls (Group II) at p<0.01

Table2. Body weight and relative organ weight of AEBN+NaCl, AEBN+NaCl+cisplatin and AEBN+NaCl +cisplatin + berberine treated Swiss albino mice.

Group (AEBN 2mg/mL)	Final body weight (g) (18 weeks)	Relative organ weight g kg^{-1} b.w.* 100 ± SEM			
		Liver	Lungs	Small Intestine	Kidney
AEBN+NaCl (Group V)	30±0.31	1.04±0.19	0.26±0.12	1.6±0.05	0.544±0.11
AEBN+NaCl+ Cisplatin (Group VI)	25±0.27	1.43±0.08	0.22±0.21	**1.7±0.01[a]**	0.536±0.18
AEBN+ NaCl+Cisplatin +Berberine (Group VII)	**29±0.04[c]**	1.88±0.06	0.22±0.22	1.8±0.03[c]	0.376±0.02[c]

Mean±SD of three independent experiments with three technical replicates each
[a] significant difference from respective controls (Group V) at p<0.05
[b] Significant difference from respective controls (Group V) at p<0.01
[c]Significant difference from respective controls (Group VI) at p<0.05
[d]Significant difference from respective controls (Group VI) at p<0.01

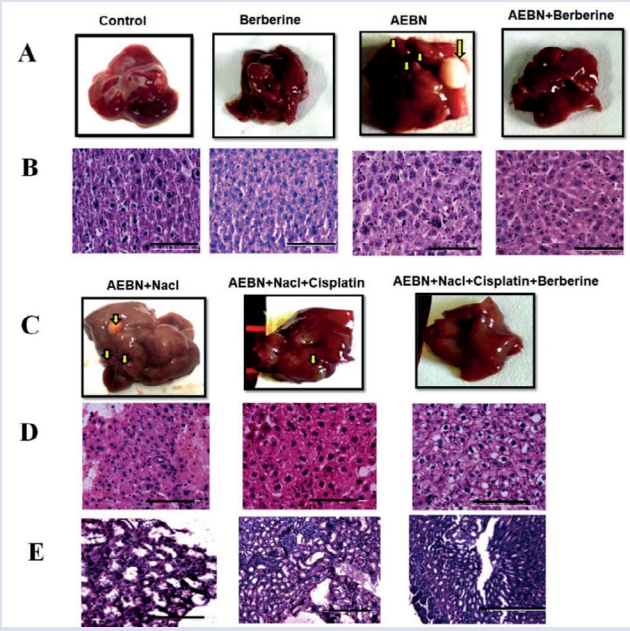

Figure 1: Berberine administered alone or in combination with cisplatin reduced liver nodulation (A) & (C) and restored restored tissue architecture of the liver (B) & (D) and the kidneys (E) in the indicated treatment groups. Scale bar = 100 µm. Number of mice, n= 30 per treatment group.

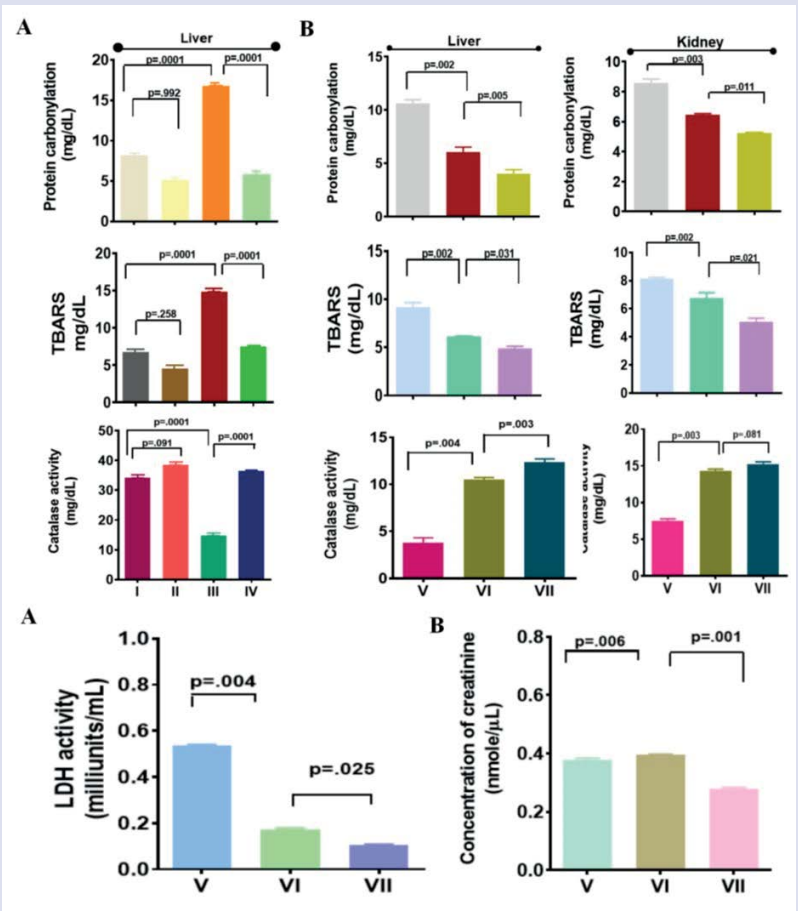

Figure 2: Berberine administered (A) alone or (B) in combination with cisplatin reduced oxidative stress and increased catalase activity (C) reduced lactate dehydrogenase (LDH) activity; and (D) reduced creatinine concentration in the tissues of AEBN exposed mice.The treatment groups indicated in the figures:I-controls given drinking water without AEBN; II-drinking water+berberine; III-drinking water+AEBN; IV-drinking water+AEBN+berberine; V-drinking water+AEBN+NaCl; VI-drinking water+AEBN+NaCl+cisplatin; VII-drinking water+AEBN+NaCl+cisplatin+berberine. Data is represented as mean ± SD of three independent experiments and *p*-values determined by one way ANOVA post hoc Tukey's Multiple Comparison Test. Number of mice, n= 30 per treatment group.

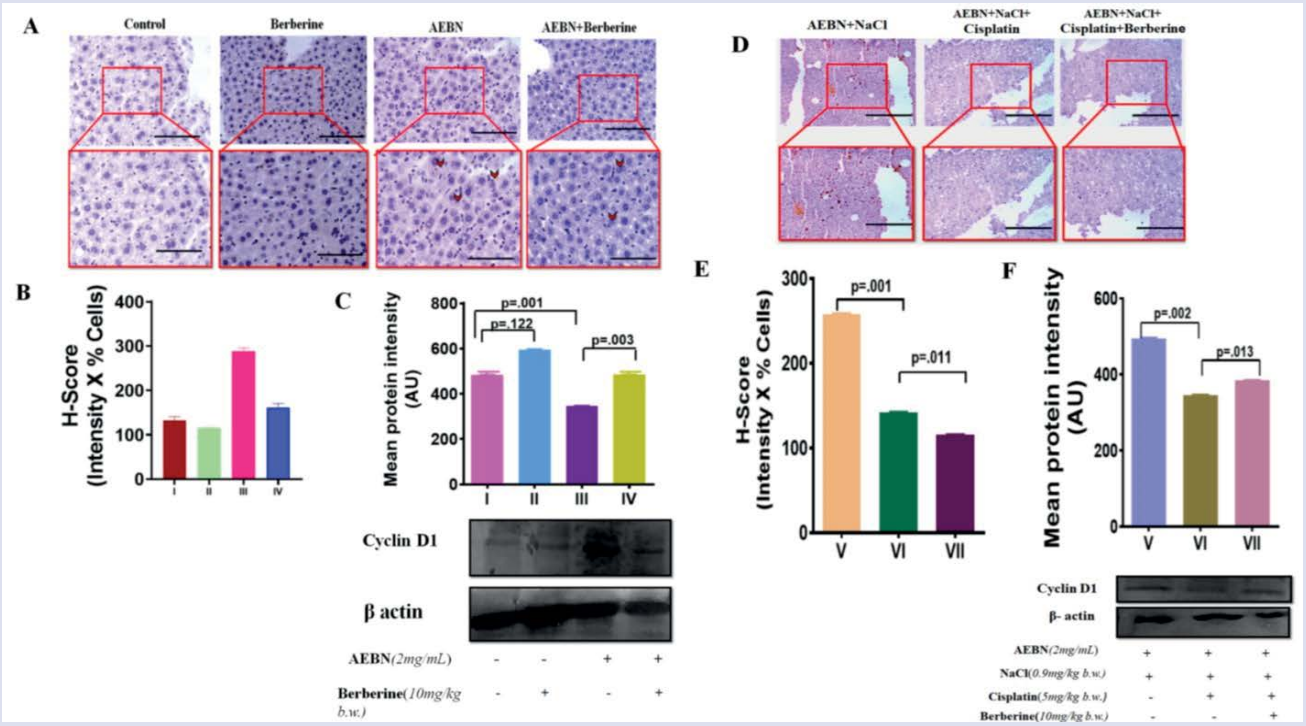

Figure 3: Berberine administered alone or in combination with cisplatin reduced the cell proliferation marker expression in the liver of AEBN and AEBN+NaCl exposed mice**.** (A), (D) ➤ indicates nuclei immunostained for Ki- 67; scale bar = 100 µm. (B), (E) H-Score obtained upon quantification of staining intensity. (C), (F) Immunoprobing for Cyclin D1 expression with β-actin serving as loading control. The treatment groups indicated in the figures:I-controls given drinking water without AEBN; II-drinking water+berberine; III-drinking water+AEBN; IV-drinking water+AEBN+berberine; V-drinking water+AEBN+NaCl;VI-drinkingwater+AEBN+NaCl+cisplatin;VII-drinking water+AEBN+NaCl+cisplatin+berberine. Data expressed as mean ± SD and *p*-values have been determined using one way ANOVA post hoc Tukey's Multiple Comparison Test. Number of mice, n= 30 per treatment group.

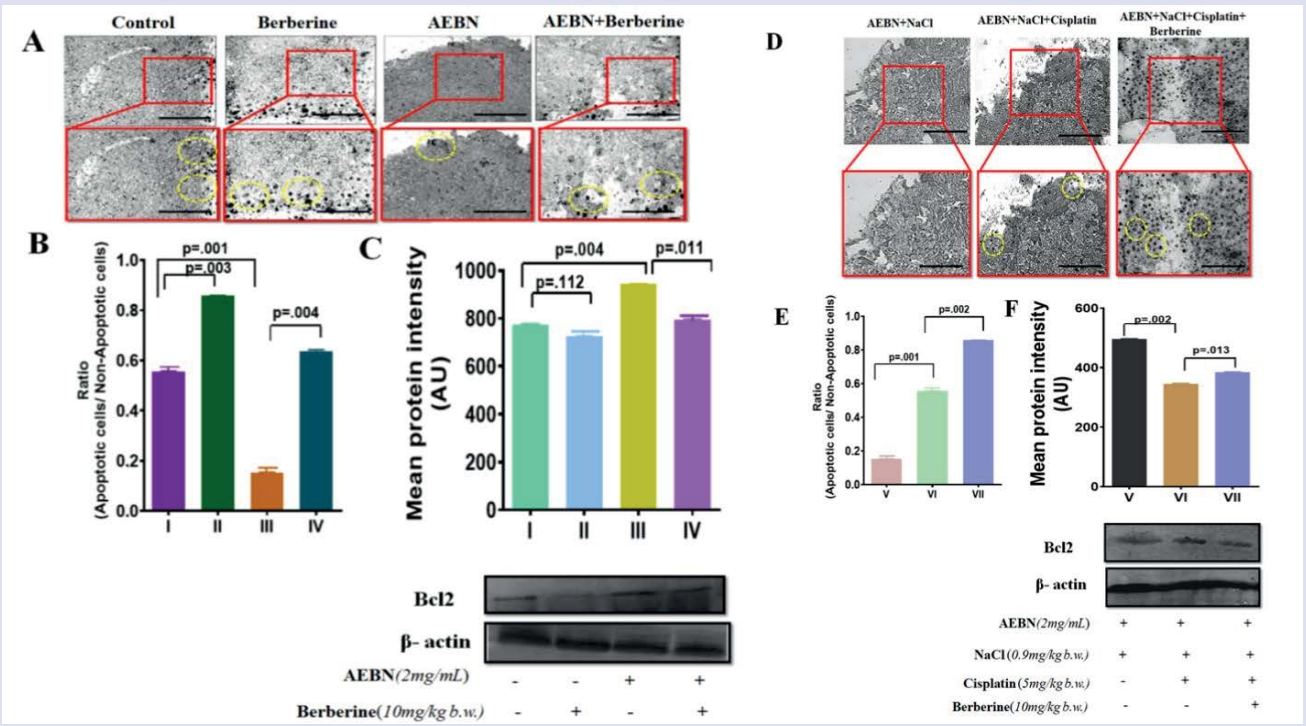

Figure 4: (A), (D) showing staining for apoptotic cells by TUNEL assay; Scale bar = (200 µm). (B), (E) Histograms showing ratio of apoptotic to non-apoptotic cells. (C), (F)Immunoprobing for Bcl2 protein expression with β-actin serving as loading control. The treatment groups indicated in the figures:I-controls given drinking water without AEBN; II-drinking water+berberine; III-drinking water+AEBN; IV-drinking water+AEBN+berberine; V-drinking water+AEBN+NaCl; VI-drinking water+AEBN+NaCl+cisplatin; VII-drinking water+AEBN+NaCl+cisplatin+berberine. Data is expressed as mean ± SD and *p*-values determined by one way ANOVA post hoc Tukey's Multiple Comparison Test. Number of mice, n= 30 per treatment group.

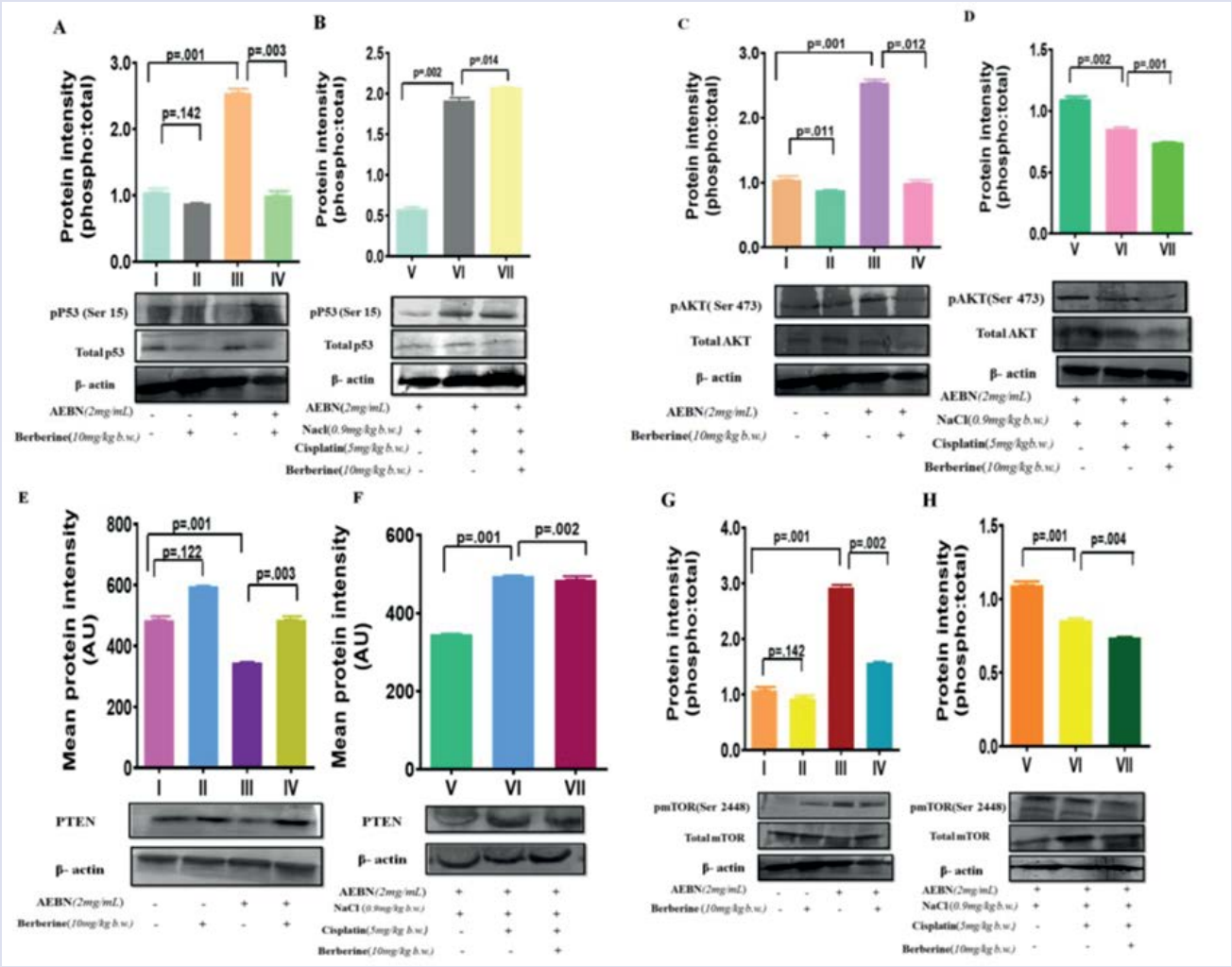

Figure 5: Berberine administered alone or in combination with cisplatin activated p53 and Akt/mTOR signaling in AEBN and AEBN+NaCl exposed mice. Histograms show ratio of protein intensity (A) pP53 (Ser-15) to tP53 (B) pAkt (Ser-473) to tAkt (C) PTEN (D) pmTOR (Ser-2448) to tmTOR and respective werstern blot in different treatment groups and immunoprobed with β-actin protein as loading control. The treatment groups indicated in the figures:I-controls given drinking water without AEBN; II-drinking water+berberine; III-drinking water+AEBN; IV-drinking water+AEBN+berberine; V-drinking water+AEBN+NaCl;VI-drinkingwater+AEBN+NaCl+cisplatin;VII-drinkingwater+AEBN+NaCl+cisplatin+berberine. Data are expressed as mean ± SD *p*-values have been determined using one way ANOVA post hoc Tukey's Multiple Comparison Test. Number of mice, n= 30 per treatment group.

activity, an antioxidant enzyme, in relation to age-matched controls (Group I). Berberine (10 mg kg-1) significantly reversed oxidative stress of the liver (Group V) (Figure 2A). Cisplatin treatment (Group VI) also reduced AEBN+NaCl induced lipid peroxidation and protein carbonylation, and improved catalase activity in both liver and kidney of Group VI mice. Interestingly, the combination of cisplatin+berberine (Group VII) significantly (p=0.001) reduced protein carbonylation and lipid peroxidation, and increased catalase activity more effectively than treatment with cisplatin alone (Group VII), indicating the higher efficacy of the combination in lowering oxidative stress (Figure 2B).

AEBN+NaCl treatment (Group V) resulted in elevated levels of lactate dehydrogenase (LDH) indicating hepato-toxicity.The combination of cisplatin and berberine (Group VII) lowered LDH levels more effectively and significantly (p=0.025) than treatment with cisplatin (Group VI) alone (Figure 2C).There was a notable increase in creatinine levels in the renal tissues of mice treated with cisplatin (Group VI) in contrast to the AEBN+NaCl treated group (Group VI), suggesting the development of nephrotoxicity due to cisplatin. Creatinine levels were significantly lowered by the combination of cisplatin and berberine (Figure 2D), indicating the efficacy of the combination in reducing cisplatin- induced nephrotoxicity.

Berberine reduced cellular proliferation and induced in situ apoptosis in AEBN exposed mice without or with cisplatin treatment

In comparison to age-matched controls (Group I), mice treated to AEBN (Group III) and AEBN+NaCl (Group V) had a substantial increase in immunoreactivity against the cellular proliferation marker, Ki-67, along with overexpression of Cyclin D1(Figure3A and 3B). Compared to Group III animals, berberine treatment (Group IV) dramatically decreased Cyclin D1 expression (p=0.021) and Ki-67 staining (p=0.001) in the liver tissue (Figure 3A). In addition, compared to Group V animals, cisplatin administration (Group VI) substantially decreased Cyclin D1 expression and Ki-67 immunoreactivity, suggesting that it is effective in reducing BN-induced carcinogenesis (Figure 3B). Interestingly, the combination of cisplatin+berberine (Group VII) showed higher efficacy in reducing Ki-67 immunoreactivity (p=0.004) in comparison to cisplatin alone (Group VI) alone though it failed to reduce Cyclin D1expression as effectively as cisplatin alone (Figure 3B).

AEBN (Group III) and AEBN+NaCl (Group V) exposed mice also showed significant decline in the ratio of apoptotic to non-apoptotic cells,concomitant with overexpression of the anti-apoptotic protein,Bcl2, in contrast to controls who are age-matched (GroupI). Berberine monotherapy (Group IV) or berberine in combination with cisplatin (Group VII) could significantly restore apoptosis by lowering Bcl2 expression, when compared to Group III and Group V mice, respectively. It is noteworthy that the combination of cisplatin with berberine (Group VII) produced a higher ratio of apoptotic to non-apoptotic cells than that produced by cisplatin alone (Figure 4).

Berberine induced p53 and Akt/mTOR dependent anti-carcinogenic impact in AEBN-treated mice when administered as monotherapy oras combination therapy with cisplatin

Mice exposed to AEBN (Group III) and AEBN+NaCl (Group V) showed significant activation of the oncogenic Akt/mTOR signaling pathway. This was evident from the notable increase in the ratios of phospho-Akt (Ser 473) to total Akt and phospho-mTOR (Ser 2448) to total mTOR, respectively.These changes were PTEN dependent, since PTEN expression was significantly downregulated in both these groups when compared to age-matched controls (Group I).In Group III and Group V mice, the phosphorylation of p53 at Ser-15 showed significant disruption compared to controls (Group I). This suggests a decline in p53-mediated tumor suppression. Cisplatin+berberine treatment (Group VII) restored normal Akt/mTOR and p53 mediated signaling more effectively than cisplatin (Group VI) alone (Figure 5), thus elucidating a mechanism for the role of berberine in augmenting cisplatin chemosensitivity.

DISCUSSION/CONCLUSION

BN ranks as the fourth most addictive drug, behind nicotine, alcohol, and caffeine. It includes alkaloids including arecoline, guvacine, arecaidine, and guvacoline, which contribute to its addictive properties and carcinogenic effects. BN chewing is a culturally acceptable habit and is widely prevalent among all social groups of South Asian countries and the Pacific islands, despite being strongly associated with cancer.[12] While chemotherapeutic drugs have been the mainstay of cancer therapy for several decades, their use is connected with a number of well investigated and documented drawbacks,[4,14] due to which there have been increasing efforts towards the development of new generation cancer therapeutics. Recognizing and reducing the harmful effects of cancer chemotherapy is believed to be a crucial aspect in deciding the effectiveness of future treatment methods. Numerous chemicals derived from natural sources, including microorganisms and plants, show great potential as potential candidates for innovative chemotherapeutic medications and/or as additional components to current medicationsdue to their efficacy in enhancing the clearance of cancer cells via many mechanisms. These natural compounds offer numerous benefits compared to synthetic drugs, including reduced cytotoxicity and cost-effectiveness.[13]

Our findings indicate that exposure to AEBN results in pronounced nodulation and dysplasia of the liver concomitant with high levels of lactate dehydrogenase (LDH), which are indicators of the progression of hepatocellular carcinoma.[14] Berberine when administered as monotherapy or in combination with cisplatin significantly reduced liver nodulation and reduced liver tissue architecture. Earlier, we reported that repurposing of the anti-diabetic drugs, metformin and vildagliptin produced similar effects in AEBN-treated mice.[1,11] However, unlike these drugs, cisplatin has been reported to cause weight loss in addition to severe toxicities.[4] In this study, we observed significant recovery of bodyweight, restoration of the coat and overall health of Group VII mice administered cisplatin in combination with berberine, alongwith significant reduction in creatinine levels and recovery of kidney architecture confirming the efficacy of berberine in reducing cisplatin-associated toxicities in BN-induced cancer. Our findings are consistent with earlier reports of the chemoprotective effects of berberine.[15,16] This may be attributed to our observation that the combination of berberine and cisplatin reduces oxidative stress more effectively than cisplatin alone, since oxidative stress is a major driver of BN-induced carcinogenesis,[1,11] promulgated by its alkaloid, arecoline.[1]

The activation of Akt and downstream signaling through mTOR is oncogenic, and promotes cellular proliferation and growth by stimulating the expressions of PTEN[17] and CyclinD1[18]. Moreover, elevated levels of oxidative stress have a role in the development of tumors by activating Akt, both via direct and indirect mechanisms. It has been observed that Akt signaling increases the production of the anti-apoptotic protein, Bcl2, resulting in cell survival[19].The results of our study suggest that AEBN stimulates the development of cancer by triggering the activation of Akt by phosphorylation at Ser 473, and mTOR phosphorylation at Ser 2448, while also causing a decrease in the expression of PTEN. The activation of mTOR signaling via phosphorylation led to a rise in the levels of CyclinD1.[18] This increase was found in both Group III and Group V animals, along with an enhanced immunoreactivity of the nuclear protein Ki-67, which plays a role in regulating cell cycle proliferation. AEBN resulted in heightened cellular proliferation and reduced apoptosis. In addition, AEBN also reduced the phosphorylation of p53 at Ser-15. The phosphorylation of p53 plays a vital role in its ability to prevent tumor growth[20] and by lowering it, AEBN would effectively reduce p53 tumor suppressor functions.[21]

We observed that the combination of berberine and cisplatin reduced CyclinD1 and Ki-67 immunoreactivity, and upregulated phospho-p53(Ser-15) levels more effectively than when cisplatin was administered. Similarly, the combination of berberine with cisplatin downregulated Bcl2 and higher ratio of apoptotic to non-apoptotic cells, significantly. Berberine in combination with cisplatin has previously been reported to induce apoptosis and suppress the growth of breast cancer.[22] It is noteworthy that berberine has also shown significant cancer mitigating efficacy in our model when administered as monotherapy, as reported earlier against colorectal cancer cells.[23] Thus, as reported earlier,[15] berberine exhibits anti-cancer potential and increases the chemosensitivity of the conventional chemotherapeutic drug in cisplatin by downregulating the oncogenic Akt/mTOR pathway to promote cancer cell death[13] and reduces its toxicity in a murine model of BN-induced carcinogenesis. As far as we know, this research is the first to describe these results. The limitation of our study is that we have not studied the absorption and bioavailability of berberine as well as its role in preventing or reducing chemoresistance in the current model. These aspects may be the subjects of future investigations.

Even though BN is strongly linked to cancer, it continues to be a popular masticatory among people of all ages, especially youngsters in underdeveloped nations. Despite its association with nephrotoxicity and hair loss, the well-known and widely used therapeutic cisplatin can successfully alleviate BN-induced cancer in our model when given intraperitoneally three times given at a dosage of 5 mg kg-1 for a duration of two weeks. Our findings suggest that berberine, when taken orally once daily at a dosage of 10 mg kg-1 for 8 weeks, may mitigate AEBN-induced carcinogenesis and hepatotoxicity via several pathways. When given in conjunction with cisplatin at a dosage of 10 mg kg-1 daily for two weeks, berberine may attenuate AEBN-induced carcinogenesis more effectively than cisplatin alone, according to all parameters investigated. Additionally, it can greatly decrease cisplatin-induced toxicities. In conclusion, berberine may be administered to Swiss albino mice as a single, safe therapeutic agent to ameliorate BN-

induced carcinogenesis; this approach yields outcomes similar to those of cisplatin therapy, but without any adverse reactions. Furthermore, the anti-cancer benefits of cisplatin are enhanced and the toxicity of cisplatin is decreased by combination therapy of cisplatin with berberine.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

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