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Chemotherapeutic effect of Farnesol against N-Diethyl Nitrosamine (DEN) Induced Experimental Hepatocellular Carcinoma in Rats

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ABSTRACT

Hepatocellular carcinoma (HCC), commonly known as liver cancer, is a most prevalent cancer which accounts for increased morbidity and mortality in developed countries. Currently available drugs for the treatment of liver cancer cause detrimental side effects. Hence search for plant - based drugs with fewer side effects continues. Farnesol which is found in the essential oils of ambrette seeds, citronella, and chamomile possess a wide range of pharmacological properties. In the present study an attempt has been made to evaluate the anticancer effect of farnesol against DEN- induced hepatocellular carcinoma in rats. Oral treatment of farnesol (25 mg/kg bw) to tumor bearing rats daily for four weeks was found to be effective against DEN- induced hepatocellular carcinoma in rats. The levels of tumor markers such as alpha fetal protein and carcinoembryonic antigen were decreased upon farnesol treatment. The increased levels of DNA and RNA were found to be decreased upon treatment with farnesol. There was a significant improvement in protein content. The increased activities of AST, ALT, ALP, ACP, 5'-ND, gamma-GT and LDH in serum of experimental rats were significantly decreased to near normal levels. Oral administration of farnesol to DEN- induced rats significantly decreased the levels of Phase I enzymes and increased the levels of Phase II enzymes in liver tissues to near normalcy. Elevated levels of glycocomponents of glycoproteins such as hexose, hexosamine and sialic acid in plasma and liver tissues were significantly decreased upon farnesol treatment. The results of the present study indicate that farnesol exerts chemotherapeutic potential in DEN- induced hepatocellular carcinoma in rats.

Keywords : Hepatocellular Carcinoma, Diethyl Nitrosamine, Farnesol, Anticancer Property.

I. INTRODUCTION

Non-communicable diseases including diabetes and cancer pose chief public health problems. Cancer is the second most common cause of morbidity and mortality in both developed and developing countries. Substantial epidemiological data on populations indicate an association between many human cancers and lifestyle/diet. Research on mutational events in human cancers has provided substantial evidence for a direct action of environmental carcinogens in the development of certain cancers [1] since these diseases are lifestyle related they require specialized infrastructure and human resource for treatment.

Hepatocellular carcinoma (HCC), commonly known as liver cancer is a most prevalent cancer not only in developed countries but also in most undeveloped countries. It is induced by toxic industrial chemicals, air and water pollutants, food additives and fungal toxins [2]. The liver is the major organ involved in the metabolism of ingested materials and it is more

susceptible to carcinogenic insult. Hepatocellular carcinoma is rarely detected at the primary stage and once detected treatment has a poor prognosis in most cases [3].

The liver plays a significant important intriguing site in the study of neoplastic diseases. As abnormal metabolism represents cancer, the liver being the major vital metabolic organ, the structural and functional abnormalities represent the diseased condition. Currently available drugs for the treatment of liver cancer cause detrimental side effects. Hence search for plant based drugs with fewer side effects continues. A large number of natural and synthetic compounds have been shown to possess anticancer activity. Plants and plant products have been shown to play an important role in the management of various liver disorders. One such compound that is known for its therapeutic value is farnesol.

Farnesol, a 15-carbon sesquiterpene (an isoprenoid intermediate of the mevalonate pathway), is produced in cells by the dephosphorylation of farnesyl pyrophosphate, a precursor of squalene generating sterols and other isoprenoid compounds [4]. Fruits such as plums, berries, apricots and peaches are the fair source of farnesol [5]. It could also be found in the essential oils of ambrette seeds, citronella, and chamomile [6].Farnesol exerts antioxidant and anti-inflammatory effects [4], [7]. In the present study, an attempt has been made to evaluate the anticancer effect of farnesol against DEN - induced hepatocellular carcinoma in rats

II. MATERIALS AND METHODS

Chemicals

N-Diethyl nitrosamines (DEN) and Farnesol were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade obtained from SRL/HIMEDIA, India.

Experimental Animals

Adult male Wistar strain albino rats weighing about 140-180 g were obtained from Fredrick Institute for Plant Protection and Toxicology, Chennai, India. The animals were fed with commercially available balanced pellet diet (Amrut laboratory Animal Feed, Bangalore, India) and water ad libitum. The animals were acclimatized for one week prior to the initiation of experiments. The experimental design was performed in accordance with the current ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India and the Institutional Animal Ethics Committee Guidelines (IAEC.No: 01/20/2018).

Experimental Design

The experimental animals were divided into four groups, each group comprising of six animals.

Group 1: Normal control rats fed with standard diet and pure drinking water for 16 weeks

Group 2: Rats were induced with hepatocellular carcinoma by providing 0.01% DEN through drinking water

Group 3: Rats treated with farnesol 25mg/kg body weight one week before the administration of 0.01% DEN and continued along with carcinogen till the end of the experiment

Group 4: Drug control (Farnesol alone treated)

All animals were fasted overnight and sacrificed by sodium pentothal anesthesia followed by cervical decapitation. Blood was collected with and without anticoagulant and the serum was centrifuged at 5000 rpm for 15 min to obtain a clear supernatant and stored at -70°C until its use for further biochemical analysis. Liver tissues from control and experimental groups of rats were immediately excised, washed in ice-cold PBS to remove the blood stains, blotted, weighed and homogenized in Tris-HCl buffer (0.1M, pH 7.4) using a Teflon homogenizer to prepare 10%

(w/v) tissue homogenate. This homogenate was centrifuged at 12,000g for 30 min at 4°C to obtain a clear supernatant. This supernatant was pooled and used for further analysis.

Evaluation of markers of tumorigenicity

Tumor markers alpha fetoprotein and carcinoembryonic antigen were quantified based on solid phase enzyme-linked immunosorbent assay method using UBI MAGIWELL (USA) enzyme immunoassay kit according to the manufacturer's instructions.

Total Protein

Total Protein was estimated according to the method of Lowry et al 1951[8].

Nucleic acids

Nucleic acids from liver tissues were extracted by the method of Schneider (1957) [9] and the DNA and RNA were quantified by the method of Burton (1956) [10] and Rawal et al. (1977)[11] respectively

Estimation of Liver marker enzymes

The activity of cytosolic marker enzymes such as AST and ALT in serum was assayed by the method of Bergmeyer et al., (1978) [12]. Alkaline phosphatase and Acid phosphatase activities were estimated by the method of King (1965a) [13] using disodium phenyl phosphate as substrate. The 5'-Nucleotidase was assayed by the method of Luly et al., (1972) [14] using 5' adenosine monophosphate as substrate and the activity ofγ-glutamyl transpeptidase was assayed by the method of Rosalki and Rau, (1972)[15]using L-γ-glutamyl-p-nitroanilide as substrate. Lactate dehydrogenase (LDH) was assayed by the method of King (1965b) [16] using lithium lactate as substrate.

Estimation of Phase I and Phase II enzymes

PhaseI detoxification enzymes such as cytochrome P450 and cytochrome b5 were assessed [17]. Phase II detoxification enzymes such as glutathione S - transferase (GST) and UDP glucuronyl transferase (QR) were also measured [19].

Glycoprotein components

The levels of glycoprotein components namely hexose, hexosamine and sialic acid in plasma and liver were estimated by the method of Niebes (1972), Wagner (1979), and Warren (1959) [20,21&22] respectively. The values are expressed as mg/dl for plasma and mg/g of defatted tissue for a tissue.

III. RESULTS AND DISCUSSION

The body weight, as well as liver weight of the experimental groups of rats, is depicted in table 1. DEN- induced hepatacellular carcinoma showed a significant decrease in the body weight with an increase in liver weight, whereas farnesol treatment to DEN- induced animals significantly improved the body weight and decreased the liver weight in tumor-bearing rats.

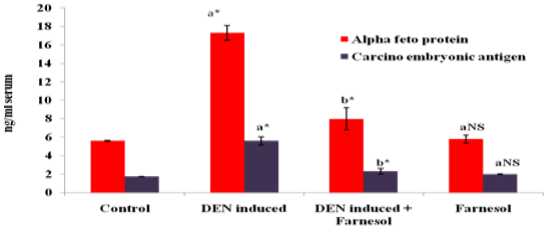
Table 1 : Effect of Farnesol on body weight and liver weight of control and experimental animals.

Groups	Body weight (g)	Liver weight (g)
Control	180.00 ± 6.45	7.00 ± 0.18
DEN induced	120.00 ±9.10*	11.30 ± 0.77
DEN induced + Farnesol	153.30 ± 9.00$^{a\#b*}$	8.20 ± 0.30
Farnesol	170.00 ± 08.00^{aNS}	7.32 ± 0.12

Values are given as mean ± S.D for six rats in each group. Statistical significance was compared within the group as follows :

A-compared with control rats b-compared with DEN INDUCED treated rats*p<0.001; @p<0.05; #p<0.01; NS-Not significant

Figure 1 shows the effect of farnesol on the levels of serum tumor markers in the control and the experimental groups of animals. The markers of tumorigenicity examined in the DMBA group showed a significant increase in the AFP, CEA levels when compared to the control group of rats. Farnesol, treatment to DEN- induced rats significantly decreased the levels of the tumor markers.



Values are given as mean ± S.D for six rats in each group. Statistical significance was compared within the group as follows: a-compared with control rats
b- compared with DEN induced rats
*p<0.001; @p<0.05; #p<0.01; NS-Not significant

Figure 1: Effect of farnesol on the levels of serum tumor markers

Table 2 depicts the activities of marker enzymes, such as transaminases, alkaline phosphatases, 5' Nucleotidase, transpeptidase, γ GT and LDH in the serum, of control and experimental group of rats. A significant increase in the levels of serum marker enzymes was observed in DEN- induced rats compared to control rats. However, upon treatment with farnesol these levels were decreased to normal in DEN- induced rats. However, there was no significant change in marker enzyme activities observed in farnesol control rats when compared to control rats demonstrating the non-toxic nature of farnesol.

Table 2 : Effect of Farnesol on the levels of marker enzymes in serum of control and experimental animals

Groups	AST	ALT	ALP	ACP	5'ND	γ GT	LDH
Control	46.02 ± 1.60	24.00 ± 0.55	54.77 ± 2.30	12.20 ± 0.38	2.50 ± 0.04	8.00 ± 0.40	79.54 ± 3.28
DEN induced	70.00±5.80^{a*}	38.60±3.88^{a*}	118.00±10.41 ^{a*}	44.40 ± 5.40 ^{a*}	6.66 ± 0.83 ^{a*}	12.24 ± 1.23 ^{a*}	129.00 ± 13.00 ^{a*}
DEN induced + Farnesol	49.00 ± 1.42 $^{a*b\#}$	32.50 ± 2.04 $^{a*b\#}$	72.85 ± 3.10$^{a\#b*}$	31.64 ± 2.11 $^{a*b\#}$	4.30 ± 0.35 $^{a*b\#}$	7.80 ± 0.24 $^{a*b\#}$	92.22 ± 7.50 $^{a*b\#}$
Farnesol	43.10 ± 0.70^{aNS}	20.58± 0.79^{aNS}	54.44± 1.32^{aNS}	12.50± 0.32^{aNS}	2.89± 0.08^{aNS}	6.80± 0.38^{aNS}	83.00± 3.45^{aNS}

Units: AST and ALT are expressed in µmoles of pyruvate liberated/mg protein/min; ALP and ACP are expressed in µmoles of p-nitrophenol liberated/mg protein/min; 5'ND is expressed in µmoles of inorganic phosphate liberated/mg protein/min, γ GT is expressed in µmoles of p-nitroanilineliberated/mg protein/min; and LDH is expressed in µmoles of pyruvate liberated/mg protein/min. AHH is µmoles offluroscent phenolic metabolites formed/mm/mg pr.

Values are given as mean ± S.D for six rats in each group. Statistical significance was compared within the group as follows: a-compared with control rats, b-compared with DEN INDUCED treated rats*p<0.001; @p<0.05; #p<0.01; NS-Not significant.

Table 3 shows the levels of nucleic acid and proteins in livers tissues of experimental groups of rats. Tumor -induced rats showed a significant increase in nucleic acid content and a decrease in protein content in liver tissue. However, upon treatment with farnesol to DEN- induced rats, the level of nucleic acids in liver tissue was found to be significantly decreased. The protein content was found to be increased. The nucleic acid and protein content remained the same in control and farnesol alone treated animals.

Table 3 : Effect of Farnesol on the levels of nucleic acid and protein level in liver tissues of control and experimental animals.

Groups	Control	DEN induced	DEN induced+Farnesol	Farnesol
DNA (mg/g wet tissue)	6.20 ± 0.05	10.26± 0.40 ^{a*}	7.00 ± 0.20 ^{a*b*}	5.80 ± 0.05^{aNS}
RNA (mg/g wet tissue)	5.20 ± 0.04	8.60± 0.35 ^{a*}	5.50 ± 0.14^{a*b*}	5.40 ± 0.06^{aNS}
Proteins (mg/gof wet tissue)	8.50 ± 0.41	5.00 ± 0.29 ^{a*}	6.20 ± 0.28 ^{a*b*}	7.14 ± 0.32 ^{aNS}

Values are given as mean ± S.D for six rats in each group. Statistical significance was compared within the group as follows: a-compared with control rats b-compared with DEN INDUCED treated rats $^{*}p<0.001$; $^{@}p<0.05$; #p<0.01; NS-Not significant

The levels of Phase I and Phase II biotransformation enzymes in the liver tissues of control and an experimental group of animals are shown in Table 4. The levels of Phase I enzymes was found to be increased significantly in the liver tissue of tumor - induced rats when compared to control animals. However, the Phase II enzymes were found to be decreased significantly in liver tissues of tumor induced rats. On the contrary, the administration of farnesol to DEN- induced rats significantly decreased the levels of Phase I enzymes and increased the levels of Phase II enzymes to near normalcy. There was no significant change in the enzyme levels in farnesol alone treated rats when compared to control animals.

Table 4 : Activities of Phase I and Phase II Biotransformation enzymes in liver tissues of control and experimental animals

Groups	Control	DEN induced	DEN induced+Farnesol	Farnesol
Phase I enzyme Cytochrome P450	0.45 ± 0.032	0.84 ± 0.02 ^{a*}	0.63 ± 0.03 ^{a*b*}	0.83 ± 0.03^{aNS}
Cytochrome b5	0.34 ± 0.040	0.74 ± 0.024 ^{a*}	0.42 ± 0.03 ^{a*b*}	0.75 ± 0.014^{aNS}
NADPH Cytochrome 'C'Reductase	12.31 ± 2.20	18.40 ± 0.83^{a*}	14.14 ± 1.06 ^{a*b*}	18.20 ± 0.23^{aNS}
Phase II Enzyme Glutathione S-transferase	3.20 ±0.71	2.87 ± 0.14^{a*}	4.05 ± 0.22 ^{a*b*}	2.76 ± 0.08^{aNS}
UDP Glucronyl tranferase	47.00 ± 3.80	37.82± 0.13 ^{a*}	44.73 ±2.60 ^{a*b*}	32.10 ± 1.80^{aNS}

Units : n moles/mg microsomal protein/min Values are given as mean ± S.D for six rats in each group.Statistical significance was compared within the group as follows: a-compared with control rats b-compared with DEN induced treated rats $^{*}p<0.001$; @p<0.05; #p<0.01; NS-Not significant

Table 5 and 6 depicts the levels of glycocomponents of glycoproteins in plasma and liver tissues of control, DEN- induced and farnesol treated rats respectively. Elevated levels of hexose, hexosamine and sialic acid in plasma and liver tissues were observed in DEN-induced rats when compared to the controls. Upon farnesol administration, levels of glycocomponents of glycoproteins were significantly decreased in a dose dependent manner when compared to control rats.

Table 5 : Effect of Farnesol on the levels of glycoproteins in plasma of control and experimental animals.

Groups	Control	DEN induced	DEN induced+Farnesol	Farnesol
Hexose	135.60±8.00	197.68±10.12 ^{a*}	153.70± 9.10 ^{a*b*}	142.50±9.50^{aNS}
Hexosamine	36.43±3.30	50.34± 4.30 ^{a*}	43.00 ± 4.00 ^{a*b*}	39.50± 3.6^{aNS}
Sialic acid	52.20±4.60	120.50± 10.00 ^{a*}	93.50±7.54 ^{a*b*}	60.11± 7.20^{aNS}

Units: mg/dl Values are given as mean ± S.D for six rats in each group. Statistical significance was compared within the group as follows: a-compared with control rats b-compared with DEN INDUCED treated rats *p<0.001; @p<0.05; #p<0.01; NS-Not significant.

Table 6: Effect of Farnesol on the levels of glycoproteins in Liver tissues of control and experimental animals.

Groups	Control	DEN induced	DEN induced+farnesol	Farnesol
Hexose	1.45±0.28	5.65±0.74 ^{a*}	3.12±0.22 ^{a*b*}	2.00±0.31^{a} NS
Hexosamine	2.50±0.08	4.20±1.00 ^{a*}	3.13±0.35 ^{a*b}	2.88±0.40^{a} NS
Sialic acid	2.02±0.20	3.30±0.42 ^{a*}	2.70±0.32 ^{a*b*}	2.52±0.25^{a} NS

Units: mg/g of defatted tissue. Values are given as mean ± S.D for six rats in each group. Statistical significance was compared within the group as follows: a-compared with control rats b-compared with DEN INDUCED treated rats *p<0.001; @p<0.05; #p<0.01; NS-Not significant

IV.DISCUSSION

Diethylnitrosamine (DEN, N-nitrosodiethylamine, and NDEA) is a well known potent hepatocarcinogenic agent present in tobacco smoke, water, curd and fried meals, cheddar cheese, agricultural chemicals, cosmetics and pharmaceutical products [23]. DEN is known to induce damage in many enzymes involved in DNA repair and it is one of the most accepted and widely used experimental models to study hepatocarcinogenesis [24]. It has been reported that DEN after its metabolic biotransformation produces the pro mutagenic adducts, O6-ethyl deoxyguanosine and O4- and O6-ethyl deoxy thymidine that may initiate liver carcinogenesis [25, 26&27].

Acute toxicity studies were conducted to evaluate the adverse effects of a drug that results either from a single or multiple exposures in a short time. Dosage fixation studies are performed to determine the optimum dosage that shows maximum activity for a particular disease in experimental animals. Acute toxicity and dosage fixation studies of farnesol were performed in control and DEN- induced rats. For the acute toxicity study, graded doses of farnesol were administered orally and the animals were continuously observed for two weeks following

administration. Change in body weight gain, food and fluid intake and psycho-motor activities were clearly monitored. The activities of liver marker enzymes such as AST, ALT and ALP were also assayed to determine the toxicity. In the acute toxicity study, farnesol did not show any toxicity nor change in behavioral patterns even at very high doses (Data not shown).

In the dosage fixation study (10, 25, 50 and 100mg/kg bw/rat/day), farnesol at a dosage of 25 mg/kg for a period of 4 weeks showed maximum activity when compared to other doses. Hence farnesol treatment at a dose of 25 mg/kg body weight for a period of 4 weeks was fixed as the optimum dosage and used for further studies to evaluate the chemotherapeutic effect in DEN-induced experimental hepatocellular carcinoma in rats.

Hepatocellular carcinoma (HCC) is a highly malignant tumor with very high morbidity and mortality and a poor prognosis [28&29]. Metabolic reprogramming occurs in tumors to foster cancer cell proliferation, survival and metastasis, but as well as a systemic level affecting the whole organism, eventually leading to cancer cachexia. Cancer cells rely on external sources of nitrogen and carbon skeleton to grow, systemic metabolic deregulation promoting tissue wasting and metabolites mobilization ultimately supports tumor growth [30].During tumor growth, liver tissue is actively co-opted to perform high-rate gluconeogenesis, using the lactate derived from tumor glycolysis [31]. Increased glycolytic process and proteolysis, decreased food intake may contribute to weight loss during cancerous condition. In DEN-induced animals there was a significant decrease in body weight and were increase in liver weight due to cancer. Treatment with farnesol increased the body weight which indicates the beneficial effect of farnesol in controlling in controlling muscle wasting.

Tumor markers are molecules that can be detected in higher or lower than normal amounts in the blood, urine, or body tissues of patients with certain types of cancer [32]. Alpha-fetoprotein (AFP) is a major plasma protein produced by the yolk sac and liver during fetal development. AFP, a tumor-associated fetal protein, has long been employed as a serum fetal tumor marker to monitor disease progression [33]. Carcinoembryonic antigen (CEA) are glycosyl phosphatidylinositol (GPI) cell surface anchored glycoproteins whose specialized glycoforms serve as functional carcinoma L-selectin and E-selectin ligands, which are involved in cancer cell adhesion and metastasis respectively. CEA is produced in gastrointestinal tissue during fetal development, but the production ceases before birth. However, the serum levels of CEA are raised in some types of cancer, which indicates its prominence as a tumor marker in clinical tests [34]. Elevation of serum AFP levels has been reported in several diseases including cancer. AFP along with CEA is most extensively used in the diagnosis of hepatocellular carcinoma [35]. There was an increase in serum AFP and CEA levels on DEN-induced rats. However, treatment with farnesol decreased the levels of tumor markers indicating its antitumor property.

The nucleic acid content of the tumor is found to be an important indicator of prognosis because it is well correlated with the size of the tumor in the cancerous condition [36]. In the diseased state, the degree of malignancy increases with the defective abnormalities in DNA. The assessment of DNA content is an index of proliferative activity in cancer conditions. An abnormally increased content of DNA may lead to an increased transcription, which in turn in reflected in increased RNA content in tumor cells. Tumor induced rats showed a significant increase in nucleic acid content in liver tissue. However, upon treatment with farnesol to DEN- induced rats, the levels of nucleic acids in liver tissue were found to be significantly decreased.

Protein synthesis is a process that is considered as important in both normal as well as in cancerous milieu. Protein waste indicates the metabolic nitrogen imbalance which is being reflected by an elevation in the apparent protein catabolism rate with no changes in apparent synthesis and thus, the body responds to the increased tumor lead by increased tissue protein breakdown [37]. The protein content was reduced in DEN-administered rats which occur as a consequence of decreased synthesis or excessive losses [38].Upon treatment with farnesol to DEN- induced rats, the catabolic rate decrease with a concomitant increase in the total protein content this indicates the beneficial effect of farnesol in controlling muscle wasting.

Aspartate transaminase (AST) and alanine transaminase (ALT) are the pathophysiological enzymes present in cytosol which serves as the marker of tissue damage. The increase in the activities of ALT, AST and ALP in hepatic cancer bearing rats may be primarily due to leakage of these enzymes from the liver cytosol into the bloodstream as a result of tissue damage. The structural integrity of the cells has been reported to be damaged in toxicity induced animals and this results in cytoplasmic leakage of enzymes into the bloodstream. Tissue damage is the vulnerable feature in a cancerous environment. Hence, elevation of these marker enzymes is an indicator of progression of tumor growth.

GGT is an enzyme embedded in the hepatocyte plasma membrane, mainly in the canalicular domain; again the liberation of this enzyme into serum indicates damage to the cell and thus injury to the liver. It is pointing out that serum GGT activity is considered to be one of the best indicators of liver damage [39].Gamma -glutamyl transferase (γ-GT) is known to protect the cells against toxins and carcinogens. Lactate dehydrogenase (LDH) is a tetrameric isoenzyme recognized as a biomarker with potential use in assessing the progression of the proliferating malignant cells. Numerous reports revealed the elevated levels of LDH in various types of cancers. The rise in LDH activity is attributed to the high glycolysis rate in the cancerous condition, which is the only energy-producing pathway for the uncontrolled proliferating malignant cells [40]. The increase in the activities of LDH in tumor induced rats could be attributed to overproduction of enzymes by proliferated cells and further release of their isoenzymes from destructed cells thus making it a sensitive marker for solid neoplasm. An increase in the AST, ALT activities in DEN- induced animals serves as an index of hepatotoxicity and carcinogenesis with the development of preneoplastic changes, thus indicating the advanced stage of liver carcinoma. In the present investigation, the increased levels of AST, ALT, ALP, 5' nucleotidase, Gamma glutamyl transferase and LDH observed in tumor induced rats were normalized upon treatment with farnesol indicating the tissue protective nature of farnesol.

Liver is a major organ involved in detoxification which is an important phenomenon for the prevention of cancer. The liver detoxifies a wide range of any foreign substances, or xenobiotics, by a complex series of chemical reactions through biotransformation of phase I and II metabolizing enzyme [41]. The Phase 1 enzymes, including the cytochrome P_{450}, cytochrome b_5 and epoxide hydrolases adds a polar functional group to the original compound producing reactive intermediates which bind to DNA and cause a mutation, or they can become a substrate for the second class of detoxification enzymes. The Phase II enzymes, including glutathione S-transferase and quinone reductase, conjugate these reactive intermediates, adding a hydrophilic functional group and making them water-soluble. These secondary products are subsequently excreted from the body.

The phase I enzymes such as cyt p450, cyt b5, NADPH Cytochrome 'C'Reductase was elevated in

DEN- induced rats. DEN, an indirect acing carcinogen requires metabolic activation to yield an ultimate carcinogen. Drugs and chemicals undergo phase I oxidative metabolic reactions resulting in the formation of more water-soluble and less toxic metabolites [42]. Genotoxic and cytotoxic carcinogen exerts more reactive electrophilic species through bioactivation, a process which is carried out by phase I cytochrome p450 complex [43]. Phase II enzymatic reactions know as conjugation reactions, involve the addition of the intracellular polar groups including glucuronate, glutathione, sulfate, glycine to the foreign molecules [42] and function to eliminate electrophiles and ROS generated by phase I reactions, thereby protecting organisms against chemical insult [44]. GST conjugation catalyzed by GST that is involved in the removal of the proximate and ultimate carcinogen through the formation of more water soluble and non-electrophilic detoxification products. There was a significant decrease in the levels of phase II enzymes of DEN- induced rats which might have resulted in an enhanced covalent binding of DEN metabolites to cellular DNA and thereby promoting carcinogenesis [45] The increase in the activities of phase II enzymes upon treatment with farnesol might be due to suppression of DEN- induced tumorigenic process. Treatment with farnesol showed high levels of phase II enzymes which might lead to an enhanced DEN detoxification, elimination as well as reduction of carcinogen-DNA adduct formation and cancer.

Glycoproteins mediate cell-cell recognition, cellular adhesion, binding and clearance of serum glycoproteins and metabolic transport among others [46]. Elevations of glycoprotein contents serve as an indicator of carcinogenesis and any alterations in these affect the rigidity of the cell membrane. Malignant transformation of the normal cell may be accompanied by changes in the carbohydrate composition of glycoproteins viz. hexose, hexosamine and sialic acid in liver tissue. Changes in surface carbohydrates during cellular differentiation and neoplastic transformation suggest their importance in physiology and behavior of the cells. Increased cell proliferation observed in DEN- induced hepatocellular carcinoma leads to the promotion of reactive oxygen-initiated cells, thereby enhancing the possibility of neoplastic changes.

During cancer, the increased levels of plasma glycoprotein components may be due to the leakage of the membrane components from dying neoplastic cells or as a consequent shedding of plasma membrane and due to increased synthesis by sequential addition of monosaccharide units to parent protein molecule catalyzed by multiple glycosyltransferases [47]. Upon treatment with farneosl the increased levels of glycoprotein components such as hexose, hexosamine and sialic acid were decreased in plasma and liver tissues indicating the cytostabilising property of farnesol.

V. CONCLUSION

The results of the current study shed light on the promising anticancer properties of farnesol on the hepatocellular carcinoma-induced experimental animal model.

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