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A STUDY ON THE EFFECT OF *RUELLIA TUBEROSA* L. (WHOLE PLANT) TREATMENT ON TOTAL BODY AND ORGAN WEIGHT IN DEN INDUCED HEPATO CARCINOMA

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ABSTRACT

The present attempt is to investigate the protective effects of ethanolic and ethyl acetate extract of *R.tuberosa* against N-Nitrosodiethylamine (DEN) induced hepatocarcinoma (HCC) in wistar albino rats. The ethyl acetate and ethanolic extract of whole plant of *R.tuberosa* were prepared and analyzed for chemopreventive effect on DEN induced liver cancer in wistar albino rats. HCC is associated with pronounced symptoms of weight loss and tissue wasting. In cancer bearing animals, there was a sharp loss in body weight but treatment with ethyl acetate and ethanolic extract of *R.tuberosa* showed improvement. The ethyl acetate and ethanolic extract of *R.tuberosa* treated animals showed a steep increase in the body weight indicating their ability to restore the body weight when compared to the DEN induced animals. Ethanolic extract had better chemopreventive effect than ethyl acetate extract. Histopathological studies further proved its hepatoprotective activity. It indicates the counteractive property of the drug against DEN-induced HCC. This study suggests that *R.tuberosa* prevents hepatic cell damage, and protects the body against DEN-induced hepatocellular carcinogenesis.

KEYWORDS: *Ruellia tuberosa,* antioxidant activity, doxorubicin, chemoprevention, organ weight, liver cancer.

Abbreviations

Human liver carcinoma cell lines (HepG2), N-Nitrosodiethylamine (DEN), Hepatocarcinoma (HCC), Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS), Tamilnadu Veterinary and animal Science University (TANUVAS), Institutional Animal Ethics Committee (IAEC), Ethylene diamine tetra acetic acid (EDTA), Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC).

1. INTRODUCTION

Cancer is a group of disease characterized by deregulated proliferation of abnormal cells that invade and disrupt the surrounding tissues.[1] Liver cancer is the 2nd most frequent cause of death throughout the world.[2] HCC is highly malignant tumor with very high morbidity and mortality and poor prognosis.[3,4] HCC is a multistep process involving genetic and epigenetic alterations of various oncogenes, proto-oncogenes, and growth factors, as well as tumor suppressors. It arises due to chronic inflammation and subsequent liver fibrosis.[5] Exposure to environmental carcinogens is an important factor leading to the formation of hepatocarcinoma. DEN, is widespread in nature such as in cheese, soybean, processed meats, alcoholic beverages, tobacco products, cosmetics and agricultural chemicals, and is one of the most important environmental carcinogens.[6-8]

DEN can induce carcinoma in all animal species as well as humans,[9] and has been used as an experimental carcinogen in many studies. HCC is associated with pronounced symptoms of weight loss and tissue wasting.[10] The conventional therapy of hepatocarcinoma including chemotherapy, radiation, surgical resection and ablation which gives little hope for restoration of health because of poor diagnosis and serious side effects. Liver transplantation is considered to be the most effective treatment for patients with hepatocarcinoma. However, low availability of organs limits the offer of this option to all candidates, and the high risk of tumor recurrence after transplantation further compromises its efficiency.[11] Therefore, developing more effective and less toxic anti-cancer agents, including natural products, is necessary to prevent or retard the process of hepatocarcinogenesis. *Ruellia tuberosa* is a tropical plant and widely distributed in South East Asia. Ethanolic and ethyl acetate extract of *Ruellia tuberosa* had very good antiproliferative, cytotoxic effect on HepG2 cells.[12] Hence the present study is intended to evaluate the chemopreventive activity of the ethyl acetate and

ethanolic extracts of *R.tuberosa* against DEN-induced hepatic cancer in rats using doxorubicin as control drug.

2. MATERIALS AND METHODS

2.1. Chemical reagents: N-Nitrosodiethylamine (DEN), Doxorubicin Hydrochloride and Phenobarbital were purchased from Sigma Chemical Company, USA. All other chemicals including solvents were of high purity and of analytical grade purchased from Glaxo Laboratories, Mumbai and Sisco Research Laboratories Pvt, Ltd, Mumbai, India.

2.2. Plant Materials and Extract Preparation: Fresh plant materials of *R.tuberosa* were collected from Tiruvallur district of Tamilnadu. The plant materials were identified and authenticated by botanist of this institute using the Flora of Presidency of Madras,[13, 14] and voucher specimen (No: 00628) was deposited in the museum of Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS), Arumbakkam, Chennai. The shade dried and coarsely powdered plant material (100g) was successively extracted with ethyl acetate and ethanol using Soxhlet apparatus, filtered and concentrated to dryness.[15] One gram of ethyl acetate and ethanolic extract from whole plant of *R.tuberosa* was weighed in dry weighing bottle. The working concentration of the each extract was diluted with Tween-80 to make a concentration of 100mg/ml and then the diluted solution was used for further chemopreventive study.

2.3. Experimental Animals: The procedure for animals experiments were reviewed and approved by Institutional Animal Ethics Committee (IAEC) of CCRIS (Approval No: 109/PHARMA/SCRI, 2011). Wistar albino male and female rats weighing 160-180g were purchased, from Tamilnadu Veterinary and animal Science University (TANUVAS), Madhavaram, Milk colony, Chennai, Tamilnadu, India, for this study. The animals were maintained under standard conditions of humidity, temperature (25 ± 2ºC) and light (12hr light and 12hr dark). The animals were acclimatized and maintained over husk bedding in polypropylene cages in central animal house facility of the institution for one week before use. The animals were fed with commercial pelleted diet (Hindustan lever Ltd, Bangalore, India, and free access to water throughout the experimental period. Experimental animals were handled according to the University and Institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental Design: The experimental animals were divided into five groups with six animals in each group as shown in the table 1.

Treatment	No. of rats	Study period
Group I - Normal control animals	(3 Male and 3 Female)	20 weeks
Group II - Animals with single intra-peritoneal injection of DEN 200mg/kg body weight followed by phenobarbital of 0.05% mixed with drinking water daily for 20 weeks.	(3 Male and 3 Female)	
Group III - Animals with single intra-peritoneal injection of DEN 200mg/kg body weight, followed by phenobarbital of 0.05% mixed with drinking water for 16 weeks and ethyl acetate extract of *R.tuberosa* 400mg/kg body weight daily for 4 weeks.	(3 Male and 3 Female)	
Group IV - Animals with single intra-peritoneal injection of DEN 200mg/kg body weight, followed by phenobarbital of 0.05% mixed with drinking water for 16 weeks and ethanolic extract of *R.tuberosa* 400mg/kg body weight daily for 4 weeks.	(3 Male and 3 Female)	
Group V - Animals with single intra-peritoneal injection of DEN 200mg/kg body weight, followed by phenobarbital of 0.05% mixed with drinking water daily for 16 weeks and standard doxorubicin drug 5mg/kg body weight of one dose per week for 4 successive weeks.	(3 Male and 3 Female)	
Total number of rats	**30**	

2.4. Tumour Induction and Drug Treatment: The experimental rats were fasted overnight and induced by a single intraperitonial injection of DEN at a dose of 200mg/kg body weight in saline to induce liver cancer. DEN was dissolved in freshly prepared saline (0.9%). The control rats were similarly injected with saline. Neither death nor any other aggressive effect was observed. Two weeks after the administration of DEN, phenobarbital at a concentration of 0.05% was incorporated into drinking water for about 14 successive weeks to promote the liver cancer.[16, 17] The changes in body weight in all groups of rats were recorded at regular intervals (every week). After the 16th week the animals with liver cancer was confirmed by testing the level α-fetoprotein measured quantitatively by solid phase enzyme linked immunosorbent assay and γ-glutamyl transferase in serum. The plant extract of 400mg/kg /body weight of ethyl acetate extract from *R.tuberosa* whole plant for (group III) animals and 400mg/kg /body weight of ethanolic extract from *R.tuberosa* whole plant for (group IV) animals were treated for about 4 weeks, the (group V) animals were treated with standard

doxorubicin 5mg/kg body weight of one dose per week for 4 successive weeks. After the end of the drug treatment the animals were fasted overnight and sacrificed by cervical dislocation. The blood/serum sample was collected with and without anticoagulant. The serum was separated and stored at -20ºC.

2.5. Collection of Blood Sample: At the end of the experimental period, the body weight of each rat was taken before sacrifice. The abdominal cavity of rats was dissected immediately after decapitation and the liver, kidney, brain, spleen and heart were rapidly removed, washed by ice-cold saline, weighed and blotted dry.

2.6. Collection of Blood Sample for Hematology: At the end of the experimental period, the animals were fasted overnight and anaesthetized with ether. Blood was collected from retro orbital vein in one tube with anticoagulant EDTA which was used for hematological studies. Enumeration of White Blood Corpuscles was counted in a hemocytometer in the Neubaur chamber according to the method of Chesbrough, (1972).[18] Differential counts of White Blood Corpuscles (Lymphocytes, Monocytes and Neutrophil) were done by the method of Leishman (1901).[19] Enumeration of Red Blood Corpuscles was done by standard method of Chesbrough, (1972).[18] Estimation of Haemoglobin (Hb) was measured by the method of Drabkin, (1932).[20] Determination of Mean Corpuscular Volume (MCV), Determination of Mean Corpuscular Haemoglobin (MCH), Determination of Mean corpuscular haemoglobin concentration (MCHC), Determination of RDW by using automatic hemocyte analyzer BC-2800Vet instrument.

2.7. Tissue Processing for Histopathological Studies: The organs like liver, kidney, brain, spleen and heart were rapidly dissected out. They were washed and dehydrated. The cleaned tissues from the control group and treated group were embedded with molten paraffin at 58ºC. Tissues were fixed in 10% formal saline, processed routinely embedded in paraffin and consecutive sections were taken at 7µ thickness using a razor blade and then stained with ordinary haematoxylin and eosin. The changes in the liver, kidney, brain, spleen and heart tissues were observed under light microscope.[21, 22]

2.8. Statistical Analysis: Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used to compare the means of different groups by using SPSS version 16.0 software (Chicago, USA), comparisons were made between DEN induced cancer group animals with other group animals. $P<0.05$ was

considered as an indicator for the significant difference between study groups. All the results were expressed as mean ± S.D for six rats in each group.

3. RESULTS AND DISCUSSION

Table 2: Effect of extract of *R.tuberosa* on initial and final stage body weight of control and experimental animals.

Groups	Body weight in gm	
	Initial stage	Final stage
Group I	250 ± 15	300 ± 20[-]
Group II DEN induced cancer	220 ± 12	200 ± 7 [c]
Group III Ethyl acetate extract	170 ± 10	280 ± 15 [c]
Group IV Ethanolic extract	190 ± 7	290 ± 17 [c]
Group V Doxorubicin	200 ± 14	295 ± 90 [c]

Each value is expressed as mean ± S.D, for six rats in each group. The body weight expressed as gm. Group I- control animals, Group II-cancer bearing animals, Group III-ethyl acetate extract 400mg/kg of body weight post treated, Group IV- ethanolic extract 400mg/kg of body weight post treated and Group V- standard Doxorubicin treated animals. Statistical significance (a = $p < 0.05$, b = < 0.01, c = $p < 0.001$) and non significant (NS), when Group II compared with Group I, when Group III, IV and V compared with Group II.

Body weight of animals was measured every week for 20 weeks before and after treatment. In general observation food and water consumption decreased after DEN treatment as evident by sudden reduction in body weight confirming the possible toxic response to hepatocarcinogen treated rats. But there was statistical difference in body and liver weights among the control and extract treated groups. Table 2 shows weight of DEN induced HCC (group II), ethyl acetate extract (group III), ethanolic extract (group IV) and standard doxorubicin (group V) treated animals. Decreased weight was due to reduced intake of food and water. The body weight of ethyl acetate extract *R.tuberosa* treated (group III) animals are significantly increased than ethanolic extract of *R.tuberosa* treated (group IV) animals. These changes in body weight in *R.tuberosa* extract treated (group III and IV) animals may be due to the improvement in metabolic activity of the system to reverse the cancerous condition to normal.

Table 3: Effect of extract of *R.tuberosa* on organ weight of control and experimental animals.

Parameters	Group I	Group II	Group III	Group IV	Group V
Liver	7.70 ± 0.3	10.50 ± 1.1 ^{c}	9.50 ± 0.7 ^{NS}	7.90 ± 0.5 ^{c}	7.80 ± 0.6 ^{c}
Kidney	1.61 ± 0.09	1.50 ± 0.07 ^{a}	1.59 ± 0.08 ^{NS}	1.55 ± 0.01 ^{NS}	1.57 ± 0.03 ^{a}
Brain	1.80 ± 0.08	1.70 ± 0.09 ^{NS}	1.70 ± 0.03 ^{NS}	1.63 ± 0.02 ^{NS}	1.75 ± 0.07 ^{a}
Spleen	0.61 ± 0.02	0.50 ± 0.03 ^{c}	0.58 ± 0.02 ^{c}	0.59 ± 0.03 ^{c}	0.60 ± 0.04 ^{c}
Heart	0.82 ± 0.03	0.80 ± 0.02 ^{NS}	0.80 ± 0.03 ^{NS}	0.80 ± 0.04 ^{NS}	0.80 ± 0.05 ^{a}

Each value is expressed as mean ± S.D, for six rats in each group. The organ weight expressed in gm. Group I- control animals, Group II- cancer bearing animals, Group III- ethyl acetate extract 400mg/kg of body weight post treated, Group IV- ethanolic extract 400mg/kg of body weight post treated and Group V- standard Doxorubicin treated animals. Statistical significance (a = $p < 0.05$, b = < 0.01, c = $p < 0.001$) and non significant (NS), when Group II compared with Group I, when Group III, IV and V compared with Group II.

The table 3 shows that the weight of liver is significantly increased ($p < 0.001$), the weight of kidney is significantly decreased ($p < 0.05$), weight of spleen is significantly decreased ($p < 0.001$), but the weight of brain and heart are not significant in DEN induced HCC (group II) animals when compared with (group I) normal animals. The weight of spleen is significantly increased ($p < 0.001$) with ethyl acetate extract *R.tuberosa* treatment but the weight of liver, kidney, brain and heart are not significant in (group III) animals when compared with (group II) animals. The weight of liver is significantly decreased ($p < 0.001$) but the weight of kidney, brain and heart are not significant with ethanolic extract *R.tuberosa* treatment (group IV) animals when compared with (group II) animals. After administration of ethanolic extract of *R.tuberosa* the weight of liver and spleen are reversed back to normal, like that of standard anticancer drug doxorubicin. This indicates that ethanolic extracts of *R.tuberosa* are more potential in reducing the HCC caused by DEN.

Table 4: Effect of extract of *R.tuberosa* on hematological parameters of control and experimental animals.

Parameters	Group I	Group II	Group III	Group IV	Group V
WBC	16.98 ± 1.1	21.1 ± 1.5 ^{c}	19.7 ± 0.7 ^{NS}	17.5 ± 1.3 ^{c}	17.1 ± 1.1 ^{c}
Lymphocyte	71.0 ± 2	50.5 ± 4 ^{c}	54 ± 1.5 ^{NS}	58 ± 2.5 ^{c}	61.1 ± 3.0 ^{c}
Monocyte	1.0 ± 0.1	0.9 ± 0.07 ^{NS}	0.81 ± 0.01 ^{a}	1.88 ± 0.02 ^{c}	1.88 ± 0.03 ^{c}
Neutrophil	28.0 ± 1	25 ± 2 ^{NS}	27 ± 1.5 ^{NS}	27.5 ± 1.8 ^{a}	27.9 ± 1.9 ^{a}

Each value is expressed as mean ± S.D, for six rats in each group. The WBC $x10^3$ cells/dL, lymphocyte, monocyte and neutrophil are in %. Group I- control animals, Group II- cancer bearing animals, Group III- ethyl acetate extract 400mg/kg of body weight post treated, Group IV- ethanolic extract 400mg/kg of body weight post treated and Group V- standard Doxorubicin treated animals. Statistical significance (a = $p < 0.05$, b = < 0.01, c = $p < 0.001$) and non significant (NS), when Group II compared with Group I, when Group III, IV and V compared with Group II.

The table 4 shows the biochemical change in blood WBC, lymphocyte, monocyte and neutrophil of control and experimental groups of animals. The level of WBC count are significantly ($p < 0.001$) increased, lymphocyte % are significantly ($p < 0.001$) reduced but the reduction of monocyte % and neutrophil % are not significant in DEN induced HCC (group II) animals when compared with (group I) normal animals. This result may be due to the damage caused by DEN in the lymphocyte along with lack of immune response against cancer cells. The criteria for good anticancer drug is to reduce the lymphocyte count. On treatment of ethyl acetate extract from *R.tuberosa* the level of WBC count, lymphocyte % and neutrophil % are reduced in (group III). On treatment of ethanolic extract of *R.tuberosa* the level of WBC count are significantly ($p < 0.001$) reduced, monocyte % are significantly ($p < 0.001$) increased, neutrophil % were significantly ($p < 0.05$) decreased but lymphocyte % were significant in (group IV) animals. White Blood Cells or leukocytes play main role in immune responses. These cells carry out the many tasks required to protect the body against disease-causing microbes and abnormal cells. Some types of leukocytes in circulation guard the diseased and damaged dead cells from the foreign invaders. These white blood cells provide a general nonspecific-level of immune protection.[23, 24] Doxorubicin induces genetic damage in human lymphocytes.[25] On treatment with ethanolic extracts of *R.tuberosa* WBC count (reduced), monocyte % (increased) and neutrophil % (increased) nearly to the standard (doxorubicin) anticancer drug. It indicates that *R.tuberosa* could improve immunity function and decrease the inflammation in DEN induced HCC rats. In the present study ethanolic extracts was highly significant than ethyl acetate extract from *R.tuberosa*.

Table 5: Effect of extract of *R.tuberosa* on hematological parameters of control and experimental animals.

Parameters	Group I	Group II	Group III	Group IV	Group V
RBC	6.29 ± 0.3	4.2 ± 0.1 ^{c}	4.9 ± 0.2 ^{c}	5.8 ± 0.2 ^{c}	6.0 ± 0.3 ^{c}
Hb	14.1 ± 1	6.8 ± 0.2 ^{c}	9.7 ± 0.6 ^{c}	11.5 ± 0.1 ^{c}	12.5 ± 0.9 ^{c}
HCT	30.14 ± 1	25 ± 1.7 ^{c}	28 ± 1.5 ^{NS}	29.1 ± 1.1 ^{c}	29 ± 1.0 ^{c}
MCV	41.4 ± 2	40 ± 3 ^{NS}	40 ± 2.1 ^{NS}	39 ± 1.5 ^{c}	40 ± 1.2 ^{NS}
MCH	21.53 ± 1	16 ± 0.7 ^{c}	18 ± 1.2 ^{a}	20 ± 1.4 ^{a}	20.5 ± 1.5 ^{c}
MCHC	43.9 ± 1.7	28 ± 0.7 ^{c}	32 ± 2.5 ^{NS}	36 ± 1.7 ^{c}	38 ± 1.5 ^{c}
RDW	14.35 ± 1.2	12 ± 0.9 ^{c}	13 ± 1.1 ^{a}	14 ± 1.5 ^{c}	14 ± 1.2 ^{c}

Each value is expressed as mean ± S.D, for six rats in each group. The RBC x10^{6} cells/dL, Hb g/dL, HCT %, MCV fL, MCH pg, MCHC g/dL and RDW % expressed as gm. Group I- control animals, Group II- cancer bearing animals, Group III- ethyl acetate extract 400mg/kg of body weight post treated, Group IV- ethanolic extract 400mg/kg of body weight post treated and Group V- standard Doxorubicin treated animals. Statistical significance (a = $p < 0.05$, b = < 0.01, c = $p < 0.001$) and non significant (NS), when Group II compared with Group I, when Group III, IV and V compared with Group II.

In cancer therapy major problem encounted are myelo suppression and anaemia due to the excess hemolysis.[26] In the present study the table 5 shows that the levels of RBC along with Hb, HCT, MCH, MCHC and RDW are significantly decreased ($p < 0.001$) in DEN induced HCC of group II animals when compared with group I normal animals. This significant decrease may be due to excess hemolysis which confirms that the anaemic condition was present in DEN induced HCC animals. On treatment with ethyl acetate extract of *R.tuberosa* the level of RBC along with Hb is highly significant ($p < 0.001$) and the level of MCH along with RDW are significant ($p < 0.05$), but the level of HCT, MCV and MCHC is not significant in group III animals. On treatment with ethanolic extract of *R.tuberosa* the level of RBC along with Hb, HCT and MCHC are highly significant ($p < 0.001$), the level of MCH and RDW is significant ($p < 0.05$), but the level of MCV is significantly reduced ($p < 0.001$). This significant change in RBC along with Hb, HCT, MCHC and RDW are near to that of the standard anticancer doxorubicin drug. It indicates that extracts of *R.tuberosa* are capable of protecting the hemopoietic system.

Histopathological Examinations (plate 1 to 5): The plate 1(a) shows the section of liver tissue with normal architecture from (group I) normal animals. The plate 1(b) shows that the sections of liver tissue with parenchyma with extensive fatty changes as indicated by clear

white cytoplasm as against pink eosinophilic cytoplasm. Hepatocytomegaly with multifocal clear cell areas with foci of necrosis were seen in liver. It also shows dysplastia and neoplastic, pleomorphic hepatocyte with nuclear enlargements, condensed chromatin, increased eosinophilia of the cytoplasm, in collectively hepatocytomegaly as detected in (group II) DEN induced HCC animals. The plate 1(c) shows that the section of liver tissue with almost uniform appearing hepatocytes with regular vesicular nuclei for (group III) ethyl acetate extract of *R.tuberosa* (400mg/kg body weight) treated animals. The plate 1(d) shows that the sections of liver tissue are normal from (group IV) ethanolic extract of *R.tuberosa* (400mg/kg body weight) treated animals. The plate 1(e) shows that the section of liver tissue as normal architecture for (group V) animals. Histopathological examinations and sections of kidney in plate 2(a-e), brain in plate 3(a-e), heart in plate 4(a-e), spleen in plate 5 for (group I), (group II), (group III), (group IV) and (group V) shows normal architecture and no abnormality was detected.

Plate 1(a-e): Histopathological sections of Liver from the control and experimental animals.

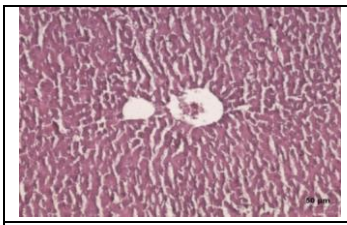	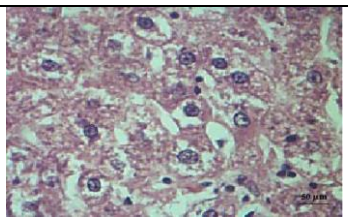	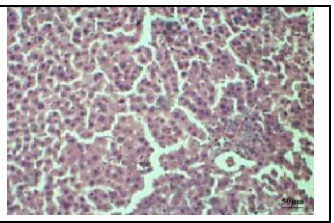
Plate 1.a: Group I	**Plate 1.b: Group II**	**Plate 1.c: Group III**
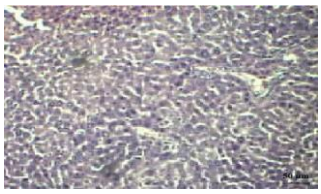	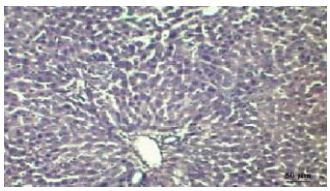	
Plate 1.d: Group IV	**Plate 1.e: Group V**	

1. Group I-Normal architecture is seen in the section of liver tissue of control animals.
2. Group II-The section of liver tissue shows parenchyma with extensive fatty changes as indicated by clear white cytoplasm as against pink eosinophilic cytoplasm. Hepatocytomegaly with multifocal clear cell areas with foci of necrosis were seen. It also shows dysplastia and neoplastic, pleomorphic hepatocyte with nuclear enlargements, condensed chromatin, increased eosinophilia of the cytoplasm, in collectively hepatocytomegaly is detected.

3. Group III-The section of liver tissue shows almost uniform appearing hepatocytes with regular vesicular nuclei.
4. Group IV-The section of liver tissue are normal.
5. Group V- The section of liver tissue shows normal architecture and no abnormalities were observed.

Plate 2(a-e): Histopathological sections of Kidney from the control and experimental animals stained with hematoxylin and eosin.

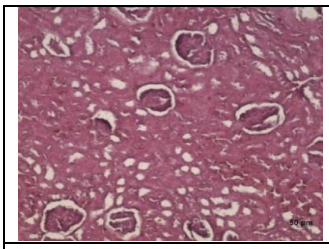	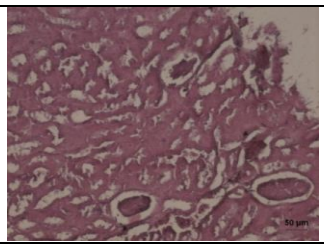	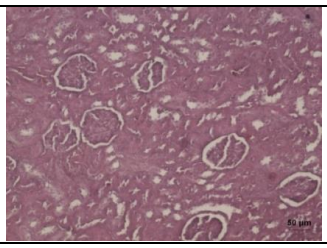
Plate 2.a: Group I	**Plate 2.b: Group II**	**Plate 2.c: Group III**
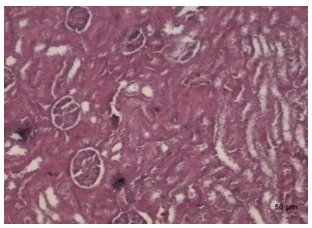	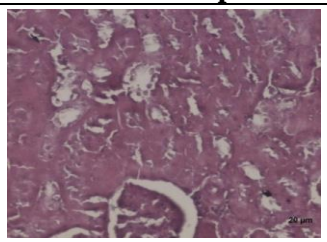	
Plate 2.d: Group IV	**Plate 2.e: Group V**	

Plate 3(a-e): Histopathological sections of Brain from the control and experimental animals stained with hematoxylin and eosin.

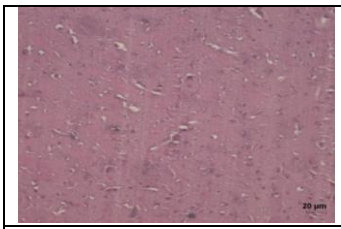	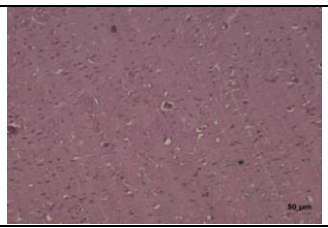	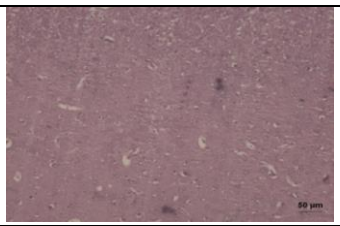
Plate 3.a: Group I	**Plate 3.b: Group II**	**Plate 3.c: Group III**
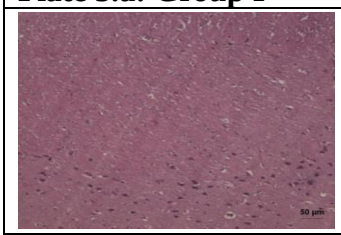	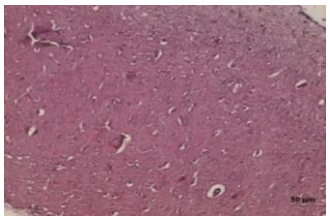	
Plate 3.d: Group IV	**Plate 3.e: Group V**	

Plate 4(a-e): Histopathological sections of Heart from the control and experimental animals stained with hematoxylin and eosin.

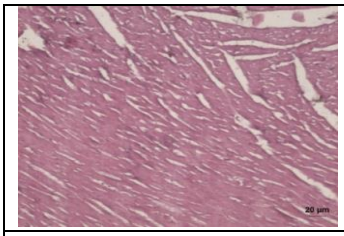	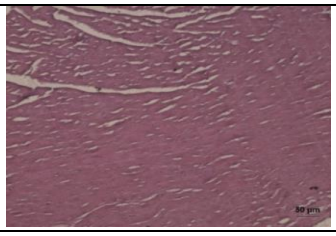	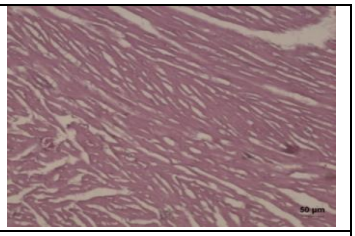
Plate 4.a: Group I	**Plate 4.b: Group II**	**Plate 4.c: Group III**
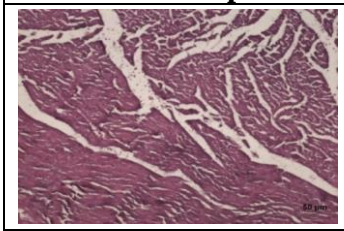	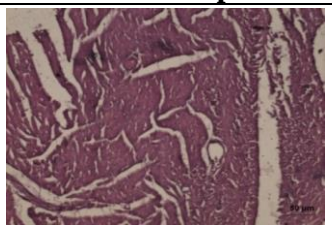	
Plate 4.d: Group IV	**Plate 4.e: Group V**	

Plate 5(a-e): Histopathological sections of Spleen from the control and experimental animals stained with hematoxylin and eosin.

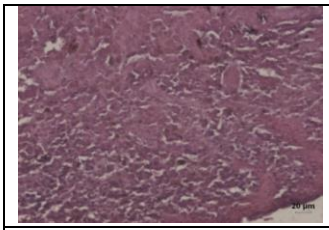	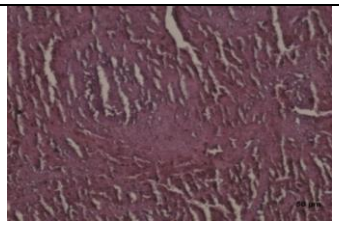	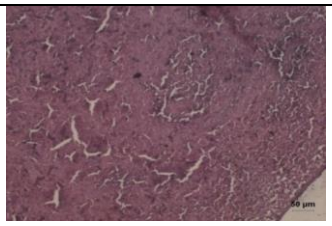
Plate 5.a: Group I	**Plate 5.b: Group II**	**Plate 5.c: Group III**
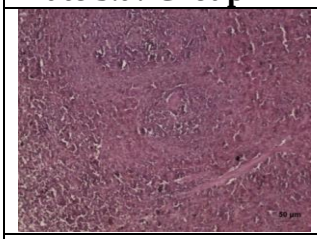	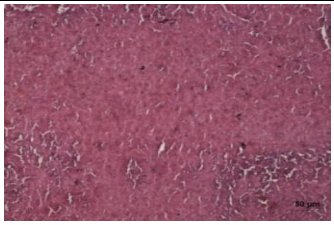	
Plate 5.d: Group IV	**Plate 5.e: Group V**	

1. Group I-Normal architecture is seen in the section of kidney, brain, heart and spleen tissue of control animals.
2. Group II- No abnormality was detected in DEN induced cancer bearing animals.
3. Group III- No abnormality was detected in ethyl acetate extract of *R.tuberosa* (400mg/Kg body weight) treated animals.
4. Group IV- No abnormality was detected in ethanolic extract of *R.tuberosa* (400mg/Kg body weight) treated animals.
5. Group V- No abnormality was detected in standard Doxorubicin treated animals.

4. SUMMARY AND CONCLUSION

Many of infectious diseases are known to be treated with herbal remedies throughout the history of mankind: even today plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. The discovery of medicinal plants in different parts of the world is important for medical sector. DEN induced HCC bearing animals showed significant decrease in body weight and increase in liver weight; these changes were reverted to near normal by treatment with ethanolic extract of *R.tuberosa*. This may be due to the improvement in metabolic activity of the system. It indicates that *R.tuberosa* has the potential chemopreventive property which in turn reduces the hepatocellular carcinoma and liver damage caused by DEN. The levels of WBC and RBC counts were effectively restored to the normal level on treatment with *R tuberosa* and thereby capable of protecting the hemopoietic system. It indicated that the *R.tuberosa* effectively blocks the secondary malignancy and thereby increases the life span and reduces the tumor growth. These changes were reverted to near normal with treatment of ethanolic extract of *R.tuberosa* and thereby liver cirrhosis was reduced and immune system was protected along with lipid peroxidation. This study has shown that the ethanolic extract of *R.tuberosa* is found to be safe in experimental wistar albino rats up to a dose of 400 mg/kg. It has shown hepatoprotective activity against DEN induced liver damage. However further studies are required to isolate the active compounds from the ethanolic extract of *R.tuberosa* to confirm their properties for safe, efficacious, cost effective and eco-friendly drug in future.

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