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IN-VIVO ANTICANCER ACTIVITY OF *TABEBUIA ROSEA* (BERTOL) DC. LEAVES ON DALTON'S ASCETIC LYMPHOMA IN MICE

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ABSTRACT

The purpose of this study was to investigate the experimentally possible antitumor effect and antioxidant role of methanolic extract of *Tabebuia rosea* leaves against Dalton's ascetic lymphoma (DAL) induced Swiss albino mice. The methanolic extract of *Tabebuia rosea* (METR) was administered at the doses of 100mg/kg, 200mg/kg in mice for 14 days after 24hours of DAL inoculation. The effects of METR on the growth of bodyweight, MST, viable and nonviable cell count, Tumor volume, Packed Cell Volume (PCV), life span of DAL bearing mice were studied. Hematological profile and liver biochemical parameters (lipid per oxidation, antioxidant enzymes) were also estimated. Treatment with METR decreased the tumor volume and viable cell count thereby increasing the lifespan of DAL bearing mice. Alteration in the Hematological parameters, protein, PCV occurred because of DLA inoculation, were almost restored in METR treated groups. The effect of METR also decreases the levels of lipid peroxidation and increases the levels of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). The histopathological results showed the loss of liver hepatocytes in DLA bearing mice. However, a mouse treated with METR extract improved the liver and rearranged more or less normal architecture. The present work revealed that the METR exhibits significant antitumor and anti-oxidant activity in vivo. These results support the traditional use of *Tabebuia rosea* and the alcoholic seed extract is a potent source of anticancer compounds that could be utilized pharmaceutically.

INTRODUCTION: Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell [1]. Lymphoma is a disease of the lymphocytes (a type of white blood cell involved in immune responses) and the lymphatic system, which includes the spleen, thymus, and liver, as well as other lymphatic tissues. Dalton's Ascites Lymphoma is transplantable, poorly differentiated malignant tumor which appeared originally as lymphocytes in a mouse. It grows in both solid and ascitic form [2].

Cancer is one of the ailments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumor are not totally free from side effects [3]. This fostered our attempts to evaluate some plant products against cancer, as they are less likely to cause serious side effects.

Many Indian plants like black pepper, asafoetida, pippali and garlic are quoted to be useful indifferent types of cancer [4, 5]. One such plant is *Tabebuia rosea*, belonging to the family Bigoniaceae.

Tabebuia rosea (Bertol.) DC. Commonly known as "Pink Trumpet Tree" can grow up to 15 meter and well known for its beautiful flowers. The timber is widely used for general construction and carpentry in many European countries. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, anti tumoural and antimicrobial agents [6].

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infection and degenerative diseases. *Tabebuia rosea* have been indicated for the treatment of several diseases, one among which is cancer. The present study was carried out to evaluate the anti tumor activity and antioxidant status of METR against DAL bearing mice.

MATERIALS AND METHODS:

Plant materials: The leaves of *Tabebuia rosea* was collected from surroundings of Seshachalam hill ranges of Triumala, Tripathi, Andhra Pradesh, India in the month of August. The plant material was identified and authenticated by Dr. Madhava Chetty, (Botanist), S.V. University, Tripathi [7] and a copy has been preserved for the future reference at the herbarium of the institute TRR College of Pharmacy (1447/PO/a/11/CPCSEA).

Preparation of the Plant Extract: The leaves of these plants were dried under shade at room temperature (27-30°C) for 15-30 days, after which the leaves of the plant were chopped and grounded into coarse powder. The powdered material (2 kg) was defatted with petroleum ether (60-80°C) in a soxhlet extraction apparatus and marc was extracted with methanol (1000 mL). Overnight, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. The crude extract was dissolved in distilled water to required concentrations and used for the experiments.

Chemicals and Reagents: Chloro-2-4-nitrobenzene (CDNB), thiobarbituric acid, 5'5 Dithio–bis-2-nitrobenzoic acid (DTNB), Nitro blue tetrazolium chloride (NBT), Trypan blue and other solvent and/or reagent obtained was used as received. The DAL cells were obtained from Amala cancer research center, Thrissur, Kerela, India. The DAL cells were maintained by intraperitoneal inoculations of $1x10^6$cells/mouse. Studies were carried out using Female Swiss albino mice weighing 20±5g. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee (IAEC).

ANIMALS: Female Swiss albino mice weighing between 18-22g were used for the present study, they were maintained under standard environmental conditions and were fed with standard laboratory diet and water *Ad libitum.*

Treatment schedule: Tumor was induced by injecting 0.2mL of $1x10^6$ mL^{-1} of DAL into peritoneal cavity of mice. The animals were divided into 5 groups (N=6). All the groups were injected with DAL cells ($1x10^6$ cells/mouse) intraperitoneally except normal group. This was taken as day 0. On the 1st day Normal saline (0.9%w/vNaCl, 5mL/kg/mouse/day) administered into normal group (Group 1) and DAL control mice (Group 2). The different doses of methanol extract of *Tabebuia rosea* (100 and 200mg/kg/day/mouse)and standard drug 5-fluorouracil (20mg/kg/i.p) were administered in groups 3, 4 and 5 respectively for 14 days intra-peritoneally. On 15th day, after the last dose and 18h fasting 6 mice from each group were sacrificed for the study of antitumor activity, Hematological and antioxidant enzyme estimation and rest of the animals of each group were kept to check the mean survival time (MST) and percentage increase in lifespan (%ILS) of the tumor bearing mice.

Tumor Growth Response: Antitumor effect of METR was assessed by observation of changes with respect to body weight, ascetic's tumor volume, packed cell volume, viable and non viable tumor cell count.MST and %ILS were also calculated. Transplantable tumor was carefully collected with the help of a sterile 3mL syringe, measured the tumor volume and the ascetic fluid was withdrawn in a graduated centrifuge tube, viable and non viable cell count of ascetic cell were stained by the tryphan blue (0.4%) in normal saline dye exclusion test and count was determined in Neubauer counting chamber.

The effect of METR on tumor growth was monitored daily by recording the mortality and %ILS was calculated using following formula

%ILS =

$$\frac{\text{(Mean survival of treated group – Mean survival of control group)}}{\text{Mean survival of control group.}}$$

Hematological Studies: Blood was collected from the Retro orbital plexus, and drawn into RBC or WBC pippetes, diluted and counted in a Neubauer counting chamber Sahli's Hemoglobin meter determined of hemoglobin concentration. Hemoglobin content [8] RBC, WBC [9] count were estimated from the peripheral blood of nomal, DAL control and extract treated groups.

Biochemical Assays: The liver was excised, rinsed in ice cold normal saline followed by cold 0.15M Tris HCl(pH 7.4) plotted and weighed. The homogenate was processed for estimation of lipid peroxidation, GSH, SOD and CAT. Assay for microsomal lipid peroxidation was carried out by the measurement of Thiobarbituric acid reactive substances (TBARS) in the tissues (Okhawa *et al.,* 1979) [10] the pink chromogen produced by the reaction of malondialdehyde, which is a secondary product of lipid peroxidation a reaction with thio barbituric acid was estimated at 532nm.Reduced glutathione (GSH) in the tissue was assayed by the method of Ellaman [11].

GSH estimation is based on the development of yellow color when 5, 5' dithiobis(2- nitrobenzoic acid) dinitrobis benzoicacid was added to coumpounds containing sulphydryl group. SOD was assayed by the method of Kakkar *et al* (1984) [12]. The assay was based on the 50%inhibition of formation of NADH –phenazine methosulphate nitroblue tetrazolium at 520nm. The activity of CAT was assayed by the method of Abei (1974) [13]. Proteins were estimated by method of Lowery *et al* (1951) [14] using bovine serum albumin as the standard.

Histopathological Analysis: A portion of the liver was cut into 2-3 pieces of approximately 3.8x 3.0x 1.5cms and fixed in 10% formaldehyde solution. After embedding in paraffin wax, thin sections of liver tissue were sliced and stained with hematoxylin–eosin. The thin sections of liver were made into permanent slides and examined under high resolution microscope with photographic facility and photomicrographs were taken.

Statistical Analysis: Total variation present in set of data was performed by using one way Analysis of variance (ANOVA) and the results are expressed as Mean±SEM.

TABLE 1: EFFECT OF METHANOL EXTRACT OF *TABEBUIA ROSEA* (METR) ON TUMOR VOLUME, VIABLE AND NON VIABLE TUMOR CELL COUNT OF DLA BEARING MICE.

Parameter	DAL control ($1x10^6$cells/mouse/mL)	METR (100mg kg^{-1}) + DAL	METR (200mg kg^{-1}) + DAL	Standard 5-FU (20mgkg^{-1}) + DAL
Body weight (g)	23.7 ± 0.473	22.3 ± 0.29	21.4 ± 0.30	15.2 ± 0.27
PCV (mL)	1.78 ± 0.058	1.46 ± 0.04	0.70 ± 0.44	0.40 ± 0.02
Tumor volume(mL)	4.95 ± 0.102	4.33 ± 0.127**	2.48 ± 0.135***	1.11 ± 0.94***
Viabletumor count (10^6cells /mL)	9.77 ± 0.17	4.58 ± 0.216**	4.03 ± 0.43**	2.28 ± 0.07***
Non Viable tumor count(10^6cells/mL)	0.84 ± 0.1	4.78 ± 0.197 **	3.68 ± 0.26**	5.75 ± 0.07***

Values are the mean±SEM. number of mice in each group (n=6). Experimental groups were compared with DAL control. (Weight of normal mice (20±5g) *.P<0.05, **P<0.01, ***P<0.001.

TABLE 2: EFFECT OF METHANOL EXTRACT OF *TABEBUIA ROSEA* (METR) ON MEAN SURVIVAL TIME AND %INCREASE IN LIFESPAN OF DLA BEARING MICE

Experiment	Mean survival time(days) Mean ± SEM	%Increase in lifespan (%ILS)
DALcontrol($1x10^6$cells/mouse/mL)	21.50 ± 0.43	-
METR (100mg kg^{-1}) + DAL	30.3 ±1.02**	41.08
METR (200mg kg^{-1}) + DAL	34.16 ± 1.92**	58.91
Standard5-FU (20mgkg^{-1}) + DAL	42.50 ± 0.76**	97.67

Values are the Mean±SEM. number of mice in each group (n=6), Experimental groups were compared with DAL control. **P<0.01

TABLE-3: EFFECT OF METHANOL EXTRACT OF *TABEBUIA ROSEA* (METR) ON HEMATOLOGICAL PARAMETERS OF DLA TREATED MICE

Parameter	Normal control	DAL control ($1x10^6$cells)	METR (100mg kg^{-1}) + DAL	METR (200mg kg^{-1}) + DAL	Standard 5-FU (20mg kg^{-1}) + DAL
Total RBC (cells/mL$x10^3$)	6.730 ± 0.07	5.855 ± 0.15***	6.67 ± 0.06***	6.63 ± 0.102***	7.55 ± 0.15***
Total WBC (cells/mL$x10^3$)	9.98 ± 1.30	14.25 ± 2.60*	12.55 ± 2.40**	12.10 ±1.92**	11.45 ± 1.90***
Hemoglobin(g%)	13.83 ± 0.07	12.05±0.514***	13.97±0.1054***	14.65±0.3354***	15.10±0.178***
Platelet count (Lakhs/cumm)	3.20 ± 0.74	1.65 ± 0.60*	2.30 ± 0.70**	2.42 ± 0.92**	2.68 ± 0.55***

Values are the mean±SEM. number of mice in each group (n=6), DAL control compared with normal group, Experimental groups was compared with DAL control.*P<0.05,**P<0.01,***P<0.001.

TABLE 4: EFFECT OF METHANOL EXTRACT OF *TABEBUIA ROSEA* (METR) ON ANTI OXIDANT PARAMETERS OF DLA TREATED MICE

Parameter	Normal control	DAL control ($1x10^6$cells)	METR (100mg kg^{-1}) + DAL	METR (200mg kg^{-1}) + DAL	Standard 5FU (20mg kg^{-1}) + DAL
LPX (nmoles MDA/gm of tissue)	0.1053 ± 0.0094	0.243 ± 0.036***	0.1127±0.0078***	0.082±0.0068***	0.149 ± 0.016**
GSH (mg/g of tissue)	0.3617±0.04012	0.1446 ± 0.021	0.344 ± 0.0424*	0.3842 ± 0.1090*	0.4518±0.1207*
SOD (unit/mg protein)	0.447 ± 0.049	0.3408 ± 0.089	0.769 ± 0.122**	0.636 ± 0.0667*	0.0668±0.0415*
CAT (units/mg tissues)	0.8406 ± 0.049	0.6305±0.0887***	0.7658 ± 0.099***	0.668±0.0817***	1.38 ± 0.1117**

Values are the mean±SEM. number of mice in each group (n=6), DAL control compared with normal group. Experimental groups were compared with DAL control. .*P<0.05, **P<0.01,***P<0.001.

RESULTS: The present investigation indicates that the METR showed significant anti-tumor and anti oxidant activity in DAL bearing mice. The effects of METR (100 and 200mgkg^{-1}) at different doses on tumor volume, viable and non viable cell count, survival time and %ILS were shown in **Table 1 and 2**. Administration of METR reduces the tumor volume, packed cell volume and viable tumor cell count in a dose dependent manner when compared to DAL control mice. In DAL control mice the mean survival time was 21.50 ± 0.43 days. Whereas, it was significantly increased mean survival time (30.3±1.02, 34.16±1.92 and 42.50±0.76 days)with different doses (100 and 200 mgkg^{-1}) of METR and standard drug (42.50±0.76) respectively.

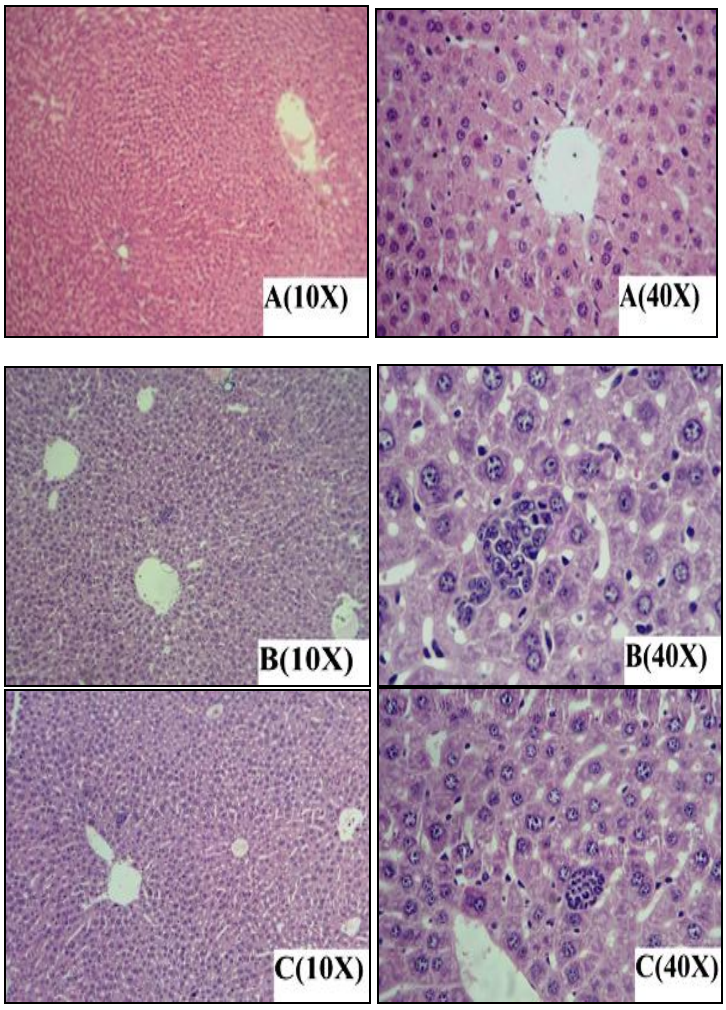
As shown in **Table 3,** the hemoglobin content in the DAL control mice (12.05 g%) was significantly decreased when compared with normal mice (13.83 g%) METR at the dose of (100 and 200 mgkg^{-1}) the hemoglobin content in DAL treated mice were increased to 13.97±0.105 and 14.65±0.335 g% moderates changes in the RBC count for also observed in extract treated mice. The total WBC counts were significantly higher in DAL treated mice when compared with normal mice. Whereas, METR treated mice significantly reduced the WBC count as compared to that of control mice.

The levels of LPX, GSH, SOD and catalase were summarized in **Table 4**, the levels of lipid per oxidation in liver tissue significantly increased in DAL control mice (0.24 n moles MDA/g of tissue) as compared to the normal mice (0.10 n moles MDA/g of tissue) Treatment with METR (100 and 200 mgkg^{-1}) were significantly decreased the LPX levels 0.112 and 0.082 n moles MDA/g of tissue in dose dependent manner.

The GSH count in liver tissues of normal mice was found to be 0.3617 mgkg^{-1} of wet tissue. Inoculation of DAL drastically decreased the GSH content to 0.14 mg kg^{-1} of wet tissue. Whereas treatment with different doses of METR, the GSH were reversed to normal level (0.344 and 0.384 mg kg^{-1} wet tissue) respectively. SOD level in the lower of DAL bearing mice was significantly decreased 3.408 units/mg proteins when compared with normal mice (0.44 units/mg proteins). Administration of the METR significantly increased the SOD levels (0.769 and 0.636 units /mg proteins in tissues) at the doses of 100 and 200 mgkg^{-1} respectively.

The CAT level was decreased in DAL control mice (0.6305 unit/mg proteins) when compared with normal mice 0.8406 units/mg protein in tissues) treatment with METR at the doses (100 and 200 $mgkg^{-1}$) brought back to normal level (0.7685 and 0.668 unit/mg proteins in tissues).

Fig. 1 shows the Histopathological observation of liver section of control and experimental animals. Control animals showed normal lobular architecture. The portal tract shows normal morphology without any inflammation or fibrosis. The hepatocytes are unremarkable. The central vein and the sinusoids are normal. There is no lobular inflammation, granuloma or fibrosis. Whereas DLA induced mice showed few foci of atypical cells with mitotic figures seen in the liver parenchyma and perivenular region. The hepatocytes show some degenerative changes. However mice treated with METR showed almost normal lobular architecture. Mice treated with 5-fluorouracilshows no inflammation or fibrosis. The liver parenchyma show many foci of collection of cells among the hepatocytes.



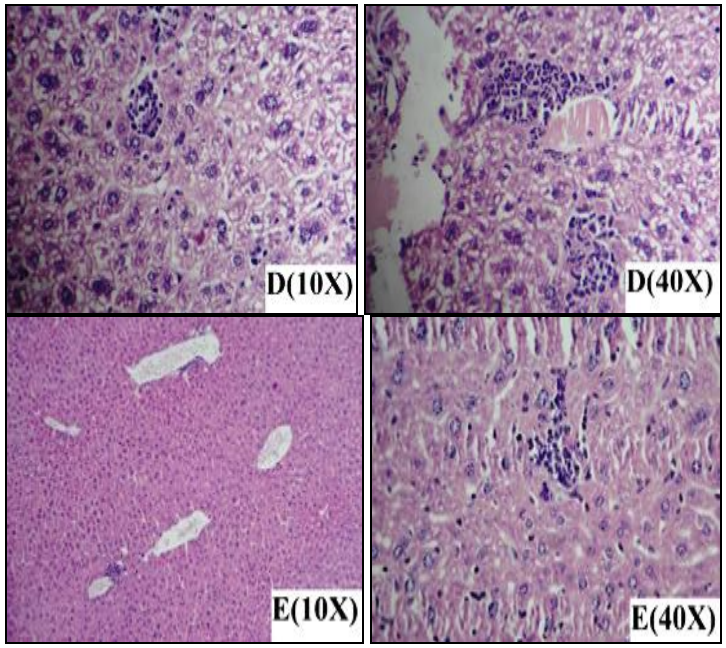


FIGURE 1: (A) HISTOLOGY OF CONTROL MICE SHOWS THE NORMAL LIVER WITH INTACT LOBULAR ARCHITECTURE, NORMAL CENTRAL VEIN AND SINUSOIDS. (B) DLA CONTROL MICE SHOWS LIVERPARENCHYMA WITH SMALL FOCUS OF ATYPICAL MALIGNANT CELLS WITH MITOSIS. (C), (D) TREATMENT GROUPS SHOWS PERIVENULAR COLLECTION OF ATYPICAL CELLS AND POLYMORPHS. (E) 5-FU TREATED MICE LIVER PARENCHYMA SHOWED COLLECTION OF CELLS (POLYMORPHS/CANCER CELL LINES).

DISCUSSION: The present study was carried out by to evaluate the effect of METR and DAL bearing mice. The METR showed anti tumor activity against the tumor. The reliable criteria for judging the value of any anti cancer drug are the prolongation of life span of animals [15] . A reduction in the number of ascetic tumor cells may indicate either an effect of METR on peritoneal macrophages or other components of the immune system [16], therefore increasing their capacity of killing the tumor cells, or a direct effect on the tumor cell growth. METR inhibiting significantly the tumor volume, viable cell count and enhancement in survival time of DAL bearing mice and there by acts as anti-neoplastic agent.

Myelosupression is a frequent and major complication of cancer chemotherapy. METR treated and subsequent tumor inhibition resulted in appreciable improvements in hemoglobin content, RBC and WBC counts (Table 3). These observations assume great significance, as anemia is common complication in cancer and the situation aggravates further during chemotherapy since a majority of antineoplastic agents exert suppressive effects on erythropoiesis [17, 18] and

thereby limiting the use of these drugs. the improvement in the hematological profile of the tumor bearing mice following the treatment with the extract could be due to the action of the different phytoconstituents present in the extracts .

Lipid per oxidation mediated by free radicals considered being a primary mechanism of cell membrane destructions and cell damage. The oxidation of unsaturated fatty acids in biological membrane leads to a reduction in membrane fluidity and disruption of membrane structure and function [19]. MDA the end point of lipid per oxidation was also reported to be higher in carcinomatous tissue than in non diseased organs [20]. Increase in the level of TBARS indicated enhanced lipid per oxidation leading to tissue injury and a failure of the anti oxidants defense mechanisms to prevent the formation of excess free radicals.

The active role of GSH against cellular lipid per oxidation has been well recognized and thereby reduces the glutathione (GSH) activity. GSH can act either Detoxify activate oxygen species such as H_2O_2. or reduce lipid per oxides themselves. In the present study, it is indicated that METR significantly reduced the elevated levels of lipid per oxidation and increased the level of glutathione (GSH) content and thereby it may act as antitumor agent.

On the other hand, SOD is a ubiquitous chain breaking oxidant and is found in all aerobic Organisms. It is a metelloprotein widely distributed in all cells and place an important protective role against ROS-induced oxidative damage. The free radicals scavenging system catalase, which are present in all major organs in the body of animals and human beings and is especially concentrated in liver and erythrocytes. Both enzymes play an important role in the elimination of ROS derived from the redox process of xenobiotic in liver tissues [21, 22]. It was suggested that catalase and SOD are easily inactivated by lipid per oxides or ROS [23]. In correlation, it has been reported that DAL bearing mice showed decreased levels of SOD activity and this may be due to loss of Mn^{++} SOD activity in, liver [24]. Inhibition of catalase activity in tumor cell line was also reported [25].

In this study, catalase and SOD were appreciably elevated by administration of METR, suggesting that it can restore the levels of SOD and catalase enzymes.

Histopathological assessment of different liver segments of the control and experimental animals by light microscope has been examined. Liver section of cell line (DLA) induced mice showed structural alteration in nucleus. The major alteration was damaged central vein, degenerative changes in hepatocytes, Atypical cells seen in liver parenchyma and perivenular region. This damage is partially reversed by the *Tabebuia rosea* leaves extract treatment.

CONCLUSION: The present study demonstrated that METR increased the life span of DAL tumor bearing mice and decreased the lipid per oxidation and thereby augmented the endogenous anti oxidant enzymes in the liver. The above parameters are responsible for the anti tumor and anti oxidant activities of *Tabebuia rosea.* Further research work is recommended**.**

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