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In-vivo anticancer effect of *Andrographis paniculate* ethanolic extract against nitrobenzene-induced hepatocarcinogenesis in albino rats

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Abstract
Andrographis paniculata plant extract is known to possess a variety of pharmacological activities. Andrographolide, the major constituent of the extract is implicated towards its pharmacological activity. We studied the cellular processes and targets modulated by andrographolide treatment in albino rats. Andrographolide treatment inhibited the *in vitro* proliferation of different tumour cell lines, representing various types of cancers. The compound exerts direct anticancer activity on cancer cells by cell-cycle arrest at the G0/G1 phase through induction of cell-cycle inhibitory protein p27 and decreased expression of cyclin-dependent kinase 4 (CDK4). Immunostimulatory activity of andrographolide is evidenced by increased proliferation of lymphocytes and production of interleukin-2. Andrographolide also enhanced the tumour necrosis factor-alpha production and CD marker expression, resulting in the increased cytotoxic activity of lymphocytes against cancer cells, which may contribute to its indirect anticancer activity. The *in vivo* anticancer activity of the compound is further substantiated against nitrobenzene-induced hepatocarcinogenesis in albino rats. These results suggest that andrographolide is an interesting pharmacophore with anticancer and immunomodulatory activities and hence has the potential to be developed as a cancer therapeutic agent.

Keywords: *Andrographis paniculate*, cancer, carcinogenesis, albino rats

1. Introduction

In terms of overall mortality rates, colorectal cancer (CRC) ranks third in males and second in females globally (Bray *et al*., 2018). According to the GLOBOCAN database, the mortality rate of CRC among females (9.5%) is higher compared with males (9.3%) in the year 2020 (Sung *et al*., 2021) [22]. In Southeast Asia, Malaysia is ranked as having the 3rd highest incidence and mortality of CRC. Specifically, the mortality and incidence of CRC were found to be increasing more among males than females, particularly in those of Chinese ethnicity (Abu Hassan *et al*., 2016) [1]. The global economic cost of CRC care approaches USD 100 billion, with medical spending alone expected to exceed USD 20 billion (Sninsky *et al*., 2022) [21]. As a result, there is a pressing requirement for a better knowledge of the pathophysiology of colorectal cancer as well as the discovery of new therapeutic methods.

There are several factors influencing CRC development such as age, sex, smoking, poor diet, genetics, and obesity (Mariotto *et al*., 2011) [13]. The most prevalent risk factor for colorectal cancer (CRC) development is a high-fat diet (HFD). The diet, which has a high proportion of fat, has been linked to obesity because it tends to promote weight gain over time (Vandevijvere *et al*., 2015) [23]. Recent epidemiological research has concluded that obesity leads to rising morbidity and mortality linked with colorectal cancer (Moghaddam *et al*., 2007) [14]. This is because a high-fat diet has been connected to pro-inflammatory potential, raising the risk of CRC. The pro-inflammatory factors associated with obesity such as adipokines (secreted by adipocytes) and cytokines will cause low-grade inflammation, which provides a favourable environment for cancer tumour growth (J. Zhang *et al*., 2019) [28]. Likewise, a previous study showed the adipose tissue secretes high levels of adipokines and cytokines, which aid in inducing CRC in high-fat-diet-fed rats (Schmoll *et al*., 2012) [18].

Surgery, chemotherapy, and radiation are the primary conventional therapies for CRC. These therapies can also be used in combination, depending on the location and course of the disease (Vodenkova *et al*., 2020) [24]. Fluorouracil (5-FU) is a fluoropyrimidine chemotherapeutic drug that has been extensively applied to treat a variety of malignant tumors, and particularly CRC, for over 50 years (Garg *et al*., 2012; Wigmore *et al*., 2010) [8, 26]. Even though 5-FU is one of the safest chemotherapy medicines, severe side and toxic effects occur in some CRC patients (Al-Henhena *et al*., 2014) [3]. The problems of conventional chemotherapies are related to their properties as anti-metabolites that disturb the formation of vital proteins, cause subsequent cell degradation, and result in long-term adverse outcomes (Aiello *et al*., 2019) [2]. Therefore, the urge to develop the safest therapy has drawn researchers' attention toward medicinal plants. Recently, significant research has been conducted on medicinal plants to understand their properties to cure certain acute diseases such as cancer (Slika *et al*., 2022) [20]. Among them, *Andrographis paniculata* is one of the medicinal plants that has been broadly studied for its anti-cancer properties.

The use of herbal extracts as complementary to modern medicine is gaining increased popularity. However, the use of herbal medicines is mainly based on anecdotal claims of their therapeutic properties in traditional medicine literature and folklore. Pharmaceutical preparations are generally, single active ingredients with defined pharmacological activities, while herbal extracts contain more than one active chemical ingredient. Also, the chemical composition of extracts varies depending on geographical distribution and climatic conditions of cultivation. Hence, to maintain the commercial value of herbal extracts, herbal manufacturers have intensified research efforts on the quantification of active ingredients to maintain consistency in their products. Identification of active pharmacophores in these extracts is also actively pursued understanding the molecular mechanism of action and in analogue synthesis in many commercial R&D centers. Notable examples of plant-derived pharma- cophores under investigation are genestein from soyabeans (Baez-Gonzalez *et al*., 2023) [4], indole 3-carbinol from cruciferous vegetables such as brussels sprout and broccoli (Chauhan, 2002) [6], curcumin from root of curcuma (Jang *et al*., 1997) [10], resveratrol from red wine (Fujiki *et al*., 2015) [7], and epigallocatechin from green tea (Okhuarobo *et al*., 2014) [17].

Andrographis paniculata is a herb indigenous to Southeast Asian countries like China and India. Extracts of whole plant have been reported to have anticancer, anti-inflammatory, anti-allergic, immunostimulatory, antithrombotic, antiviral, hypoglycemic and hypotensive (Shen *et al*., 2002) [19] activities. Andrographolide extracts are sold as healthcare products under the names Remdex, Kalmegh, and Restenoril. The beneficial effects of this herbal extract in cancer, HIV, and restenosis patients have been reported. Andrographis plant extract is known to contain diterpenoids, flavonoids, and steroids. However, the main components of andro-graphics are the diterpene lactones of which andrographolide is the major component. Andrographolide, unsaturated lactone is reported to have anticancer activity (C. Zhang & Tan, 1996) [27]. However, its molecular mechanism of action has not been fully defined. Hence, in this study, we evaluated the anticancer and immunomodulatory activities of andrographolide on human cancer and immune cells.

Moreover, prior research investigated the potential chemopreventive activities of *A. paniculata* against colorectal cancer. However, there is little understanding concerning the effect of *A. paniculata* on CRC under HFD conditions. This study's goal was to examine the anti-cancer properties of *A. paniculata* ethanolic extract on nitrobenzene-induced colon cancer in albino rats.

2. Materials and Methods

2.1. Collection of Plant: The medicinal plant selected for the present investigation *Andrographis paniculata* was collected from G.P. Siddha Ayurvedic Clinic of Pollachi. (Figure. 1)

2.2. Preparation of Ethanol extract: About 100g of the air-dried, powdered plant material was suspended in 500 ml of 99% ethanol for 60 hours. The extract was concentrated to 1/4th of its volume by evaporation at room temperature.

2.3. Phytochemical Screening Tests

2.3.1. Test for Alkaloids: 0.5 ml aliquot of the extract was treated with the following reagents to test the presence or absence of alkaloids.

Reagent Positive result

(a) Dragendroff's reagent: Orange or orange-red precipitate

(b) Mayer's reagent: White precipitate.

Fig 1: *Andrographis paniculata* (Burm. f.) Nees

Division : Angiosperms
Class : Dicotyledonae
Subclass : Gamopetalae
Series : Bicarpellatae
Order : Personales
Tribe : Justicieae
Family : Acanthaceae
Genus : *Andrographis*

2.3.2. Test for Steroids and Sterols: (a) Salkowski's Test**:** The extract was dissolved in 1 or 2 ml of chloroform and an equal volume of concentrated sulphuric acid was added through the sides of the test tube. The upper layer turns red and the sulphuric acid layer shows yellow with green fluorescence which represents the presence of steroids or sterol compounds in the extract. (b) Libermann-Burchard Test: 1.0 ml of the extract was dissolved in 1.0 ml of chloroform and 2-3 ml of acetic anhydrate was added followed by 1-2 drops of concentrated sulphuric acid.

2.3.3. Test for Flavonoids: (a) Shindo test**:** 1.0 ml of the extract was treated with magnesium turnings and 1-2 drops of concentrated hydrochloric acid. The formation of pink or red colour shows the presence of flavonoids. (b) 1.0 ml of the extract was treated with 1.0 ml of ferric chloride. The formation of brown colour confirms the presence of flavonoids.

2.3.4. Test for Tannins and Phenolic compounds: (a) 1.0 ml of the extract was treated with a few ml of 5% neutral ferric chloride.

The formation of a dark blue or bluish-black colour shows the presence of tannins. (b) 1.0 ml extract was treated with a few ml of gelatin solution; a white precipitate revealed the presence of tannins and phenolic compounds. (c) 1.0 ml of the extract was treated with lead tetra acetate solution, and the production of precipitate shows the presence of tannins and phenolic compounds.

2.3.5. Test for Terpenoids: 1.0 ml of the extract was treated with 2.0 ml of tri-chloroacetic acid solution and the formation of a yellow colour which turns red indicates the presence of terpenoids.

2.3.6. Test for Glycosides: Keller Killani Test**:** Dissolve the extract in acetic acid containing traces of ferric chloride and transfer it to a test tube containing sulphuric acid. The formation of a reddish-brown colour at the junction which gradually becomes blue confirms the presence of glycosides.

2.3.7. Test for Saponins: About 1.0 ml of the alcoholic extract was diluted separately with 20.0 ml of distilled water and shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of saponins. To 1.0 ml of the extract add alcoholic vanillin solution and a few drops of concentrated sulphuric acid were added. A deep violet colour confirms the presence of saponins.

2.3.8. Test for Carbohydrates: (a) Fehling's test**:** Add 5.0 ml of Fehling's solution to the extract and keep it in a boiling water bath. The production of yellow or red precipitate indicates the presence of reducing sugars. (b)Benedict's test**:** Add 5.0 ml of Benedict's solution to the extract and keep it in a boiling water bath. Red, yellow or green precipitate indicates the presence of reducing sugars.

2.3.9. Test for Amino acids and Proteins: Ninhydrin Test: Dissolve a small amount of extract and add a few ml of water and 1.0 ml of ninhydrin solution and heat. The blue or violet colour indicates the presence of amino acids. Add 1.0 ml of 40% sodium hydroxide and 2 -3 drops of 1% copper sulphate solution to a small amount of extract and the appearance of violet colour indicates the presence of proteins.

2.4. Experimental Design
The animals were divided into four groups. A control group of animals, a Nitrobenzene-induced group of animals, a Nitrobenzene-induced group of animals treated with an ethanolic extract from *Andrographis paniculate* and a group of animals fed with Ethanolic extract of *Andrographis paniculata* alone

2.5. Animals Used
The Wistar strain of albino rats of both sexes weighing between 140-160 g was obtained from the Animal House of Karpagam Arts and Science College, Coimbatore. The animals were housed in large spacious cages and they were given food and water at libitum during the course of the experiment. The animal room was well-ventilated and the animals had a 10± 1-hour night schedule, throughout the experimental period. The atmospheric temperature in Coimbatore remained between 20° to 37 °C with only a 5 °C difference between day and night throughout the year. No special arrangements were made for heating, cooling or lighting in the animal room. The animals had wakefulness and sleep periods during the day and night.

2.6. Diet Used
The commercial pelleted animal food marketed by M/s. Hindustan Lever Limited, Mumbai, India under the name "Gold Mohur rat feed" was used.

2.7. Induction of Liver Carcinogenesis
Nitrobenzene (E. Merck (India) Limited, Mumbai) was given orally at a single dose of 1000 mg/kg body weight of this dose is known to cause hepatotoxicity in rats. Alanine amino transferase, aspartate amino transferase was used as Tumor Marker Enzymes

2.8. Treatment of Liver Carcinogenesis
On the day after the oral dosage of nitrobenzene, the treatments were started. The drug, ethanolic extract of *Andrographis paniculata* was given to Group III orally at a dose of 30mg/kg for seven successive days. Group IV was given an ethanolic extract of *Andrographis paniculata* at a dose of 30mg/kg orally for seven successive days. Standard rat feed and clean drinking water were provided *at the libitum* to all the animals.

2.9. Preparation of sample
On the eighth day the animals were sacrificed, and their blood and liver were collected. From the blood, the serum was separated and given to Microlab, R.S Puram, Coimbatore for estimation of SGOT, SGPT and Alkaline phosphatase. The liver sample was taken and homogenized and the assay of the following enzymes was conducted. Estimation of protein was also done.

3. Results
3.1 Phytochemical Tests
The phytochemical screening test was done by the method of Trease and Evans (1978) and Harborne (1984). Ethanolic extract of *Andrographis paniculata* showed the presence of alkaloids, steroids, flavonoids, tannins, saponins, aminoacids proteins and carbohydrates. The results are shown in Table 1.

Table 1: Phytochemical test of *Andrographis paniculate.* AL- Alkaloids, ST-Steroids, GY- Glycoside, FV- Flavanoids, TER- Terpenoids, T-Tannins, SA- Saponins, AA & PROT- Aminoacids and Proteins, CH-Carbohydrates

Extract	AL	ST	GY	FV	TER	T	SA	AA & PROT	CHO
Ethanol	+	+	-	+	-	+	+	+	+

3.2 Experimental Animals and Design
After the experimental period, the rats were killed by cervical decapitation, blood was taken. The liver was immediately removed, washed in ice-cold saline and homogenized in 0.1m Tris-HCl buffer pH 7.4. From blood, serum was separated.

3.3 Cellular Constituents
Table 2 shows the effect of the ethanolic extract of *Andrographis paniculata* on the protein content in nitrobenzene-induced hepatocarcinogenesis.
Protein level was found to be significantly decreased in Group II. Nitrobenzene induced experimental animals. But protein level is

improved and restored to almost normal in Groups III and IV which was treated with ethanolic extract of *Andrographis paniculata* when compared to Group I.

Table 2: Effect of Ethanolic extract of *Andrographis paniculata* on the Protein Content in Nitrobenzene Induced Hepatocarcinogenesis. a –Group -I compared with Group- II, b - Group- II compared with Group -III, c – Group- II compared with Group -IV

Particular	Group-I	Group-II	Group-III	Group-IV
Protein (mg/ gm)	190.06± 15.43	123.52 ±10.18 (a*)	155.24±18.32 (b*)	162.31±15.23 (c*)

3.4 Tumor Marker Enzymes

Table 3 and 4 shows the activities of liver marker enzymes like ALT and AST on ethanolic extract of *Andrographis paniculata* on nitrobenzene-induced hepatocarcinogenesis in rats.

Table 3: The Activity of Liver Marker Enzyme ALT on ethanolic Extract of *Andrographis paniculata* on Nitrobenzene Induced Hepatocarcinogenesis. a –Group -I compared with Group- II, b - Group- II compared with Group -III, c – Group- II compared with Group -IV

Particular	Group-I	Group-II	Group-III	Group-IV
Alanine Transaminase (μ moles of pyruvate liberated per mg protein)	8.03±0.84	17.23 ±1.23 (a*)	11.42±1.84 (b*)	9.04±0.85 (c*)

Table 4: The Activity of Liver Marker Enzyme AST on Ethanolic Extract of *Andrographis paniculata* on Induced Nitrobenzene Hepatocarcinogenesis. a –Group -I compared with Group- II, b - Group- II compared with Group -III, c – Group- II compared with Group -IV

Particular	Group-I	Group-II	Group-III	Group-IV
Aspartate Transaminase (μ moles of pyruvate liberated per mg protein)	2.14± 0.33	7.55± 0.23 (a*)	4.04±0.53 (b*)	2.43±0.24 (c*)

Table 5 shows the activities of enzymes like ALT, AST and ALP in serum on ethanolic extract of *Andrographis paniculata* on nitrobenzene-induced hepatocarcinogenesis in rats.

Table 5: The Activity of Serum Liver Marker Enzymes AST, ALT and ALP on Ethanolic Extract of *Andrographis paniculata* on Nitrobenzene Induced Hepatocarcinogenesis. a –Group -I compared with Group- II, b - Group- II compared with Group -III, c – Group- II compared with Group -IV

Particular	Group-I	Group-II	Group-III	Group-IV
Alanine Transaminase (U/L)	130.32±10.47	200.34±19.82 (a*)	146.80±15.63 (b*)	166.20±15.90 (c*)
Aspartate Transaminase (U/L)	43.83±3.61	71.71±8.64 (a*)	39.42±3.54 (b*)	56.8±5.21 (c*)
Alkaline phosphatase (U/L)	275.42±26.84	424.91±40.81 (a*)	228.14±24.68 (b*)	79.8±21.84 (c*)

Alanine transaminase level is increased both in liver tissue and serum in Group II when compared to Group I control. The elevated level comes down in Group III (treated with ethanolic extract of *Andrographis paniculata*) when compared to Group II.

Aspartate transaminase level is increased both in liver tissue and serum in nitrobenzene-induced Group II experimental animals when compared to Group I animals. This highly elevated level of AST was brought down by treatment with ethanolic extract of *Andrographis paniculata* comparatively than Group II.

Alkaline phosphatase level is increased in serum in nitrobenzene-induced Group II animals than in Group I control animals. Successive seven days of treatment of ethanolic extract of *Andrographis paniculata* in Group III and Group IV respectively have reduced the values making them range to normal control animals of Group I.

3.5 Lipid Peroxidation

Basal lipid peroxidation shows a much more significant increase in nitrobenzene-induced Group II animals than in Group I control animals (Table 6). The elevated levels were decreased in Group III and Group IV when compared to Group II by treating them with ethanolic extract of *Andrographis paniculata* successively for seven days.

Table 6: The Activity of Lipid peroxidation on Ethanolic Extract of *Andrographis paniculata* on Nitrobenzene induced Hepatocarcinogenesis. a –Group -I compared with Group- II, b - Group- II compared with Group -III, c – Group- II compared with Group -IV

PARTICULAR	GROUP-I	GROUP-II	GROUP-III	GROUP-IV
Lipid peroxidation (n moles of MDA formed per mg protein)	0.96± 0.07	3.82 ±0.25 (a*)	2.02±0.24 (b*)	1.28±0.13 (c*)

3.6 Antioxidant Enzymes

The Table 7, 8, and 9 shows that the activities of antioxidant enzymes like catalase, superoxide dismutase and glutathione peroxidase respectively on ethanolic extract ethanolic extract of *Andrographis paniculata* against nitrobenzene- induced hepatocarcinogenesis in rats.

Table 7: The Activity of Antioxidant Enzyme Catalase on Ethanolic Extract of *Andrographis paniculata* on Nitrobenzene Induced Hepatocarcinogenesis. a –Group -I compared with Group- II, b - Group- II compared with Group -III, c – Group- II compared with Group -IV

Particular	Group-I	Group-II	Group-III	Group-IV
Catalase (μ moles of H_2 O_2 utilized per minute per mg protein)	50.85± 4.63	26.43± 2.25 (a*)	44.43±3.74 (b*)	51.23±3.65 (c*)

Table 8: The Activity of Antioxidant Enzyme Superoxide Dismuatse on Ethanolic Extract of *Andrographis paniculata* on perchloroethylene Induced Hepatocarcinogenesis. a –Group -I compared with Group- II, b - Group- II compared with Group -III, c – Group- II compared with Group -IV

Particular	Group-I	Group-II	Group-III	Group-IV
Superoxide dismutase (units per mg protein)	5.34±0.37	3.76±0.25 (a*)	4.82±0.42 (b*)	5.91±0.48 (c*)

Table 9: The Activity of Antioxidant Enzyme Glutathione peroxidase on Ethanolic Extract of *Andrographis paniculata* on Nitrobenzene Induced Hepatocarcinogenesis. a –Group -I compared with Group- II, b - Group- II compared with Group -III, c – Group- II compared with Group -IV

Particular	Group-I	Group-II	Group-III	Group-IV
Glutathione peroxidase (μ moles of GSH utilized per minute mg per protein)	6.20±0.35	4.93±0.52 (a*)	5.81±0.48 (b*)	6.84±0.57 (c*)

Catalase level was significantly decreased in Group II animals when compared to control animals of Group I. The level of the antioxidant enzyme, catalase was increased in Group III and Group IV when compared to Group II by administering the animals with ethanolic extract of *Andrographis paniculata.* The level of catalase is ranging to normal when compared with Group I. Superoxide dismutase level decreases in nitrobenzene-induced Group II animals when compared with Group I. The level of the enzyme was increased in Group III when compared with Group II by treating with ethanolic extract of *Andrographis paniculata*. In Group IV, the level of enzyme nears to control animal of Group I by treating with ethanolic extract of *Andrographis paniculata*.

Glutathione peroxidase enzyme level drastically falls in nitrobenzene-induced Group-II when it is compared with Group-I. The level of enzyme is somewhat restored to normal control level of Group-I when Group-IV animals are treated with ethanolic extract of *Andrographis paniculata*. The level of Group –III enzymes is increased than Group-II when treated with ethanolic extract of *Andrographis paniculata*.

3.7 Histopathological study

The results of the histopathological study of Groups I, II, III and IV are shown in Figure 2.

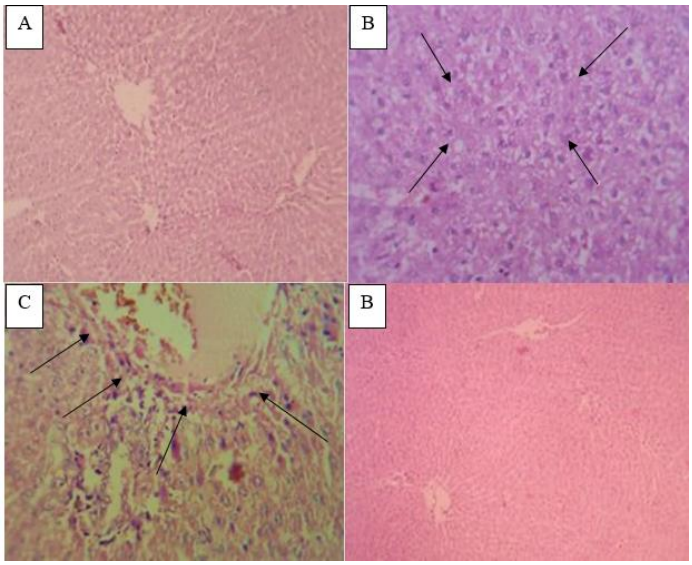

Fig 2: A). Control, B). Nitrobenzene induced, C). Nitrobenzene+ Ethanolic Extract and D). Ethanolic extract alone

4. Discussion

Cancer is a Worldwide problem and is emerging as a major killer of the modern era. Natural products of plant origin and numerous non-nutritive dietary constituents have been shown to play a significant role in cancer chemoprevention. It is reported that extracts of some spices were found to inhibit the tissue-cultured cell lines as well as being cytotoxic to tumour cell lines. The present study aims to evaluate the chemopreventive efficacy of the ethanolic extract of *Andrographis paniculata* against hepatocarcinogenesis. The liver is the largest glandula organ in the body and has more function than any other human organ. Liver function can be related to most complaints accompanied by inflammation and infection. Hepatocarcinogenesis is the cancer of the liver. The liver is a frequent site for the development of chemically induced cancer in rodents (Vogelstein & Kinzler, 1993). Hepatocarcinogenesis can be induced by various chemical carcinogens. In the present study, nitrobenzene is used to induce hepatocarcinogenesis in albino rats (1000 mg/kg body weight). *Andrographis paniculata* also known as "King of Bitters", has been used throughout the centuries against different diseases, especially as a hepatoprotective agent (Hossain *et al*., 2014) [9].

Medicinal plants have pharmacological properties due to the presence of some secondary metabolites. Table 1 ethanolic extract of *Andrographis paniculata* showed the presence of alkaloids, steroids, flavonoids, tannins, saponins, carbohydrates, proteins and amino acids. Table 2 shows the concentration of protein in the liver of control and experimental animals. The protein level was found to be significantly decreased in Group II animals treated with Nitrobenzene. Administration of ethanolic extract of *Andrographis paniculata* to Group III and Group IV has restored the values to almost normal.

In the present investigation, the alanine and aspartate aminotransferases were significantly increased in tissue and serum samples in nitrobenzene-induced animals (Group II) along with an increase in alkaline phosphatase in serum. When treated with ethanolic extract of *Andrographis paniculata* the level was significantly reduced. The hepatoprotective effects of bergenin, a major constituent of *Mallotus japonicus*, were evaluated against carbon tetrachloride (CCl_4)-induced liver damage in rats. The substantially elevated enzymatic activities of alanine/aspartate aminotransferase were restored towards normalization (Lim *et al*., 2000) [11]. Manjunatha (2006) [12] also reported that there is an increase in serum alanine transaminase (1413.00±1.99), aspartate transaminase (2213.50±32.79) and alkaline phosphatase (444.33±1.56) in CCl_4 treated control group which were resorted towards normalization when treated with extracts of *Pterocarpus santalinus.*

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process where free radicals " steal" electrons from the lipids in the cell membrane, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. In the present investigation, the lipid peroxidation levels were found to be significantly increased in nitrobenzene-induced animals. The observed increase in lipid peroxidation rate may be due to its poor

antioxidant defence as well as either leak of MDA from tissue or inactivation of the antioxidant system in hepatocarcinogenic conditions. By treatment with the ethanolic extract of *Andrographis paniculata,* the lipid peroxidation levels were found to be significantly normal. (Nevin & Vijayammal, (2005) [16] also reported that CCl_4 administration increased lipid peroxidation in the liver and microsomal fraction. When compared to a normal animal, pretreatment with *Aerva lanata* showed a significant reduction in lipid peroxidation in the liver and microsomes.

Intracellular lipid peroxidation is regulated by antioxidant enzymes like catalase, superoxide dismutase and glutathione peroxidase. All these enzymes are significantly decreased in nitrobenzene-induced rats. Enzyme activities were restored to near normal by treating them with water extract and ethanolic extract of *Andrographis paniculata* to Group III and Group IV. Naaz *et al*., (2007) [15] reported that aflatoxin-induced hepatic damage reduced the levels of antioxidant enzymes. Ethanolic extract of *Phyllanthus amarus* was found to show a hepatoprotective effect by enhancing the levels of antioxidant enzymes GPx, SOD, CAT and GST.

Histology of the liver sections of control animals (Group I) showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus and visible central veins. The liver sections of nitrobenzene-intoxicated rats (Group II) showed massive fatty changes, necrosis, ballooning degeneration broad infiltration of the lymphocytes and loss of cellular boundaries. The histological architecture of the liver section of the rats treated with ethanolic extracts (Group III) showed a more or less normal lobular pattern with a mild degree of fatty changes, necrosis and lymphocyte infiltration almost comparable to the control. Histopathological analysis of the *Andrographis paniculata* alone treated animals (Group IV) showed the same result as that of the control group. Histopathological analysis of liver samples showed focal areas of necrosis with periportal chronic necrosis in aflatoxin treated liver of mice, while mice treated with aflatoxin and *Phyllanthus amarus* extracts showed kupffer cells hyperplasia and regeneration activities in cells (Naaz *et al*., 2007) [15]

5. Summary and Conclusion

Cancer is not a single disease. It is a large and complex family of malignancies that can affect virtually every organ in the body. The liver is the largest organ in the body and is an extremely important organ that has many functions. Hepatocellular carcinoma is a primary malignancy (cancer) of the liver. Carcinogenic response was observed after exposure to nitrobenzene in rats and mice. Here nitrobenzene is used to induce hepatocarcinoma. The dosage given was 1000 mg/kg body weight. *Andrographis paniculata* is the herbal drug used here to treat the hepatocarcinogen in rats. *Andrographis paniculata* also known as the “King of Bitters”, has been used throughout the centuries against different diseases, especially as a hepatoprotective agent. Phytochemical screening of the plant revealed the presence of alkaloids, steroids, flavonoids, tannins, saponins, carbohydrates, proteins and amino acids. In the homogenate of the liver, the concentration of protein levels was found to be significantly decreased in group II animals. The level again rose to almost normal when group III animals were treated with ethanolic extract of *Andrographis paniculata*. In group IV animals the level is near normal as compared to group II because they were being treated with ethanolic extract of *Andrographis paniculata*. Amino transferases (alanine and aspartate) were found to be significantly increased in group II both in liver homogenate and serum. The level of Alkaline phosphatase was also elevated in serum in group II. The elevated levels were lowered in group III (ethanolic extract of *Andrographis paniculata*). In group IV, the levels are near to normal by treating them with ethanolic extract of *Andrographis paniculata*. Lipid peroxidation is one of the primary events in tumour development as well as in aging. The basal lipid peroxidation is significantly increased in nitrobenzene-induced animals. The values are lowered in group III and group IV by treating them with ethanolic extract of *Andrographis paniculata*. The antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidases were significantly lowered in nitrobenzene-induced animals. The levels came down by treating animals with ethanolic extract of *Andrographis paniculata.* The histopathological study also supported the hepatoprotective action of *Andrographis paniculata.* Finally, it can be concluded that *Andrographis paniculata* has anticancer activity against nitrobenzene-induced hepatocarcinogenesis.

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