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THERAPEUTIC USES OF THE POLYHERBAL DRUG TRIPHALA IN GERIATRIC DISEASES

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ABSTRACT

Degenerative physiological changes related to old-age are increasing world over. These geriatric diseases affect almost all vital body systems. The rejuvenating and preventive therapy called Rasayana therapy in Ayurvedic system of Indian medicine deals with prevention, amelioration and cure of geriatric ailments by increasing overall body immunity, fighting infections & antigens, and preventing carcinogenic mutations. A specific polyherbal preparation called Triphala, which consists of equal amounts of fruits of three plants namely Terminalia chebula Retz., Terminalia bellirica Roxb. and Emblica officinalis Gaertn. in fine powder form, has been specifically mentioned in traditional Ayurvedic texts for its beneficial effects in geriatric diseases. It contains tannins, phenols and glycosides which are responsible for its strong antioxidant activity apart from its immunomodulatory, anti-inflammatory, analgesic and antimutagenic properties. These attributes make Triphala an effective remedy for geriatric degenerative diseases.

KEY WORDS

geriatric, polyherbal, antioxidant

INTRODUCTION

Globally, a significant increase in the life expectancy has been observed. The combination of low fertility and declining mortality rates has resulted in large and rapid increases in the elderly population. The proportion of the elderly in the world population is expected to increase rapidly from 10.0% in 2000 to 15.0% in 2025 and 21.1% in 2050. The growth in the ageing population in India has been faster than in other developing countries. The absolute number of the elderly in India is projected to reach 137 million by the year 2021, a drastic increase from 81 million in 2002. Ageing puts an increased burden on the social, economic, and health care demands of any country. A challenge to ensuring the quality of life of the ageing population is the double burden of diseases and disability, especially in developing countries. These countries still struggle with infectious diseases and malnutrition along with the recent, rapid growth of non-communicable diseases such as diabetes, cardiovascular diseases and hypertension, as well as disability caused by age-related changes in physical health, including mobility and ability to perform activities of daily living [1].

Physiological changes related to ageing

It has been reported that between the ages of 25 and 65, the total body water can decrease by 15-20% and the extra cellular fluid by approximately 35-40%.; however, the fat weight to body weight ratio increase by 25- 45%. These changes in the body compartment with advancing age can affect pharmacokinetic parameters.

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The entire gastrointestinal tract undergoes changes caused by the effect of ageing. With advancing age there is frequent loss of teeth, and such loss of teeth seems to cause a reduction in salivary flow. There is also a significant reduction in salivary ptyalin content and the absorbing capacity of the oral mucosa with advancing age. With advancing age cardiac output diminishes, which alters blood distribution to various organs; in order to distribute blood effectively in the presence of diminishing cardiac output, the brain, heart and skeletal muscles preferentially receive more of the diminished cardiac output than do the liver and kidney. Both structure and function of the liver are affected by the ageing process. Since the liver has the highest capacity to metabolize drugs for their elimination from the body, any dysfunction of this vital organ during the ageing process is expected to prolong their half-life. Therefore, the enzymatic activities of the enzymes may decrease during the ageing process because of the gradual death of hepatocytes and reduced blood flow to this organ. The age induced loss of hepatocytes is compensated for by an increased activity of the surviving cells. An age related deterioration of renal function occurs because the kidney loses 20% of its weight. The weight loss involves a gradual reduction in the number of nephrons due to decreased renal blood flow per unit tissue mass.

The most common physiological changes which take place in the human body with ageing can, therefore, be summarized as follows:

a. ***Nervous system:*** neuronal loss, impairment of memory, hearing and vision/sense of balance

b. ***Respiratory system:*** reduced lung elasticity, chronic bronchitis, emphysema, recurrent respiratory infections

c. ***Cardiovascular system:*** reduced myocytes, dilated aorta, narrowed coronaries, ischemic heart disease, cardiomyopathy

d. ***Digestive system:*** degenerative changes in inner lining, reduced digestive capacity, dyspepsia, chronic constipation, heartburn

e. ***Renal system:*** progressive loss of nephrons, reduced glomerular filtration rate, rising creatinine

f. ***Endocrine system:*** reduced sensitivity to insulin, glucose intolerance, overt diabetes

g. ***Skeletal system:*** reduced bone mineral density, osteoporosis, fractures on minor trauma, degenerative arthritis & loco motor disability

RASAYANA THERAPY: AN AYURVEDIC APPROACH FOR GERIATRIC PROBLEMS

Most of the drugs used today are obtained from natural sources or semi synthetic derivatives of natural products as mentioned and used in the traditional systems of medicine. Hence, it is a logical approach for drug discovery to screen traditional natural products instead of randomly synthesized chemical compounds. This

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methodology was put into medicinal practice by the Indian scholars of the ancient era whose dedicated efforts gave birth to the Ayurvedic system of medicine. The Ayurvedic system is based upon the Dosh (metabolic factors), Dhatu (constitution, body tissue) and Mala (excretory matters) factors which are found at equilibrium in the healthy person but any disturbance occurring in their composition produces diseases. These factors depend upon the food habits, daily routine, work environment and life style of the individual personality. If any changes occur in these factors which increase or decrease the amount of any of the three triodes of life – Vata (nervous activities), Pitta (digestion and metabolism) or Kapha (stability and immunity), it may lead to disease. The inadequate digestion of food due to improper food and irregular routine produces undigested food particles which are circulated throughout the various body parts. These can obstruct the various body channels and produce disease due to malnutrition of specific body parts.

Rejuvenating and preventive therapy called Rasayana therapy has been used since ancient times to provide vital capacity and long life of specific body parts by using appropriate medicines and balanced diet. The word Rasayana can be related to the modern word Geriatric which is closely linked with the Sanskrit word Geeryadi, which means degenerative changes in old age linked to diseases such as hypertension, diabetic mellitus, osteoporosis, metabolic diseases, mental diseases, cancer, etc. Rasayana refers to substances that stimulate the production of the subtle essence of the immune system (Ojas) due to complete digestion of the food. Low levels of these essences make the immune system weak which facilitates the development of chronic and degenerative diseases. Rasayana Therapy arrests aging, enhances intelligence, memory, strength, youth, luster, sweetness of voice and vigor. It is supposed to nourish blood, lymph, flesh, adipose tissue and semen and thus prevent degenerative changes and illness. It improves the overall metabolic process and builds natural resistance against infection[2,3] . There are many types of rasayana and many herbs have been mentioned according to their role in preventing degenerative changes in the specific organ systems.

INGREDIENTS OF TRIPHALA: THEIR PROPERTIES AND THERAPEUTIC ACTIONS

The drug Triphala is a combination of the fruits of Terminalia chebula Retz., Terminalia bellirica Roxb. and Emblica officinalis Gaertn. in equal amounts in fine powder form. Here one fruit of Terminalia chebula, two fruits of Terminalia bellirica and four fruits of Emblica officinalis are used for the preparation of medicine. Terminalia chebula and Terminalia bellirica have a warm energy, while Emblica officinalis is cool in nature. Triphala, being a combination of all three, is therefore balanced, making it useful as an internal cleansing, detoxifying formula for everyone. Triphala is regarded as an important *rasayana* and good purgative in Ayurvedic medicine. Recipe for this traditional herbal supplement is described in the traditional Indian texts, the Charak Samhita and Susruta Samhita which date back to 1500 B.C. Triphala is considered a 'tridoshic rasayan' having balancing and rejuvenating effects on the three constitutional elements that govern human life: Vata which regulates the nervous system, Pitta which maintains metabolic processes, and Kapha which supports structural integrity.

The Ayurvedic and modern properties of individual ingredients of Triphala are given in table 1 [4, 5]:

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Table 1:
Ayurvedic and modern properties of individual ingredients of Triphala

Local name	Haritaki	Amlaki	Vibhitak
Scientific name	Terminalia chebula Retz.	Emblica officinalis Gaertn.	Terminalia bellirica Roxb.
Family	Combretaceae	Euphorbiaceae	Combretaceae
Parts used	fruits	fruits	fruits
Properties			
Guna	Laghu (Lightness), Ruksha (Roughness)	Guru (Heavy), Ruksha (Roughness), Sheet(Cold)	Ruksha (Roughness) Laghu (Lightness)
Rasa	Pancharas (Kashay (Astrigent) predominant)	Panchrasa (Amla (Sour) predominant)	Kashay (Astringent)
Vipak	Madhur (Sweet)	Madhur (Sweet)	Madhur (Sweet)
Virya	Ushna (Hot)	Sheet (Cold)	Ushna (Hot)
Doshakarma	Tridoshahar, especially Vatahar	Tridoshahar, especially Pittahar	Tridoshahar, especially Kaphahar
Actions-	Deepan (Appetizer), Shothahar (Anti-inflammatory), Vranashodhan (Wound healing), Vednasthapan (Analgesic) Mriduvirechan (Mild Laxative, Rasayan (Rejuvenator), Vrishay (Aphrodisiac),	Rasayan (Rejuvenator) Dahaprashaman (Refrigerant), Jwarhar (Antipyretic), Medhya (Brain tonic), Rochan, Deepan (Appetizer), Amlatanashak (Antiulcer), Vrishay (Aphrodisiac),	Vednasthapan (Analgesic), Deepan (Appetizer), Krimighan (Anthelmintic), Kasahar (Anticough), Swasahar (Anti-asthmatic)
Chemical compound	Tannin, Chebulagic acid, Anthraquinone, Chebulinic acid ,Glycosides	Phyllembic acid , Tannin, Procynidin, Vit C, Ellagic acid, Carotene, Phyllantine Ribflavin, Polyphenols	Gallic acid, Ellagic acid, Chebulagic acid, B-Sitosterol, Tannin, Bellericanin
Pharmacological activities	Antimicrobial, Antistress, Purgative, Anthelmintic, Hypolipidaemic, Antifungal	Antioxidant, Antiulcer, Antimicrobial, Imm uno-modulatory, Antitumor, Hypolipidaemic, Anti-inflammatory	Antihistaminic, Antistress, Purgative, Antibacterial, Antispasmodic, Anti-asthmatic

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PHYTOCHEMICAL CONSTITUENTS OF TRIPHALA

The common chemical compounds on review present in this drug are Tannin, Gallic acid, Chebulagic acid, Ellagic acid, Phenols and Glycosides. Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts. The total Phenolic content in Triphala using spectrophotometric methods has been evaluated and the phytochemical analysis showed that Triphala is rich in phenols/polyphenols (38.3%) and tannins (35.□3%), while flavonoids were found to be absent. HPLC analysis (column, C18 PCX 500; mobile phase, aqueous acetonitrile (10%)–HCl (0.05 M)–KCl (0.1 M); detection, absorbance at 260 nm) showed that Triphala contains 73.5 mg Gallic acid per gram of Triphala, which was found to increase to 150.5 mg/g upon acid hydrolysis. Tannins are naturally occurring, high molecular weight plant polyphenols. They are usually subdivided into two groups, hydrolysable tannins and condensed tannins. The total tannin content present in Triphala was measured using a colorimetric Folin–Denis method. The measurements were compared with standard tannic acid sample and results expressed in terms of % tannic acid equivalents. The tannin content in Triphala was found to be 35.3%. Analysis of total flavonoid content in Triphala has been done using colorimetric method and quercetin as standard flavonoid. The results showed that Triphala did not contain any significant amount of flavonoids [6].

PHARMACOLOGICAL ACTIVITIES OF TRIPHALA

According to Ayurvedic practitioners, daily use of Triphala promotes appetite, ensures good digestion, increases red blood cells and hemoglobin, and helps in removal of undesirable fat. Triphala creates a favorable chemical environment for the proliferation of beneficial intestinal bacteria and an unfavorable environment for non-beneficial intestinal bacteria. Triphala as a bowel regulator is considered as safe as food and is not habit forming, even when taken on a daily basis. Since Triphala is a tonic, cleanser and blood purifier, it is also beneficial in ailments related to eyes, namely cataracts, conjunctivitis and glaucoma. Triphala can be used as daily eyewash to strengthen vision, counteract many common eye defects and eliminate their redness and soreness. Conditions for which Triphala is employed also include headache, dyspepsia, constipation, liver conditions, ascites, and leucorrhoea. It is also used as a blood purifier and a purgative and to improve the mental faculties and is reported to possess anti-inflammatory, analgesic, anti-arthritic, hypoglycemic, and anti-aging properties. Its purgative action is due to the presence of Chebulagic acid and its antioxidant, immunomodulatory, anti-inflammatory, analgesic and anti-ageing action is due to the presence of Phenolic-glycoside compounds and tannin.

Antibacterial activity

The antibacterial activities of aqueous and ethanol extracts of Triphala and its individual components were tested against certain bacterial isolates (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella sonnei*, *S. flexneri*, *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella paratyphi-B*, *Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhi*) obtained from HIV infected patients using Kirby-Bauer's disk diffusion and minimum inhibitory concentration (MIC) methods. Most of the bacterial isolates were inhibited by the ethanol and aqueous extracts of *T. chebula* followed by *T. belerica* and *E. officinalis* by both disk diffusion and MIC methods[7].

Anti-infective property

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Infection is a major problem in the management of wounds. Even though the development of synthetic antimicrobial agents persists, drug resistance and toxicity hinder their way. Many plants with multi-potent pharmaceutical activities may offer better treatment options, and alcoholic extract of Triphala has shown *in vitro* antimicrobial activity against wound pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*. An ointment prepared from the Triphala extract (10% w/w) was assessed for *in vivo* wound healing on infected rat model by rate of healing, bacterial count, biochemical analysis, and expression of matrix metalloproteinases. The treated group showed significantly improved wound closure. Assessment of granulation tissue on every fourth day showed significant reduction in bacterial count with significant level of collagen, hexosamine, uronic acid, and super oxide dismutase in the treated group ($P < 0.01$). Reduction of matrix metalloproteinase expression observed in the treated group by gelatin zymography and immunoblotting confirmed the *in vivo* assessment. The above results showed the antibacterial, wound healing, and antioxidant activities of Triphala ointment, necessary for the management of infected wounds [8].

Antioxidant activity

The evaluation of the in vitro antioxidant activity of aqueous extract of the fruits of Emblica officinalis, Terminalia chebula and Terminalia belerica and their equi-proportional mixture, Triphala, has indicated their strong ability to scavenge free radicals such as DPPH and super oxide. Results of DPPH reduction have shown that Triphala had a synergistic effect, compared to each individual constituent, and it may be useful for free radical induced disorders such as paracetamol toxicity, heavy metal and radiation. As the phenolic compounds present in these extracts are mostly responsible for their radical scavenging activity, the total phenolic content present in these extracts has been determined and found to vary from 33% to 44% in terms of Gallic acid equivalents. These studies revealed that all three constituents of Triphala are active and they exhibit slightly different activities under different conditions. Emblica officinalis shows greater efficiency in lipid peroxidation and plasmid DNA assay, while Terminalia chebula has greater radical scavenging activity. Thus their mixture, Triphala, is expected to be more efficient due to the combined activity of the individual components [9, 10].

The methanolic extracts (75%) of *Terminalia chebula*, *Terminalia belerica*, *Emblica officinalis* and their combination named Triphala were found to inhibit lipid peroxide formation and to scavenge hydroxyl and super oxide radicals in vitro. The concentration of plant extracts that inhibited 50% of lipid peroxidation induced with Fe^{2+}/ascorbate were found to be 85.5, 27, 74 and 69 µg/ml, respectively. The concentration needed for the inhibition of hydroxyl radical scavenging were 165, 71, 155.5 and 151 µg/ml, and that for super oxide scavenging activity were found to be 20.5, 40.5, 6.5 and 12.5 µg/ml, respectively[11].

The effects of 10 mg/kg of Triphala extract (TE) was studied on radiation-induced sickness and mortality in mice exposed to 7-12 Gray (Gy) of gamma-irradiation. Treatment of mice with Triphala once daily for 5 consecutive days before irradiation delayed the onset of mortality and reduced the symptoms of radiation sickness when compared with the non-drug double distilled water treated irradiated controls (DDW). Triphala provided protection against both gastrointestinal and hemopoetic death. However, animals of both the TE + irradiation and DDW +

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irradiation groups did not survive up to 30 days post-irradiation beyond 11 Gy irradiation. The LD50/30 was found to be 8.6 Gy for the DDW + irradiation group and 9.9 Gy for TE + irradiation group. The administration of Triphala resulted in an increase in the radiation tolerance by 1.4 Gy, and the dose reduction factor was found to be 1.15. To understand the mechanism of action of Triphala, the free radical scavenging activity of the drug was evaluated. Triphala was found to scavenge (.)OH, O(2) (.) 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate) diammonium salt (ABTS)(.+) and NO(.) radicals in a dose dependent manner [12].

Protection against whole body gamma-irradiation (WBI) of Swiss mice orally fed with Triphala (TPL), an Ayurvedic formulation, in terms of mortality of irradiated animals as well as DNA damage at cellular level has been investigated. It has been found that radiation induced mortality was reduced by 60% in mice fed with Triphala (1g/kg body weight/day) orally for 7 days prior to WBI at 7.5 Gy followed by post-irradiation feeding for 7 days. An increase in xanthine oxidoreductase activity and decrease in super oxide dismutase activity was observed in the intestine of mice exposed to WBI, which, however, reverted back to those levels of sham-irradiated controls, when animals were fed with Triphala for 7 days prior to irradiation. These data have suggested the prevention of oxidative damage caused by whole body radiation exposure after feeding of animals with Triphala [13].

Analgesic, antipyretic and ulcerogenic activities

Most of the presently available anti-inflammatory drugs show analgesic, antipyretic effect associated with gastric damage. Therefore, an attempt was made to ascertain whether Triphala exhibits analgesic and antipyretic activities without any gastric damage. Increased body temperature and pain are known as the main reactions of the body against an inflammatory stimulation. Therefore, it is generally essential to possess analgesic and antipyretic activities for an anti-inflammatory compound [14]. The analgesic, antipyretic and ulcerogenic activities of Triphala (500/1000 mg/kg body wt) were compared with the non-steroidal anti-inflammatory drug Indomethacin (10 mg/kg body wt) on the experimental models in mice and it was found that Triphala at both the dose levels produced excellent analgesic and antipyretic effect, with the absence of gastric damage [15]. Induction of the acetic acid writhing in mice is an effect of the acute inflammatory reaction related to the increase in the level of prostaglandin E2 and F2a in the peritoneal fluid [16]. Acetic acid acts indirectly by inducing the release of endogenous mediators of pain sensitive to non steroidal anti-inflammatory drug and opioids. The mechanism of analgesic action of the Triphala could probably be due to the blockade of the effect or the release of the endogenous substances that excite pain nerve ending similarly to non steroidal anti inflammatory drugs [17]. The Triphala ointment showed strong antibacterial, wound healing, and antioxidant activities during the management of infected wounds [18]

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Anticancer activity

The use of Triphala in diet has been shown to significantly reduce the benzo(a)pyrene [B(a)P] induced fore stomach papillomagenesis in mice, thus establishing the cancer chemo preventive potential of Triphala. In the short term treatment groups, the tumor incidences were lowered to 77.77% by both doses of Triphala mixed diet. In the case of long-term treatment, the tumor incidences were reduced to 66.66% and 62.50% respectively by 2.5% and 5% Triphala containing diet. Tumor burden was 7.27 +/- 1.16 in the B(a)P treated control group, whereas it

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reduced to 3.00 +/- 0.82 ($p < 0.005$) by 2.5% dose and 2.33 +/- 1.03 ($p < 0.001$) by 5% dose of Triphala. In long-term studies the tumor burden was reduced to 2.17 +/- 0.75 ($p < 0.001$) and 2.00 +/- 0.71 ($p < 0.001$) by 2.5% and 5% diet of Triphala, respectively. It was important to observe that Triphala was more effective in reducing tumor incidences compared to its individual constituents. Triphala also significantly increased the antioxidant status of animals which might have contributed to the chemoprevention. It was inferred that the concomitant use of multiple agents seemed to have a high degree of chemoprevention potential [19].

The in vitro antimutagenic activity of Triphala was extended to test its cytotoxic effects on cancer cell-lines using Shionogi 115 (S115) and MCF-7 breast cancer cells and PC-3 and DU-145 prostate cancer cells as models. The results revealed that acetone extract of "Triphala" showed a significant cytotoxic effect on these cancer cell-lines and the effect was similar on all cancer cell lines. The major phenolic compounds in the most potent acetone extracts were isolated and purified. Structural analysis was conducted using spectroscopic techniques including mass spectroscopy, nuclear magnetic resonance (NMR) and infrared (IR) which showed Gallic acid as the major component. The suppression of the growth of cancer cells in cytotoxic assays may be due to the Gallic acid—a major polyphenol observed in Triphala [20].

A 70% methanol extract of *Terminalia chebula* fruit was studied for its effects on growth in several malignant cell lines including a human (MCF-7) and mouse (S115) breast cancer cell line, a human osteosarcoma cell line (HOS-1), a human prostate cancer cell line (PC-3) and a non-tumorigenic, immortalized human prostate cell line (PNT1A) using assays for proliferation ([^{3}H]-thymidine incorporation and coulter counting), cell viability (ATP determination) and cell death (flow cytometry and Hoechst DNA staining). In all cell lines studied, the extract decreased cell viability, inhibited cell proliferation, and induced cell death in a dose dependent manner. Flow cytometry and other analyses showed that some apoptosis was induced by the extract at lower concentrations, but at higher concentrations, necrosis was the major mechanism of cell death. ATP assay guided chromatographic fractionation of the extract yielded Ellagic acid, 2,4-chebulyl-β-D-glucopyranose (a new natural product), and chebulinic acid which were tested by ATP assay on HOS-1 cell line in comparison to three known antigrowth phenolics of *Terminalia*, Gallic acid, ethyl gallate, luteolin, and tannic acid. Chebulinic acid (IC_{50}=53.2 μM±0.16)>tannic acid (IC_{50}=59.0 μg/ml±0.19)> and Ellagic acid (IC_{50}=78.5 μM±0.24), were the most growth inhibitory phenolics of *T. chebula* fruit in the study [21].

Action on human breast cancer cells

The cytotoxic effects of aqueous extract of Triphala have also been investigated on human breast cancer cell line (MCF/7) and a transplantable mouse thymic lymphoma (barcl/95) which suggests that Triphala possesses the ability to induce cytotoxicity in tumor cells but spares the normal cells. The differential effect of Triphala on normal and tumor cells seems to be related to its ability to evoke differential response in intracellular ROS generation [22].

The cytotoxic effects of Triphala (TPL) have been investigated on two human breast cancer cell lines differing in their p53 status. In vitro studies showed that MCF 7 with wild type p53 was more sensitive to TPL than T 47 D, which is p53 negative. TPL induced loss of cell viability was determined by

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MTT assay. After 72h incubation, the IC 50 values for MCF 7 was found to be approximately 8 microg/ml and that for T 47 D was approximately 26 microg/ml. Moreover, TPL inhibited the clonogenic growth of MCF 7 cells, which was significantly recovered by pifithrin-alpha, the p53 inhibitor. However, pifithrin-alpha, did not modify TPL induced cytotoxicity in T 47 D cells. Exogenous addition of antioxidants, glutathione (GSH) and N-Acetyl-Cysteine (NAC) inhibited the anti-proliferative ability of TPL in both MCF 7 and T47 D. Annexin-V and propidium iodide double staining of cells treated with TPL for 2h revealed that TPL induced significant apoptosis in both the cell lines in a dose dependant manner but magnitude of apoptosis was significantly higher in MCF 7 than in T 47-D cells. TPL was also found to induce dose and time dependent increase in intracellular reactive oxygen species in both the cell lines. Present results have demonstrated that MCF 7 and T 47 D cells exhibited differential sensitivity to TPL, which seems to be dependant on their p53 status. Inhibition of anti-proliferative ability of TPL by antioxidants suggests a role for TPL induced ROS in the induction of apoptosis. It is concluded that p53 status of cancer cells formed an important factor in predicting the response of cancer cells to prooxidant drugs [23].

Antidiabetic activity

The oral administration of Triphala extract (100 mg/kg body weight) has reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats significantly within 4 hours and continued daily administration of the drug produced a sustained antidiabetic effect [11].

Immunomodulatory activity

Immune activation is an effective as well as protective approach against emerging infectious diseases. The immunomodulatory activities of Triphala were assessed by testing the various neutrophil functions like adherence, phagocytosis (phagocytic index (P.I) and avidity index (A.I)) and nitro blue tetrazolium (NBT) reduction in albino rats. Noise (100dB) stress for 4h/d for 15d, was employed to alter the neutrophil functions. The neutrophil function tests and corticosterone levels were carried out in eight different groups of animals, namely control, Triphala, noise-stress, Triphala noise-stress, and corresponding immunized groups were used. Sheep red blood cells (SRBC 5×10^9 cells per ml) were used for immunizing the animals that belongs to immunized groups. In Triphala administration (1g/kg/d for 48d), A.I was found to be significantly enhanced in the Triphala group, while the remaining neutrophil functions and steroid levels were not altered significantly. However the neutrophil functions were significantly enhanced in the Triphala immunized group with a significant decrease in corticosterone level was observed. Upon exposure to the noise-stress, the neutrophil functions were significantly suppressed and followed by a significant increase in the corticosterone levels were observed in both the noise-stress and the noise-stress immunized groups. These noise-stress-induced changes were significantly prevented by Triphala administration in both the Triphala noise-stress and the Triphala noise-stress immunized groups. Thus oral administration of Triphala appears to stimulate the neutrophil functions in the immunized rats and stress induced suppression in the neutrophil functions were significantly prevented by Triphala [24].

Antimutagenic activity

The antimutagenic potential of chloroform and acetone extracts of Triphala has been evaluated in an Ames histidine reversion assay using TA98 and TA100 tester strains of Salmonella typhimurium

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against the direct/acting mutagens, 4/nitro/o/ phenylenediamine (NPD) and sodium azide, and the indirect/acting promutagen, 2/aminofluorene (2AF), in the presence of phenobarbitone/ induced rat hepatic S9. The study revealed that chloroform and acetone extracts showed inhibition of mutagenicity induced by both direct and S9/dependent mutagens. A significant inhibition of 98.7% was observed with acetone extract against the revertants induced by S9/dependent mutagen, 2AF, in co/incubation mode of treatment [25].

Antitumor activity

Growth-inhibitory effects of Triphala were evaluated in Capan-2, BxPC-3 and HPDE-6 cells by Sulphoradamine-B assay. Apoptosis was determined by cell death assay and western blotting. Triphala was administered orally to nude mice implanted with Capan-2 xenograft. Tumors were analyzed by immunohistochemistry and western blotting. Exposure of Capan-2 cells to the aqueous extract of Triphala for 24 h resulted in the significant decrease in the survival of cells in a dose-dependent manner with an IC50 of about 50 µg/ml. Triphala-mediated reduced cell survival correlated with induction of apoptosis, which was associated with reactive oxygen species (ROS) generation. Triphala-induced apoptosis was linked with phosphorylation of p53 at Ser-15 and ERK at Thr-202/Tyr-204 in Capan-2 cells. Above mentioned effects were significantly blocked when the cells were pretreated with an antioxidant N-acetylcysteine (NAC), suggesting the involvement of ROS generation. Pretreatment of cells with pifithrin-α or U0126, specific inhibitors of p53 or MEK-1/2, significantly attenuated Triphala-induced apoptosis. Moreover, NAC or U0126 pretreatment significantly attenuated Triphala-induced p53 transcriptional activity. Similarly, Triphala induced apoptosis in another pancreatic cancer cell line BxPC-3 by activating ERK. On the other hand, Triphala failed to induce apoptosis or activate ERK or p53 in normal human pancreatic ductal epithelial (HPDE-6) cells. Further, oral administration of 50 mg/kg or 100 mg/kg Triphala in PBS, 5 days/week significantly suppressed the growth of Capan-2 pancreatic tumor-xenograft. Reduced tumor-growth in Triphala fed mice was due to increased apoptosis in the tumors cells, which was associated with increased activation of p53 and ERK [26].

Hypolipidaemic activity

Hypercholesteremia is one of the risk factors for coronary artery disease. The efficacy of Triphala on total cholesterol, Low density lipoprotein (LDL), Very low density lipoprotein (VLDL), High density lipoprotein (HDL) and free fatty acid was studied in experimentally induced hypercholesteremic rats. Four groups of rats were employed namely control, Triphala treated, hypercholesterolemia rats (4% Cholesterol+1% cholic acid + egg yolk) and Triphala pre-treatment in hypercholesteremic rats. Results showed significant increase in the total cholesterol, LDL, VLDL, and free fatty acid in hypercholesteremic rats were significantly reduced in Triphala treated hypercholesteremic rats. The data demonstrated that Triphala formulation was associated with Hypolipidaemic effects on the experimentally induced hypercholesteremic rats[27].

DISCUSSION

Aging is considered to be a passive process that results from two opposite phenomenon: a deterioration process occurring at molecular, cellular and organism levels due to exposure to damaging agents on the one hand, and the action of many enzymatic and non-enzymatic systems that attempt to counteract such deterioration on the other. The more efficient the system dealing with the deteriorative

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process, the longer will be the life of the organism and the slower will be the ageing process. As mentioned above, Triphala possesses strong anti-oxidant and anti-inflammatory properties which possibly can counteract the two most important causes of ageing, namely inflammation and oxidative stress. Triphala can thus be considered as a possible tool to delay ageing as well as the onset of many age-related diseases.

Oxidant by-products of normal metabolism cause extensive damage to DNA, protein, and lipid which is a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts. The free radical theory of ageing suggests that the presence of free radicals causes extensive damages to our body cells. Antioxidants are chemical substances that donate an electron to the free radical and convert it to a harmless molecule. Antioxidants intercept free radicals and protect cells from the oxidative damage that leads to aging and disease. Antioxidants prevent injury to blood vessel membranes, helping to optimize blood flow to the heart and brain, defend against cancer-causing DNA damage, and help lower the risk of cardiovascular disease and dementia, including Alzheimer's disease.

The polyphenols are thought to be responsible for many of Triphala's effects. Gallic acid, a major polyphenol in Triphala, has antioxidant property. Triphala also increased the reactive oxygen species (ROS) in breast cancer cells (MCF-7 and T-47D), resulting in apoptosis. Terminalia chebula, one of the components of Triphala, was shown to be a potent hyaluronidase and collagenase inhibitor that prevented degradation of cartilage. Triphala also protected mice from radiation-induced mortality. Oral administration of Triphala enhanced the immune functions in rats.

The presence of a number of known antioxidants such as flavonoids, tannins and glycosides in Triphala could be a primary reason for its antioxidant properties. The antioxidant and anti-inflammatory bioflavonoids promote the health of the circulatory system by reducing platelet aggregation, strengthening vascular membranes and protecting cell membranes. Triphala, having potent antioxidant and radioprotecting ability due to the presence of polyphenols, has been found to be an excellent scavenger of hydroxyl radicals and super oxide radicals, whose excessive formation is implicated in oxidative stress and exposure to radiation.

Many scientific studies have indicated that an association exists between inadequate antioxidant status and increased risk for or poor outcome of several age-related diseases, including Alzheimer's Disease, stroke, Cardio Vascular disorders, osteoporosis, cancer, osteoarthritis, degenerative diseases of the eye, and peripheral arterial disease [28, 29, 30]. The strong antioxidant properties of Triphala combined with its analgesic, antipyretic, chemo preventive, antidiabetic, antimutagenic and wound healing properties play a very important role in the prevention, interception and repair of the effects of many of these age-related diseases. Therefore, the drug Triphala can be used for both preventive and curative purposes in the management of many common geriatric diseases very effectively because of the abovementioned pharmacological and therapeutic properties. In fact, it has been traditionally used for these purposes since ancient times in the Ayurvedic system of medicine.

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