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Anticancer Activity in Lawson lainnermis (Henna)

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Abstract – Every year cancer cases are increasing in leaps and bounds. No country is spared from its deadly paw. Cancer treatment is expensive; its medicines are costly and sometimes scarce in the market. Hence, the invention of alternative treatments and discovery of naturally available medicines are going on. Discovery of anti-cancer alkaloids like vinblastine, vincristine, and podophyllotoxins in the 1950s helped the researchers to develop anticancer agents from naturally available products. Mehandi or Lawsonia inermis (L.) is a popular cosmetic product whose usage is known to Asians especially in the Indian subcontinent for centuries. But, Henna has a number of medicinal properties too. It is anti-carcinogenic, antimicrobial, anti-inflammatory, analgesic, antipyretic, antituberculostatic, and hepatoprotective. Many researchers have discussed about the anticancer and chemo-preventive features of Henna. One of the major constituent of Henna is Lawsone. It is used as an introductory material in the systhesis of a number of anticancer drugs such as atovaquone, lapachol, and dichloroallyl lawsone. Henna also contains quite a few other important chemicals such as isoplumbagin, apigenin, apigenin glyco-sides, luteolin, luteolin-7 glucosides, p-coumarin and lupeol among which many are reported for their chemopreventive and cytotoxicity action against different kinds of cancer cell. In this review, we are providing a report on palingenesis of information on the anticancer potential of Henna or Mehndi. We have gathered information related to in vivo and in vitro studies. We have used the internet, articles, text books, and research papers as resources for information in this regard. This present review summarizes some significant findings on the anticancer prospective of Mehndi or Henna. We think more works are necessary in this regard. Future research works on novel molecules extracted from Mehndi or Henna may offer great expectation for discovering new cancer chemo-therapeutic and/or chemo-preventive agents from this extraordinary plant.

Keywords: Anticancer, Chemoprevention, Henna, Lawsone, Lawsonia Inermis, Photochemistry.

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I. INTRODUCTION

The occurrence of cancer is promptly mounting worldwide. Presently, it rants second cardiovascular or heart diseases in terms of number of incidents registered worldwide. Both developed and developing countries are experiencing the ill-effects of this deadly disease. Inadequate supply of anticancer drugs, high-priced treatments, and fatal adverse effects of a number of available drugs in this genre has shown the way to take on complementary and different medicines for the treatment and/or prevention of this deadly disease [1]. More than 25% of the medicines used these days are formulated from natural sources. For example, taxol from Taxusbaccata (Yew), vincristine and vinblastine from Catharanthusroseus (Sadabahar), podophyllotoxin from Podophyllumpeltatum (Mayapple), aspirin from Sahennax species (Willow bark), digitalis from Digitalis purpurea (Foxglove), pilocarpine from Pilocarpus jaborandi (Jaborandi or Indian hemp) [2].

Plants are necessary component of human society. It is extensively used in traditional medicines for the treatment of many diseases. As a result, hundreds of species of plants have become a part and parcel of several from of indigenous treatments such as Ayurveda, Unani, Siddha, and Homeopathy which is popularly known as AYUSH Plants. These plants are rich in secondary metabolites and vital oils have been studied during the last thirty years or more for finding their practicability as the potential sources of drugs for different diseases [3].

Lawsonia inermis L. (Lythraceae), a monotypic genus, commonly recognized as ‘Mehndi’ or ‘Henna’ is popular as a cosmetic as well as medicinal agent for centuries. The extracts and purified ingredients of Henna in traditional medicines accounts for a wide range of activities including antibacterial [3], antifungal, antioxidant, and immunomodulatory, [4] hepatoprotective [5] analgesic, anti-inflammatory and anti-pyretic and

cytotoxic [6]. The bactericidal and fungicidal action of this plant has been attributed through its tanning effect [7]. It has also been established that Henna has no side-effects. It is not an allergen or a carcinogen [8]. The key colouring agent present in Henna leaves is a red-orange pigment known as lawsone (2-hydroxy-1, 4-naphthoquinone) [9], which makes this plant functional for dying of hair, as well as to colour different parts of the body such as fingers, fingernails, and soles [10]. The pigment, Lawsone is also an appropriate reagent for uncovering latent finger marks on paper in criminology [11]. Lawsone (2-hydroxy-1, 4-naphthoquinone) is a preliminary material in the synthesis of many clinically useful anti-cancer compounds such as atovaquone, lapachol and dichloroallyl lawsone [12].

Both the genres of Unani medicine and that of Ayurveda has clearly emphasized that Henna can be a great source to derive therapeutic agents that can work great for cancer, blood tonic requirements, infectious diseases, tuberculosis, inflammation and also tumors. However the needs to be given in single decoction or henna extract that might be blended with other molecules as well. Converting henna compounds into modern drugs has given rise to especially effective therapeutic agents. The lawsone derivates that has been described earlier has quantified proving item affectivity against a number of types of cancers. Lawsone is an element that has certain inherent antioxidant and anti-inflammatory properties that can be a major anti-cancer drug.

The Lawsonia inermis is a plant with medicinal properties found in various parts of the world (Lythracea). The leaves of henna sap can be made into a powder that is used for staining, nails, hair and beard [13]. The leaves of thus plant is also used for treating problems of poliomyelitis and the ailments of measles amongst the people of Yoruba tribe found in South Western Nigeria [14]. The seeds of the plant is also said to have deodorant properties that are used for the purpose of treating gynecological disorders such as vaginal discharge, meorrhagia and leucorrhea [15]. Tribes of Andhra Pradesh in India makes a paste out of the leaves of henna that is mixed with proportions of Hibiscus rosa-sinensis, Ecliptaprostrataand seeds of Abrusprecatorius so that a nutritious paste for the hair can be made. Henna is the extracts of Lawsonia plant is a great source of air dye and also a nail dye that is used in many parts of the world such as Turkey. It is one of the most popular sources of dyeing in the global cosmetic industry. Additionally the methanolic roots that is extracted from the Lawsonia plant is not just used for cosmetic purpose in Nigeria but at the same time it also has a number of other properties such as used in ant malarial treatments and also for abortifacient purposes [16]. The seeds of the plant when roasted and then crushed into a powder can be mixed with gingerly oil to make a great treatment option for ringworms. The leaves can be made into a decoction for cleaning wounds as an antiseptic. Its use as a blood tonic is also much popular [17]. The Lawsonia inermis is a plant that has a branched glabrous look. It can often come in the form of a small tree no taller than 2 to 6 meters in its height. It can also have a spiny look. The young branches coming out of the tee has a quadrangular look with a green shade. However these branches often turn red with age. The leaves of the tree are opposite in nature with an entire and sub sessile look. It has an 1.5-5 x 0.5 – 2 cm with a glabrous look. The vein that appears on the upper surface has a depressed appearance. The tree often has white small flowers that can be numerous in their volume and might appear in a pyramidal terminal. They are fragrant in their nature and are of the dimension of about 1 cm if measures across their four petals often crumpled at the stage of a bud. It has a 2 mm calyx tube and its spread lobes comes to a measure of 3mm. the petals can be of a ovate shape and also orbicular occasionally. They can be red or white in color and they have 8 stamens that are inserted in pairs around the region of the calyx tube rim. The ovary is divided into 4 cells and the style is often of the height of 5mm. these plants have small brown color fruits with a capsule of 4- 8mm diameter. They are often many seeded that has irregular opening and are split in 4 sections. The seams are often 3 mm in diameter and have an angular shape with a thick coat. These Lawsonian inermis plants can be found in various parts of Central Africa and Sahel. You can also find them in the Middle East. These plants can be found in waterways and also in semi-arid regions and can adapt to a large number of environmental conditions. The plant can survive extreme conditions like drought and low air humidity. The seeds of the plant germinate best in high temperatures [18].

Although it has been by and far agreed that henna extracts has a number of medicinal qualities, very less has been done to find out and explore about its anti-cancer properties. In the course of this discussion we make an attempt of the same through an analysis of the available vitro and vivo studies on the said domain.

A. Information Retrieval

In the course of this investigation various sources like articles, texts, research papers, summaries, notes, books, per reviews, abreacts are used to get all the literature and information through them. These information has been categorically found by looking for information under classified actions of the internet gaunt and also other literature sources using the keywords ‘Lawsonia inermis’, ‘Mehndi and/or ‘Henna as anticancer agent’, ‘anticancer activity of mehndi/henna’, ‘cytotoxicity of mehndi/henna’, ‘Ex-tracts/compounds in Mehndi/henna as anticancer agent’ ‘Anticancer/cytotoxicity activities of mehndi/henna extract and essential oil’, ‘Mehndi/henna and

cancer cell lines', 'Mehndi/henna and cancer/tumor', 'chemopreventive properties of mehndi/henna'. Much information has also been derived using the sources and the information available from the large data gamut of NISCAIR, SCIELO, PUBMED, SCOPUS, INFLIBNET, Sci-Finder, Science Direct and Google Scholar.

B. Phytochemistry

The inherent properties that can be found in henna is due to the presence of the chemical, lawsone. There are other forms of molecules that are also available from this plant such as qui-nones, phenylpropanoids, flavonoids, terpenoids, phenolics, fatty acids, carbohydrates, proteins, tannins, alkaloids, xanthones, coumarin, glucosides, naphthoquinone, saponins, triterpenoids, sterols and dioxin derivatives. Other elements like isoplumpagin (a naphthaquinone from bark), lupeol, 30-norlupan-3-ol-20-one, betuhennan, betuhennanic acid and n-tridecanoate (bark), phenolic glycosides, lawsoniaside, β-sitosterol and stigmasterol is also available from this group of plants, 24-beta ethyl cholest-4-en-3-beta-ol have also been reported from the roots of henna (Gupta et al., 1992). The seeds of Mehndi/Henna contain ~7.5 % viscous oil possessing palmitic, behenic, arachidic, stearic, oleic and linolenic acids. Bioactivity guided fractionation of methanolic seed extract lead to isolation of two new triterpenoidslawnermis acid and its methyl ester. The leaves of Mehndi/Henna also contain apigenin-7-glucoside, apigenin-4-glycoside, luteo-hennan-7-glucoside luteolin-3-glucoside [19]. The essential oil of Lawsonia inermis seeds contains about 23 components revealed in GC-MS analysis.

The principal components were Tridecane (7.7%), phytol (10.30%), Hexadecane (14.88%), Tetradecane (16.77%), Heptad cane (23.48%). Structures of the reported anticancer molecules from Lawsonia inermis and some of the derivatives of lawsone are given in Figure below.

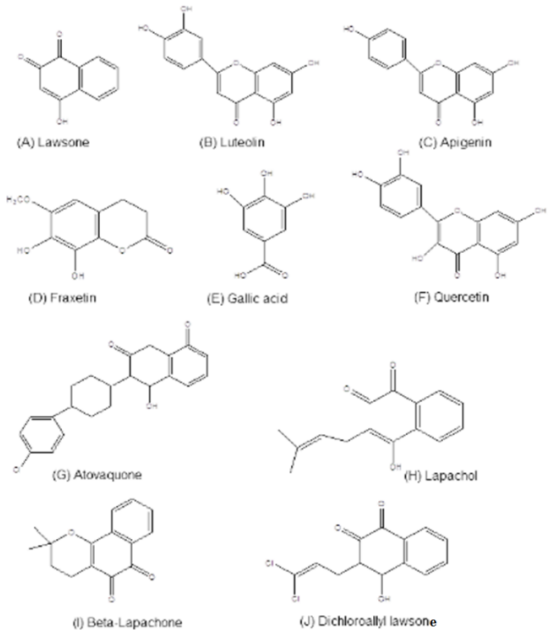

Figure 1: Reported anticancer molecules from Lawsonia inermis and some derivatives of lawsone. (A) Lawsone, (B) Luteolin, (C) Apigenin, (D) Fraxetin, (E) Gallic acid, (F) Quercetin, (G) Atovaquone, (H) Lapachol, (I) β-lapachone, (J) Dichloroallyllawsone.

II. ANTICANCER/CYTOTOXICITY ACTIVITIES OF LAWSONIA INERMIS EXTRACT AND ESSENTIAL OIL

It has been seen that Lawsonia inermis or the plant of henna has certain and definite anti-cancer properties which is why it can be a major drug in the treatment of various types of cancer. Let us take a detailed look at this part of the research.

A. Vitro Study On Different Cancer Cell Lines

New researches done into the domain of apoptic pathway and oncogene have revealed the fact that genetic basis and molecular aspects can play a vital role in finding suitable drugs for cancer treatment, where the drugs will be targeting the cancer cells and will leave out the normal ones [20]. A study has been conducted on the human liver cells that has revealed the anti-cancer properties of mehendi leaves which exhibited a concentration of 20 and 30 mg/ml. the essential oils as derived from the henna leaves shows the presence of cytotoxicity on HepG2 with an IC50 value of 24µg/mL in MTT assay. Similarly, in vivo experiment revealed that Henna reduces chemical-induced hepatocarcinogenesis in rat model. Likewise, chloroform extract of Mehndi leaves showed cytotoxicity on HepG2 and MCF-7 (hormone-dependent breast cancer cell line) with an IC50 value of 0.3 and 24.8µg/mL respectively. The effect of Mehndi/Henna extract on expression of c-myc gene was also studied and it was observed that the

gene was not expressed in cell (HepG2 and MCF-7) treated with 20 and 30µg/mL of crude extract [21].

B. In Vivo Study In Different Tumour Model Of Rodent

Ozaslan et al (2009) has clearly pointed out the effect of henna powder on the cells of cancer in body, and the effect they have on intracellular free radical elements on the body and the influence on the hydrogen peroxide level of the body. It has been seen that cancerous cells requires a high H= concentration which is reduced by the henna powder due to its oxidative nature. The ethanolic extracts from the roots of henna has antitumoral activities as introduced and explained by the Swiss albino mice model. The body weight, tumor volume, packed cell volume and viable cells were brought back to basal level which was comparable to vincristine (1mg/kg bw), a potent anticancer drug. The experimental finding of the study revealed the reversal of the immunological and pathological abnormalities like increased WBC, platelet, lymphocytes, ALT, AST, ALP, LDH & decreased level of Hb, RBC, PCV, monocytes and differential count in treated mice compared to control group. Investigations have revealed the fact that extracts of the henna increases the life span of the DLA tumor ad enhances the level of antioxidants and also brings a check in the lipid profile [21]. Dasgupta, (2003) has also done an investigation on the modulatory effect of henna leaves and the enzymes that has been derived from them. 200 to 400 mg/kgbw 80% ethanolic extracts that has been derived from henna leaves have been used to observe the metabolization effects in I and II phases on the liver of a six week old Swiss albino mice. The anti-cancerous effects of the henna leaves was also investigated using the benzo protocol (a) pyrene-induced fore stomach and 7, 12 dimethylbenz (a) anthracene (DMBA)-initiated and croton oil-promoted skin papillomagenesis. The outcomes showed that in the phase II detoxification of the liver carcinogen in the mice had taken place that reveals the duel acting nature of the henna extracts. A decrease in the tumor incidence has also been observed. It also signified the cancer chemo preventive nature of the plant extracts [22].

III. ANTICANCER ACTIVITIES OF LAWSONE AND ITS DERIVATIVES

Lawsonia inermis has 2-hydroxy-1, 4-naphthoquinone which is key molecules of the plant extracts that can be used in the process of making various types of anticancer medicines and drugs such as Atovaquone, Lapachol and Dichloroallyllawsone [23]. Juglone and lawsone is said check the growth of human colon cancer cells through doing and effective blocking of the S-phase of cell cycle. This has been observed during the cytometric study. Furano-1, 2- naphthoquinone synthesized from 2-hydroxy-1, 4-naphthoquinone and chloroacetaldehyde promotes a check of the lung cancer cells that happens through meditating of the G92) M cell cycle [24].

Further researchers on mice with carcinogenesis model has revealed that henna leaf extracts seemed to have a much better effect on the growth of the tumors. It has been seen that tumor incidence seemed to go down by a total 66% and multiplicity by 40%. This happens to be much better than what has been received after an application of radiation. Similar positive effects have also been revealed when henna powder was topically applied on mouse skin with carcinogenesis [25]. Amino derivatives from lawsone and lapachol were found to be much effective against the cells of leukemia. It seemed to have beneficial anti-cancer properties that could work well to reduce tumors. Henna leaves extracts also seems to have chemotherapeutic agents.

Table 1: Reported anticancer/cytotoxicity mechanism of Lawson iainermis extracts, Lawsone and its derivatives

Type of extract/compound	Model/method of study	Type of Cancer	Result	Probable mechanism	References
Ethyl acetate and petroleum ether extract of leaves	*In vitro*; cytotoxicity assay by (3H)-hypoxanthine incorporation assay	Breast cancer (MCF-7 cell lines)	Active against MCF-7 cell line at dose of 22 and 27 mg/mL	Not mentioned	(Babli et al., 2013)
Essential oil and chloroform leaf extract	*In vitro* cell viability assay by tryptan blue and tunel assay for apoptosis	Liver cell (HepG2)	Henna extract and essential oil lead to shrinkage of cell, condensed nucleus and also produced apoptotic bodies.	Induction of apoptosis	(Endrini et al., 2011)
Ethanolic root extract	*In vivo*; Dalton's lymphoma ascites model in swiss albino mice at dose of 180 mg/kg bw	Lymphatic cancer	Significant antitumor activity	Due to antioxidant activity and reduced the lipid profile	(Priya et al., 2011)
Henna leaf powder	*In vivo*; Ehrlich ascites tumour model in swiss albino mice at dose of 0.3% w/w	Gluteal sarcoma	Lawsonia inermis suppresses tumour and delayed survival time in mice.	Promotion of apoptosis due to oxidant effect by enhancing intracellular level of H_2O_2 and free raicals.	(Ozaslan et al., 2009)
Leaf extract	*In vitro*; exposure of broad spectrum light on human leukaemia cell-line HL60 at dose of 20 µg/mL	Human promyelocytic leukemia cells	Significantly effective in protection of phototoxicity	Not mentioned	(Ong et al., 2009)
Chloroform extract of dried leaves	*In vitro*; Microculture tetrazolium salt assay (MTT)	Liver (HepG2) and Human breast (MCF-7) cancer cell lines	Henna was found to be cytotoxic to liver cancer cell line and hormone dependent breast cancer cell MCF-7 (IC_{50} - 0.3 and 24.85 µg/mL respectively)	Cytotoxicity was found to be mediated by the down regulation of c-myc expression	(Endrini et al., 2007)
Essential oil from leaf	*In vitro* cell cytotoxicity assay by MTT	Liver (HepG2), breast (MDA-MB-231, MCF-7) and colon ($CaCO_2$). cell lines	Strong cytotoxicity on HepG2 (liver cancer) cells with an IC_{50} value of 24 µg /mL	High antioxidant activity speculated due presence of terpenoids like phytol and hexahydrofernysyl acetone.	(Rahmat et al., 2006)
80% ethanolic leaf extract	*In vivo* by Benzo (a) pyrene-induced forestomach and 7,12 dimethylbenz (a) anthracene-initiated and croton oil-promoted skin papillomagenesis in swiss albino mice	Skin cancer	Chemopreventive potential of henna was confirmed by significant inhibition of tumor burden and reduction of tumor incidence in both the model.	Selective activation of Phase-II and inhibition of phase I metabolic enzymes. Naphthaquinone is the major constituen of henna and might be involved in induction of DT-diaphorase.	(Dasgupta et al., 2003)

Type of extract/compound	Model/method of study	Type of Cancer	Result	Probable mechanism	References
Lawsone	*In vitro*; Inhibition of EBV-EA activation assay, and cell viability assay	Skin Cancer	Lawsone was found effective in reducing the effect of DMBA induced, TPA promoted mouse skin carcinogenesis. It also demonstrated chemopreventive potential against skin tumors induced by UV-B radiation, chemical carcinogen, DMBA and PON.	Inhibitor of TPA induced EBV-EA activation in cultures of Raji cells and suppress the tumor promotion.	(Kapadia et al., 2013)
	In vitro; Cytotoxicity assay on HCT-15 and flow cytometric anlysis	Colon cancer	IC_{50} ~ 12.5μg/mL was repored; anthraquinones with 2 or 3 OH groups were found to better than with no OH group	Inhibition of S-phase of cell cycle	(Kamei et al., 1998)
Furano-1, 2-naphthoquinone	*In vitro*; cell viability assay and flow cytometric detection	Lung cancer	Significant cytotoxicity of A549 cell and promote apoptosis	Cytotoxicity was mediated with the G2/M cell cycle arrest and apoptosis vta inactivation of EGFR-mediated signaling pathway and up-regulation of Bax and down-regulation of Bcl-2	(Chien et al., 2010)
	In vitro; cell vtability assay and flow cytometric detection	eukemia /*metabolter	Altered mitochondrial membrane potential, released of cytochrome C and activation of caspases 3 and 9.	Bring back apoptosis in Ca9-22 cells via inactivation of the EGF receptor	(Chien et al., 2010)
Atovaquone derivative	*In vitro*; MTT cytotoxicity assay on Du145 human prostate cancercell line	Prostate cancer	Showed significant cytotoxicity against and promote apoptosis in prostate cancer cell line	Tempted apoptosis vta activation of pro-apoptotic caspases 9 and 3.	(Zhou et al., 2009)
Lapachol	Chick embryo chorioallantoic membrane model	Cervical cancer (HeLa cells)	At concentration of 400ug/ml, lapachol changes the protein profile and inhibited the invasiveness of HeLa cells in CAM model.	It reduces cancer metastasis in HeLa cells.	(Balassiano et al., 2005)
Dichloroallyl lawsone	Two-dimensional chromatographic procedure.	Mouse lymphocytic leukemia (L1210) cells	Powerful inhibitors of nucleotide bio-synthesis	It prohibited the conversion of UMP UDP by potent inhibition of dihydroorotate – orotat	(Kemp et al., 1986)

IV. REVIEWS

Henna is best known for being a natural coloring agent that gives a brown dye. This dye is attained from the leaves of the tree of lawsonia inermis. 2-hydroxy-1, 4-naphthoquinone is the main ingredient in henna in lawsonia inermis that gives off the active brown dye. This is a dye that is used in both the Hindu and the Islamic cultures for the purpose of dyeing the hair and coloring and decorating the palms, the feet and the nails. This is a great ingredient that can be used for the purpose of making temporary tattoos on the skin. Henna is seldom known to cause any allergy for any type of skin or any part of the human body. Even if you do get an allergic reaction you can be sure of the fact that this is not due to the natural qualities and the features henna as an element but due to the chemical additives that are mixed into the powder of the henna leaves so that a darker more vivid color can be attained. Some of the common additives that are often mixed with henna powered are diaminobenzenes and daiminotoluenes. In some rare cases it has been seen that allergic reactions can arise out of contact between henna powders with that if dermatitis which was actually used to get some relief from rheumatic pain [26]. Use of henna for the purpose of creating temporary tattoos have been quite popular in the recent times henna leaves comes from Lawsonia inermis that comes from a plant belongs to the Lythraceae family. For henna tattoo a dye of henna powder is used. In its raw form the powder of henna leaves comes in the form of a dark green color. In the current times of commercialism the substance f-parar-phenylenediamine is mixed with the dry powder of henna leaves so that a darker color can be attained so that the whole mixture can dry up fast as possible, PPD can be one of the major elements that can cause allergy on the skin. There has been a case where a nine year old boy has been reported to have skin allergy after he got a tattooing done with the help of henna powder. Later it was found that the reaction occurred due to PPD contact with the skin. After the treatment with topical corticosteroid and oral antihistamines, the lesion cleared with discrete residual hypopigmentation [27].

Lawsonia inermis is a many branched small tree or a glabrous shrub that is mainly cultivated for its leaves. However the roots, the stem bark, the flowers and even the seeds of the plan have been used from ancient times for the purpose of making medicines. The plant is reported to contain nutrients like proteins,. Carbohydrates, flavonoids, proteins, genomic compounds, tannins, alkaloids, terpenoids, quinines, coumarins, xanthones and fatty acids. The plant has been reported to have analgesic, hepatoprotective, hypoglycemic, anti-inflammatory, antibacterial, immunostimulant, antimicrobial, , antiviral, antifungal, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic and anticancer properties. Henna is known to be one of the most helpful elements from which a number of medicines and drugs can be developed that can be used to fight several types of ailments and diseases [28].

V. CONCLUSION

Although henna is mainly used as a cosmetic element that is used for the purpose of dye and coloring hair and making tattoos and decorating various parts of the body it has a number of anti-cancer properties. This current research has been induced to make a detailed research into these domain further needs to be looked into so that more exploration can be made and more substantial information can be found in this domain. This is a domain that requires more work so better cancer treatments can be found in the future.

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