Available online at www.jobpcr.com

ISSN-2394:3076
CODEN(USA) : JBPCBK

Journal of Biological Pharmaceutical And Chemical Research , 2016,3(2): 1-12

(http://www.jobpcr.com/arhcive.php)

Nanotech Combination Chemoprevention Study Against Balb/c Mice Implanted with Sarcoma 180 Cancer Cells

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ABSTRACT

Cancer is one of the most dreaded diseases in 21st century. Nanoparticle based therapeutics exhibit more benefits when compared to its active phytoconstituent alone. It also widely used as an efficient chemotherapeutic agent for the treatment of cancer and related diseases. The present work has designed to test in vivo anticancer activity of gold nanoparticle embeded 3,6-dihydroxyflavone (GNDHF); lutein (LUT) and selenium methyl selenocysteine (MSC) in single and combination against sarcoma 180 cancer cells induced in female inbred Balb/c mice strain. In vivo anticancer activity was evaluated by antigenotoxic (Chromosomal aberrations test, Micronucleus test) and Physical observation ((Body weight, tumor volume and tumor delay time)) of Balb/c mice strain. The antioxidant assays were determined by reduced glutathione (GSH) and Lipid Peroxidation (LPO) assay. Among the various treatment considered for the study, combination of (GNDHF: LUT: MSC) 5mg/Kg body weight dose exhibited optimum antioxidant activity (0.11±0.54 µM) GSH per mg protein while (0.187±0.74 nM) MDA per mg wet weight and rendered significant protection against oxidative stress induced by sarcoma 180 cancer cells in liver tissues. Combination (GNDHF: LUT: MSC) significantly suppresses the tumor growth in terms of body weight: 28.12 gm±1.04; tumor volume; 70.12 mm^3 (42 % inhibition) at 5mg/Kg body weight dose. The study highlights that the combination of (GNDHF: LUT: MSC) is more promising cytotoxic bioagent against sarcoma 180 cancer cells induced in female Balb/c mice compared to the single compound and has enough potential for clinical applications.

Key words: Nanoparticle; Combination study; *in vivo;* Oxidative stress.

INTRODUCTION

Various etiological factors like genetics, family history, age, etc. and exposure to chemicals, including pesticides is associated with increased incidences of cancer. The present trend in the management of cancer development involves either reduction of the exposure of an individual to known carcinogen to the extent possible and or seeking advantage of the inhibitors of carcinogenesis for their eventual application as anticancer agents. Since exposure to the environmental carcinogens is often unavoidable, the latter field has been widely explored.

In view of the side effects and development of resistance against conventional therapeutic agents, cancer is considered to be a complex disease to which combination therapy including the use of

phytochemicals might be more encouraging. The pharmacological efficacy of plant based products has created a revolutionary interest and awareness among the medical community. Several biologically active compounds in a plant work together to produce greater effect than single chemical and take care of other health aspects like short term energy, long term endurance or weight control also. Chemical partnership in the plant extract is the reason to believe that plant constituents might inhibit cancer growth when used in combination. The synergistic effect of various components of plant material may enhance the therapeutic effect simultaneously reducing the side effects [Lal et al., 2011; Ulrich-Merzenich et al., 2010].

Experimental breakthrough involving the use of two or more bio agents have been recognized recently, providing enhanced therapeutic bioefficacy with reduced side effects [Bloland et al.,2000; Braham, 2011]. Multiple active phytochemicals into synergism were quite effective than that of single compound [Ojeswi et al., 2009]. The developments in combination therapies involving nano size enhanced therapeutic activities have shown enough scope of applications in medical sciences [Zhang et al., 2008; Katti et al., 2009; Medina-Ramirez et al., 2012]. The bioactive phytochemical embedded with gold nano particles have an emerging interdisciplinary area with potential applications of nano composites in therapeutic applications [Joshi et al., 2008; Nune et al., 2009].

In recent perspectives, cancer is considered a complex disease which requires a combination therapy. Flavonoids, a large group of natural polyphenolic compounds, are ubiquitously present in the human diet [Kok et ai., 2008] and belong to a chemically heterogeneous group of small molecules with chemopreventive activity [Balasundrama et al., 2010]. Selenium compounds act as sensitizer affecting various cellular pathways of inducing apoptosis [Emmanua et al., 2005; Fazaludeen et al., 2012]. Organically bound selenium enhances the efficacy and selectivity of anticancer drug against tumor [Clark et al., 1996; Chintala et al., 20-10]. The potential role of antioxidants to enhance lymphocyte proliferation, lowering of lipid peroxidation and suppression of tumor growth has occupied a significant position in combination mode of cancer therapy [Fleet, 1997]. Chemical partnership of biomolecules like flavonoid, selenium compound and antioxidant might believe to enhance anti cancer activity when used in combination.

As the use of *in vitro* results applicable for *in vivo* study [Chou, 2010], present paper describes the effect of combination and nanotech reinforcement of **dietary flavonoid** (3,6-dihydroxyflavone), **sensitizer** (selenium methyl selenocysteine) and **antioxidant** (lutein) for the enhancement in anticancer activity against sarcoma 180 cancer cell induced in female Balb/c mice strain.

Our recent studies shown that combination of GNDHF: LUT: MSC, significantly inhibits survival of MCF-7 and MDA-MB-468 breast cancer cell line, whereas proliferation of a normal human lymphocyte is minimally affected by combination of GNDHF: LUT: MSC even at concentrations that are highly cytotoxic to breast cancer cells. Based on these observations, we predicted that above combination administration could inhibit growth of sarcoma 180 cancer cell induced in female inbred Balb/c mice. Furthermore, the results of the present study indicate that the combination of GNDHF: LUT: MSC is associated with increased apoptosis and reduced mitotic activity. To the best of our knowledge, the present study is the first published report to indicate *in vivo* anticancer activity of GNDHF: LUT: MSC combination in sarcoma 180 cancer cell model induced in female inbred Balb/c mouse strain.

MATERIALS AND METHOD

Animal and cell line

Female Balb/c mice of 8-10 weeks old age weighing 20±5 were maintained in ventilated animal house at Deshpande's laboratory, Bhopal (India). All the mice were kept at controlled

environmental condition (22±2 °C, 60±5 % humidity) with 12h light/dark cycle. They were provided with standard pallet diet and water *ad libitum*. Sarcoma 180 cancer cells were obtained from the National Centre for Cell Sciences, Pune. These cells were maintained in Dulbecco's Modified Essential Medium supplemented with antibiotics, L-glutamine and Fetal Calf Serum. All the chemicals and reagents used were of high purity. The animal experiments were carried out under the guidelines of Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Animal experiment, treatment and monitoring

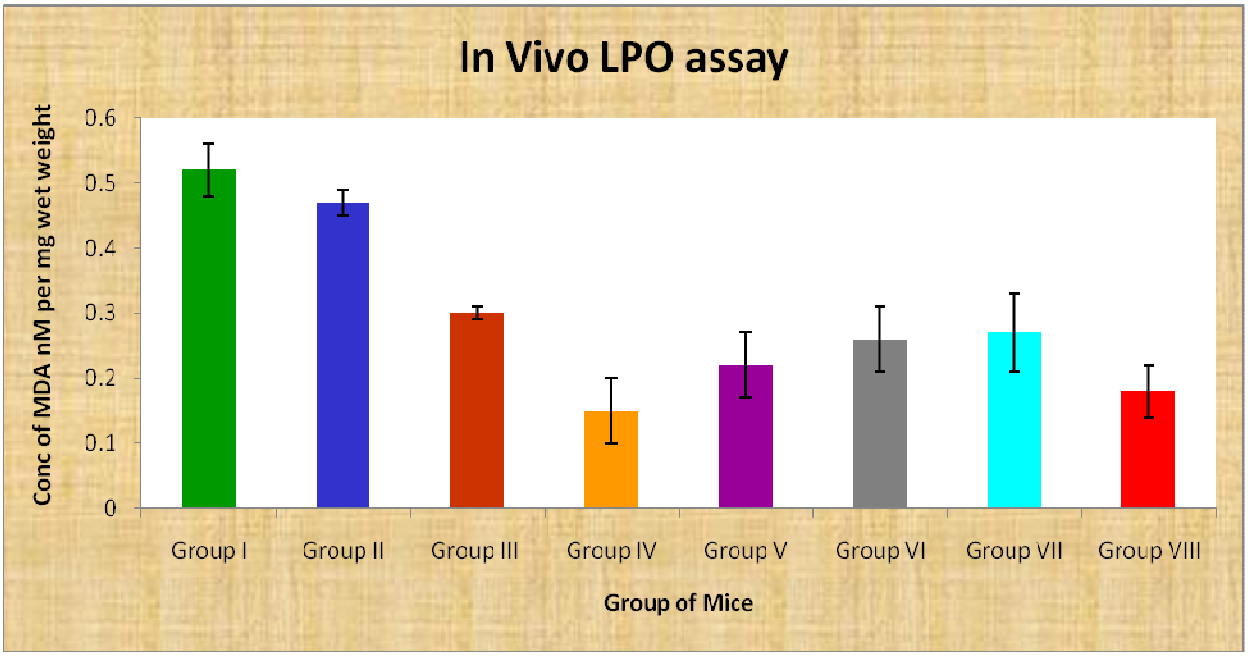
To investigate the effect of test compounds against reference anticancer drug (doxorubicin), mice were randomized and divided in to 8 groups of 6 animals each. Group I kept as normal control group and Group II-VIII were injected with Sarcoma 180 cancer cells (4×10^5) subcutaneously (s.c.) into the right flank using a 21 G injector of day zero. Mice were injected intraperitoneally with Group II: saline; Group III: 2 % dimethyl sulphoxide; Group IV: doxorubicin (1 mg/Kg body weight in 6 doses on the 1^{st}, 5^{th}, 9^{th}, 13^{th}, 18^{th} and 23^{rd} day of treatment); Group V: GNDHF ; Group VI: LUT ; Group VII: MSC and Group VIII: [GNDHF: LUT: MSC] (5 mg/Kg body weight). The incorporation of (4×10^5) viable cells (highly proliferative and metastatic cancer cells) is likely to complete one mitotic cycle with in 48 h and develop significant tumor within 5 – 6 days [Annual report, 2009]. Therefore, this regimen was considered for screening of anticancer activity. Doxorubicin induces apoptosis by induction of DNA fragmentation and cell shrinkage in tumor cells and has been in use for more than 30 years in treating a variety of malignancies [Gewirtz, 1999; Elmore et al., 2002; Skladanowski & Konopa, 1993] therefore, has been considered reference drug for the present study. Local tumor growth was determined by measuring diameter with calipers every other day, starting with the day when tumor became palpable. Sarcoma 180 cancer cells injected subcutaneously into mice when grew to average size of tumor volume 2000 mm^3 in the control group. Tumor volume (mm^3) was estimated by the formula: $4/3 \times \pi \times [(1/2 \times \text{smaller diameter}) 2 \times (1/2 \times \text{larger diameter})]$ [Feleszko et al., 2002]. Tumor growth delay was determined [Corbett et al., 1997] and was calculated as follows: Tumor growth delay = T−C, where T represents median time (in days) required for the treatment group tumors to reach a volume of 100 mm^3 and C represents median time (in days) required for the control group tumors to reach the same size. Body weights (b.w.) of all animals were measured every alternative day during treatment period to detect life threatening toxicity by test samples and reference drug. Mice in all groups were observed daily for survival and sacrificed at 29^{th} day after the experimental schedule. The tumors were dissected, weighed and stored at −80 °C until analysis was completed. To examine the histopathology, tumors from each group of animals were removed and fixed in 10 % formalin solution for 24 h. Tissues were then embedded into paraffin. A section (4 μm) was stained with haematoxylin and eosin and examined under a microscope [Chen et al., 2005].

Assay for reduced glutathione (GSH)

The liver were dissected, weighed and homogenized in saline (154 mM, Potassium chloride) to give a 10 % homogenate (w/v). The crude homogenate was centrifuged at 2000 rpm for 15 minutes and supernatant was collected. Phosphate ethylene diamine tetraacetate buffer (0.9 ml) and 5, 5'-dithio-bis-(2-nitrobenzoic acid) solution (0.050 ml) was added to supernatant (0.050 ml) making the solution 1.0 ml. The reaction mixture was incubated at room temperature for 20 min and the optical density was measured at 410 nm. The GSH levels were monitored by the reduction of 5, 5'-dithio-bis-(2-nitrobenzoic acid) DTNB to 5-thio-2-nitrobenzoate (TNB) [Brown & Jeffries, 1975; Beutler, 1984].

Assay for Lipid Peroxidation (LPO)

After euthanasia of mouse, the liver tissue was homogenised in 1.15 % potassium chloride solution by homogeniser (1 gm of tissue in 9ml of 1.15 % potassium chloride solution). Sodium Dodecyl Sulphate (8.1 %) was added to 0.2 ml of sample in test tube and pH was adjusted to 3.5 with 5N sodium hydroxide. To this, 1.5 ml of 0.8 % aqueous solution of thiobarbituric acid was added and mixture made up of 4 ml with distilled water and heated at 95 °C for 60 minutes. After cooling under tap water, 1 ml of distilled water and 5 ml of mixture of n-butanol and pyridine (15:1) were added and shaken vigorously [Ohkawa et al. 1979]. The solution was centrifuged at 3900 rpm for 10 minutes. Upper organic layer was removed and absorbance was measured at 532 nm using UV-Visible Spectrophotometer.



Each value is mean ± SD (n=6). $p > 0.01$ vs. tumor control, $p < 0.05$ vs. normal control. NC: Normal Control, TC: Tumor Control, DOXO: Doxorubicin, GNDHF: Gold nanoparticle embedded 3,6-dihydroxyflavone, LUT: lutein, MSC: selenium methyl selenocysteine DMSO: Dimethyl sulphoxide.

Fig. 1 *In vivo* LPO study-effect of the gold nanoparticle embedded 3,6 dihydroxyflavone with lutein and selenium methyl selenocysteine (single and combination), against doxorubicin on conc of MDA nM per mg wet weight in normal and cancer tumor bearing mice at 29th day.

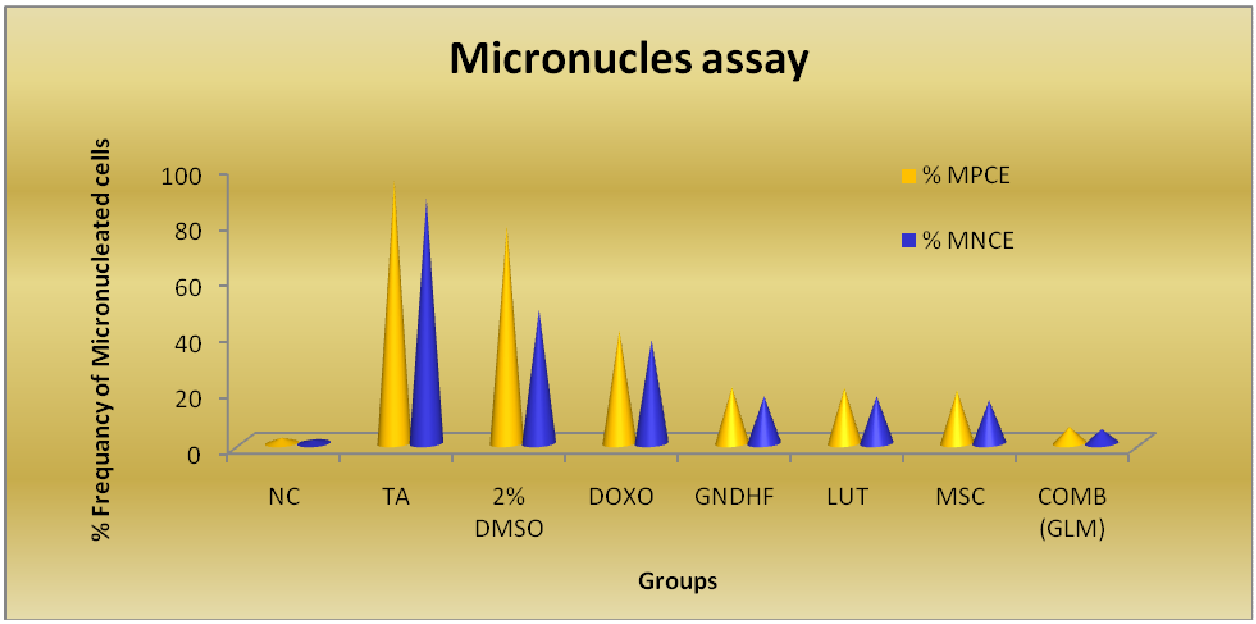
Chromosomal aberrations test

Cytogenetic damage in the bone marrow cells was studied by chromosomal aberration analysis at the end of experiment. All the animals were injected 0.025 % colchicine intraperitoneally and sacrificed 2 h later to arrest the cells in metaphase by cervical dislocation. The femurs were dissected and cleaned to remove adherent muscles. The bone marrow was flushed into centrifuge tubes through repeated aspirations with 2 ml medium using fine needle. After sampling of bone marrow from femurs of the animals, the cells were centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded completely and pellet was suspended in hypotonic solution (5 ml, 0.56 % Potassium chloride). These tubes were kept in water bath at 37 °C for 20 minutes. After incubation, cells were re-centrifuged at 1400 rpm for 5 minutes, the supernatant was discarded and the pellet was re-suspended in freshly prepared chilled Cornoy's fixative solution (2 ml, methanol : glacial acetic acid mixture in 3:1 ratio) and again centrifuged at 1400 rpm for 10 minutes [Maistro et al., 2010]. The pellet was re-suspended in fresh fixative and the process was repeated 2-3 times.

Cells kept for overnight fixation (4°C) were centrifuged for 1400 rpm for 10 minute again and the pellet was re-dispersed in fresh fixative (0.7-1 ml) depending on the amount of pellet. The cells were agitated and mixed thoroughly using Pasteur pipette and dropped on to the pre-cleaned chilled slides from a distance of 30-40 cm. The slides were left for air dry. The slides were dipped in 5 % giemsa solution for 10 minute and rinsed in double distilled water (DDW) and air dried. A total of 100 well spread metaphase plates/animal were analyzed for different types of chromosomal damage including breaks, fragments, exchanges and multiple aberrations including pulverizations at a magnification of (100X × 10X) for all treated groups. The slides prepared were used for the counting of Mitotic Index. Metaphase plates were prepared by the air drying method [Obe et al., 2002].

Assay for Micronucleus test

The bone marrow was flushed out using minimum essential medium, centrifuged and the pellet was resuspended in few drops of fetal bovine serum. Smears were prepared on pre-clean glass slides [Schmid, 1975], stained with May-Grawnwald (5 min) and followed by Giemsa stain (5% giemsa solution for 10 minute), rinsed in DDW and air dried. The number of (polychromatic erythrocytes) PCEs and (normochromatic erythrocytes) NCEs and the frequency of micronucleated PCEs and NCEs were recorded at a magnification of (100X × 10X) for all treated groups.



Each value is mean±SD (n=6). $p > 0.01$ vs. tumor control, $p < 0.05$ vs. normal control. NC: Normal Control, TC: Tumor Control,) DOXO treated, GNDHF, LUT, MSC, COMB (GLM) - GNDHF:LUT:MSC.

Fig. 2 Combination effect of gold nanoparticle embedded 3,6-dihydroxyflavone with lutein and selenium methyl selenocysteine and doxorubicin on percentage Micronucleated polychromatic and Normochromatic erythrocytes in normal and cancer tumor bearing mice at 29th day.

Statistical analysis

All experimental data were given as mean ± SD. Statistical analysis was carried out using the one-way Analysis of Variance (ANOVA). Post Dunnett test was applied between control, reference drug and test samples using Graph Pad Prism software. Probability values were found to be less than 0.05 ($p < 0.05$).

RESULT AND DISCUSSION

Cancer mediated modulation of GSH level

The down regulation level of reduced glutathione in liver of experimental mice was investigated to determine the antioxidative effect of test groups against the oxidative stress induced by sarcoma 180 cancer cells. After induction of sarcoma 180 cancer cells, the weights and the level of GSH of liver tissues of experimental mice were recorded. The liver weight of combination of GNDHF: LUT: MSC treated treated mice (1.16 gm) was found similar to the normal control group mice (1.23 gm) as compared to the tumor control mice (1.54 gm). Decreased concentration of reduced GSH (0.07 μM) per mg protein in tumor control group has been observed compared to normal control 0.27 μM. The reduced GSH level of test samples were as follows: GNDHF –0.12 μM; LUT – 0.10 μM; MSC – 0.09; (GNDHF: LUT: MSC) – 0.15 μM against the reference drug doxorubicin 0.22 μM (Table 1).

Subcutaneous induction of sarcoma 180 cancer cell showed a significant lowering of reduced glutathione in liver compared to normal and reduced the scavenging of reactive oxygen species. Among the groups studied, optimum value of reduced GSH per mg protein is found to be in the order: Doxo reference > GNDHF: LUT: MSC combination > GNDHF > LUT > MSC.

Increased concentration of malonaldehyde (MDA) 0.47 nM per mg wet weight has been observed compared to normal control 0.52 ± 0.04 nM. Concentration of MDA per mg wet weight were as follows: GNDHF – 0.22 ± 0.05 nM; LUT – 0.26 ± 0.05 nM; MSC – 0.27 ± 0.06 nM; (GNDHF: LUT: MSC) – 0.18 ± 0.03 nM against the reference drug doxorubicin 0.15 nM. Among the groups studied, optimum value of MDA per mg wet weight is found to be in the order: Doxo reference < GNDHF: LUT: MSC combination < GNDHF < LUT < MSC. Based on our observation, GNDHF: LUT: MSC combination exhibited optimum antioxidant activity and rendered significant protection against oxidative stress induced by sarcoma 180 cancer cells in liver tissues (Fig. 1).

In vivo anti-cancer activity

Injection of sarcoma 180 cancer cells subcutaneously into mice was followed by 29 days observation, monitoring the mean body weight, tumor volume and tumor growth delay of all the experimental groups (Table 2). The body weights of the control and treated mice were determined periodically to assess non-specific toxicity of test samples. The average body weights of the control and GNDHF, LUT and MSC (single and combination) treated mice did not differ significantly by one-way ANOVA suggesting that GNDHF, LUT and MSC administration did not cause weight loss. The mice in GNDHF, LUT and MSC treated group appeared healthy and did not show any other sign of non-specific toxicity, such as food and water withdrawal and impaired movement. Average tumor volume in the tumor control group and test samples treated group are depicted, and can be ordered as: Doxo reference < GNDHF: LUT: MSC combination < GNDHF < LUT < MSC < tumor control. Similar trend was also observed when tumor delay time in all the experimental groups has been studied. Overall, significant reduction in tumor volume and tumor delay time was found in GNDHF: LUT: MSC combination treated mice.

Antigenotoxic and antimutagenic activities

The effect of the test samples on percent of chromosomal aberration was measured in terms of chromatid breaks, centric rings, acrocentric association, acentric fragments, intracalary deletion, minutes and total abnormal metaphases (Table 3). Percent of aberrant metaphase in various groups were in the range as follows: cancerous control (77 %), doxorubicin treated (69 %), GNDHF: 31 %, LUT: 45 %, MSC : 57 %, GNDHF: LUT: MSC combination : 17 %, providing the following order: GNDHF: LUT: MSC > GNDHF > LUT > MSC > DOXO > cancerous control group.

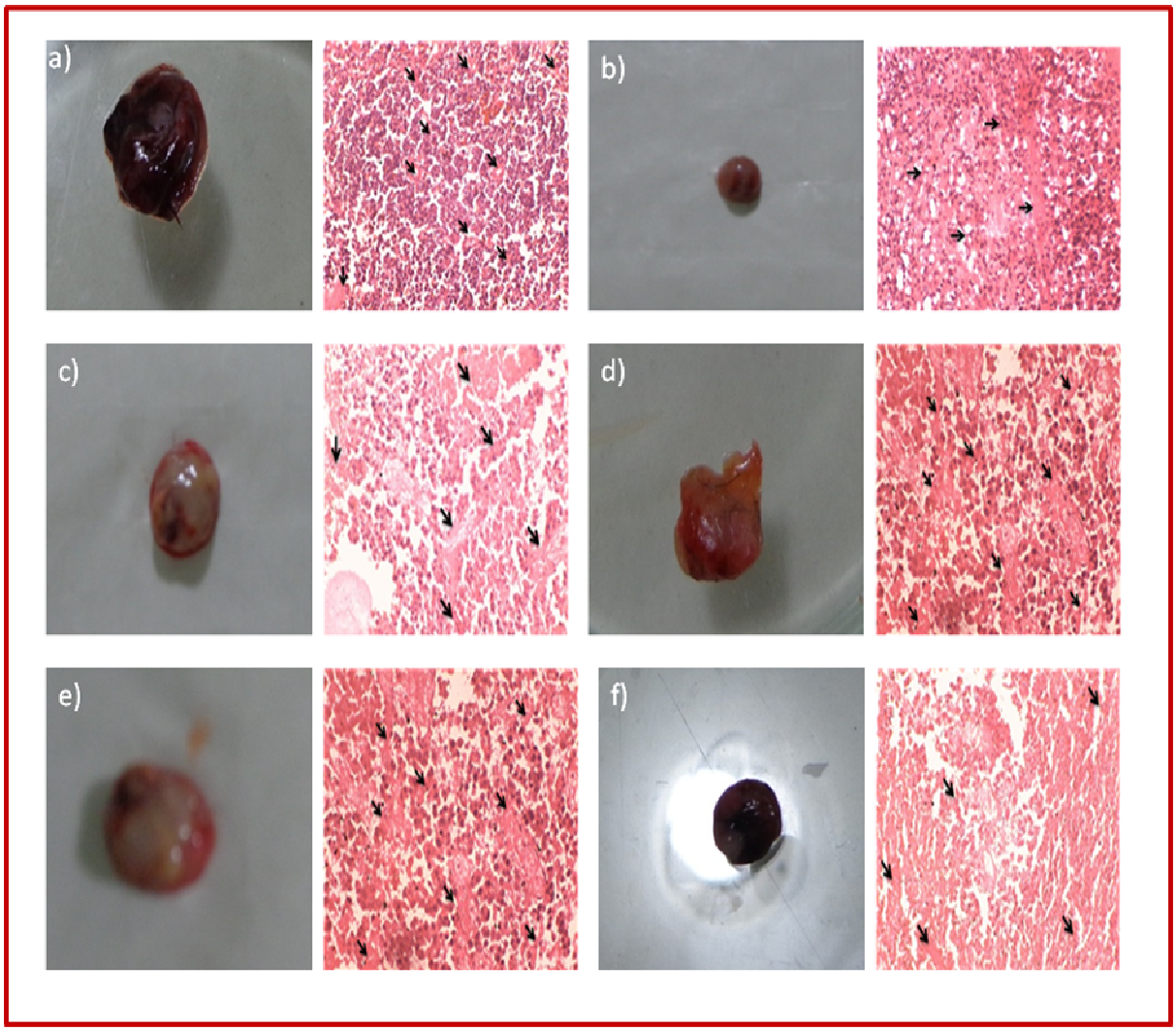
The combination of GNDHF: LUT: MSC is found to exhibit maximum reduction in all the chromosomal aberrations studied in bone marrow cells compared to tumor control and standard doxorubicin. The effect of various treatments on sarcoma 180 cancer induced mice was determined in terms of micronucleated polychromatic erythrocytes (MNPCEs) and normochromatic erythrocytes (MNCEs) per 1000 cells.

Percent of MPCE and MNCE in various groups were in the range as follows: cancerous control (87.7 - 94.5 %), Doxo treated (37.1 - 40.6 %), GNDHF: 16.8-20.2 %, LUT: 16.9-19.9 %, MSC: 15.4-19.1 %, and GNDHF: LUT: MSC: 5.1-5.9% (Fig. 2). GNDHF: LUT: MSC combination treated mice were significantly ($p > 0.01$) reduced the micronuclei in PCEs and NCEs comparable with tumor control and doxorubicin treated group. The combination of GNDHF: LUT: MSC reduces the frequency of micronuclei per polychromatic (PCEs) and normochromatic erythrocytes (NCEs) compared to standard drug and tumor control group.

Analysis of histological studies dealing with tumor micro vessel density evaluation

Accumulating evidences [Folkman, 1975; Saaristo et al., 2000., Folkman 2002., Chekenya et al., 2002] demonstrate that tumor growth and lethality are dependent on angiogenesis. An observation of histological slides (Fig. 3) exhibits the decrease in tumor growth in mice by the GNDHF, LUT and MSC treatment which may be attributed to decreased host angiogenesis. A marked and dense microvasculature was observed in the control tumors. Tumors treated with GNDHF: LUT: MSC combination (29.19 ± 4.41%) and doxorubicin (23.2 ± 2.63%) had significantly fewer micro-vessels compared with the GNDHF (41.23 ±4.64%), LUT (43.4 ± 4.30%), MSC (38.31 ± 4.38%) and tumor control (58.2 ± 4.3%). Angiogenesis inhibition observed with combination treatment is indicative of drug accumulation in the tumor and decreased tumor micro-vessel density which is further associated to the suppression of angiogenic vascularization, inhibited tumor cell proliferation and increased tumor cell apoptosis.

The *in vivo* study highlights the effectiveness of gold nanoparticle embeded with 3,6-dihydroxyflavone with lutein and selenium methyl selenocysteine in combination capable of significant reduction of cancer tumor against reference drug doxorubicin. The results indicate that the combination of compounds is a better cytotoxic bioagent against sarcoma 180 cancer cells induced in female Balb/c mice compared to the individual compounds. It is worth mentioning that neither life threatening toxicity nor a loss of body weight during the nanotech combination treatment was observed compare to the normal control. Overall, the results nicely complement other investigations depicting the safe and health promoting value of nanotechnology with dietary compounds, highlighting its potential for clinical applications and lend support to its use in traditional medicine.



Where DOXO: Doxorubicin, GNDHF: Gold nanoparticle embedded 3,6-dihydroxyflavone, LUT: lutein, MSC: selenium methyl selenocysteine.

Fig. 3 Tumor histology of a) Tumor control, b) DOXO treated, c)GNDHF, d)LUT, e)MSC, f) GNDHF: LUT : MSC.

Table 1 Combination effect of the gold nanoparticle embedded 3,6-dihydroxyflavone with lutein and selenium methyl selenocysteine against standard doxorubicin on level of GSH per mg protein (µM) in normal and melanoma tumor bearing mice at 29th day.

Sr. No.	Groups	µM GSH per mg protein ± SD
1.	Group I: NC	0.27±0.03
2.	Group II: TC	0.07±0.02
3.	Group III: 2% DMSO	0.17±0.08
4.	Group IV: DOXO (1mg/Kg b.w.)	0.22±0.05

5.	Group V: GNDHF (5mg/Kg b.w.)	0.12±0.08
6.	Group VI: LUT (5mg/Kg b.w.)	0.10±0.05
7.	Group VII: MSC (5mg/Kg b.w.)	0.09±0.09
8.	Group VIII: GNEF+LUT+MSC (5mg/Kg b.w.)	0.15±0.07

Each value is mean±SD (n=6). $p > 0.01$ vs. tumor control, $p < 0.05$ vs. normal control. NC: Saline treated normal control, TC: Tumor Control, DMSO: Dimethyl sulphoxide, DOXO: Doxorubicin, GNDHF: Gold nanoparticle embedded 3,6 dihydroxyflavone, LUT: lutein, MSC: Selenium methyl selenocysteine.

Table 2 Single and combination effect of gold nanoparticle embedded 3,6-dihydroxyflavone with lutein and selenium methyl selenocysteine against standard doxorubicin on body weight, tumor volume and tumor delay time in normal and sarcoma 180 cancer tumor bearing mice at 29th day.

Groups	**Body weight ±SD(gm)**	**Tumor Volume ± SD (mm^3)**	**Tumor delay time**
Group I: NC	30.4±1.07	-	-
Group II: TC	28.0±1.20	126.9±1.14	0
Group III: 2% DMSO	31.2±1.12	121.2±1.05	0
Group IV: DOXO (1mg/Kg b.w.)	25.6±1.17	004.0±1.15	7
Group V: GNDHF (5mg/Kg b.w.)	27.05±1.18	104.7±1.16	3
Group VI: LUT (5mg/Kg b.w.)	28.02±1.19	109.2±1.16	3
Group VII: MSC (5mg/Kg b.w.)	28.27±1.09	112.1±1.15	3
Group VIII: GNEF+LUT+MSC (5mg/Kg b.w.)	29.17±1.16	091.0±1.19	5

Each value is mean±SD (n=6). $p > 0.01$ vs. tumor control, $p < 0.05$ vs. tumor control. NC: Normal Control, TC: Tumor Control, GNDHF: Gold nanoparticle embedded 3,6 dihydroxyflavone, LUT: lutein, MSC: selenium methyl selenocysteine, DMSO: Dimethyl sulphoxide and DOXO: Doxorubicin.

Table 3 Combination effect of Gold nanoparticle embedded 3,6-dihydroxyflavone with lutein and selenium methyl selenocysteine against standard doxorubicin on percent of aberrant metaphases in the bone marrow of Balb/C mice after induction of sarcoma 180 cancer cell.

S No	**Group**	**CB**	**CR**	**FR**	**ACA**	**ICD**	**AC**	**MIN**
1	NC	12.50 ±1.14	0 ± 0.0	25.00 ± 1.13	12.50 ± 1.02	0.0 ± 0.0	12.50 ± 1.05	0.0 ± 0.0
2	TA	12.59 ±1.85	8.14 ± 1.85	17.03 ± 2.09	38.51 ± 2.14	11.85 ± 1.98	9.62 ± 1.65	2.96 ± 1.74

3	2%DMSO	21.93 ±1.78	7.74 ± 1.98	9.03 ± 2.41	29.03 ± 2.10	9.67 ± 2.14	9.67 ± 1.65	5.64 ± 1.57
4	DX	25.11 ±1.85	7.44 ± 1.65	8.37 ± 1.84	31.62 ± 1.78	9.30 ± .98	10.23 ± 1.56	5.11 ± 1.64
5	GNDHF	12.63 ±0.94	5.26 ± 0.68	7.36 ± 0.84	16.84 ± 0.98	6.31 ±0.75	10.52 ± 0.72	5.26 ± 0.86
6	LUT	14.14 ±0.68	5.05 ± 0.92	7.07 ± 0.84	18.18 ± 0.65	6.06 ±0.94	11.11 ± 0.81	5.05 ± 0.75
7	MSC	14.70 ±1.78	3.92 ± 1.58	7.84 ± 1.46	19.60 ± 1.34	7.84 ±1.52	12.74 ± 1.64	4.9 ± 1.74
8	GNDHF+LUT+MSC	7.69 ±1.78	4.32 ± 1.95	6.69 ± 2.1	15.38 ± 2.15	5.98 ±2.08	5.76 ± 1.80	3.84 ± 1.71

Each value is mean±SD (n=6). $p > 0.01$ vs. tumor control, $p < 0.05$ vs. normal control. NC: Normal Control, TC: Tumor Control, GNDHF: gold nanoparticle embedded 3,6 dihydroxyflavone, LUT: lutein, MSC: Selenium methyl selenocysteine, DMSO: Dimethyl sulphoxide, DOXO: Doxorubicin, CB: Chromatid Breaks, CR: Chromatid Rings, FR: Fragments, ACA: Acrocentric Association, ICD: Intercalary Deletion, AC: Acentric Association, MIN: Minutes

Scientific Justification

1. What is the main claim of the paper?

The study claims that chemopreventive role of Nanotech combination which is performed by using Balb/ c mice

2. What is the novel about it?

To prepare gold nanoparticle (along with antioxidant and sensitizer), this enhanced the anticancer property.

3. Why it is relevant and how the result advances the field.

The in vivo study highlights the effectiveness of gold nanoparticle embedded with 3,6-dihydroxyflavone with lutein and selenium methyl selenocysteine in combination capable of significant reduction of cancer tumor against reference drug doxorubicin.

The results indicate that the combination of compounds is a better cytotoxic bioagent against sarcoma 180 cancer cells induced in female Balb/c mice compared to the individual compounds.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Prof. V.G. Das, Director, Dayalbagh Educational Institute, Dayalbagh, Agra for providing the necessary research facilities. Sharad Medhe one of the author is gratefully acknowledges Department of Atomic Energy, Board of Research in Nuclear Sciences for rendering financial assistance. All the persons have informed consent prior to their inclusion in the study.

Conflict of interest

There is no conflict of interest.

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