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MODERATING IMPACTS OF ANTIOXIDANT PROPERTIES OF GINSENG ON STANNOUS CHLORIDE INDUCED HEMATOLOGICAL PARAMETER IN RABBITS

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ABSTRACT

Panax ginseng has been used for centuries in the Orient for medicinal purposes since the 18th century. For more than 2000-year ginseng has been used in Asian countries, including Chine, Korea and Japan. stannous chloride, $SnCl_2$, is widely used in daily human life to con-serve soft drinks, in food manufacturing, processing and packaging, and in biocidal preparations. Five rabbits per group were assigned to 1 of 4 treatment groups:0 mg ginseng and 0 mg $SnCl_2$/kg BW (control); 100 mg Gs/kg BW; 20 mg $SnCl_2$/kg BW; 100mg Gs/kg Bw plus 20 mg $SnCl_2$. Results indicated that treatment with ginseng alone caused significant ($P<0.05$) decrease in BW and relative weight of liver, kidney, spleen, testes, and heart compared to control animals. On the other hand, the BW and relative weight of liver, kidney, lung, spleen, testes, and heart were significantly ($P<0.05$) increased in rabbits treated with ginseng alone as compared to control animals. treatment with ginseng caused significant increase in WBCs and significant increase in Hb, RBCs, PCV and platelets compared to control. On the other hand, stannous chloride ($SnCl_2$) caused significant increase in white blood cells (WBCs) and insignificant decrease in haemoglobin (Hb), red blood cells (RBCs), packed cell volume (PCV) and platelets.

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INTRODUCTION

Stannous is used for the treatment of rheumatoid arthritis and other inflammatory diseases **[1]**. With the use of stannous ointment, the local metal exposure can reach 0.15 g day_1 [2]. People are uncovered to stannous generally through tin-lined cans but too through toothpaste, fragrances and nourishment added substances or to organotin compounds through plastic channels, paints and pesticides. Stannous overload can cause anemia as well as liver and kidney problems [3] Stannous chloride induced a dose-dependent increase in the MN frequency in peripheral erythrocytes of adult zebra fish (Daniorerio) after 120 h exposure to doses ≥ 50 lM **[4]**. The use of herbal products is gaining popularity around the world, as they are considered to be effectual and to have few side-effects [5]. Variation inc blood constituents gives a rapid and good picture reflecting the physiological status of the organism because these changes develop more quickly in response to toxicants than any apparent morphological changes [6]. **[7]** investigated the effect of stannous chloride at 0,250,500 ppm Sn^{+2}. They found that hemoglobin decreased significantly. [8] studied the anti nutritive effect of dietary tin. He reported that a variety of effects of tin including variations in the activity of heme oxygenause and δ-aminolevulinic acid dehydratase and also tin has adverse effects on metabolism of essential trace minerals including iron. Organotin compounds were also reported to have adverse effects on blood and blood forming organs. [9] studied the disturbances in heme biosynthesis in rabbits after administration of low doses of tin. They measured the activity of δ-aminolevulinic acid dehydratase (δ- ALAD) in the whole blood, liver, kidney, brain, spleen, and bone marrow, concentration of free erythrocyte protoporphyrins after the treatment of rabbit with 17 µ mole $SnCl_2$ $2H_2O$. kg/ day for 5days. Tin doses did not affect the process of heme biosynthesis in rabbits. [10] determined the effect of tin on heme biosynthesis in rabbits. Female rabbits received single doses of 10,100, and 200 mg/kg body weight of $SnCl_2$. The

activities of δ- aminolevulinic acid dehydratase in the whole blood, free erythrocyte protoporphyrins, urine δ-aminolevulinic acid (δ-ALA-U) and coproporphyrin (CP- U) were determined. The activity of the enzyme decreased by about 80 % and two fold increase in the ALA and CP concentration in urine were observed in animals received 100 mg Sn/ kg body weight.[11] also mentidned many hematological changes compressing anemia, Iymphocytopenia, and thrombocytosis in rats treated with bis tri- n- butyltin oxide(TBTO) at a dietary level of 50 mg/ kg for 106 weeks. **[12]** found that both hemoglobin concentration and hematocrit value were decrease in a dose- related fashion in both sexes of rats treated with triphenyltin acetat (TPTA) compared to the control groups. Hemoglobin concentration was decreased in female rats received 2.8 and14.2 mg tin/ kg body weight after 4 and 8 weeks. In male rats, hemoglobin concentration was decreased at the 0.8 mg tin/ kg body weight level after 4, 8, and 12 weeks, and at the 3.9 mg tin /kg body weight level after 4, 8, 12, and 16 weeks. Hematocrit value decreased in female rats given 2.8 mg tin/ kg body weight level after 4, 8 and 12 weeks and in female rats given 14.2 mg tin/kg body weight after 4 and 8 weeks. Hematocrit value was not significantly different from control value in male rats given 0.8 mg tin/ kg body weight. Significant decrease in hematocrit value was seen in male rats given 3.9 mg tin/ kg body weight after 4, 8and 16 weeks. Rats examined 6 weeks after the initiation of treatment with 20 or 80 ppm TBTO in the diet exhibited increases in both the reticulocyte fraction and isocitrate dehydrogenase activity, as well as decreases in both serum iron concentration and splenic hemosiderin content. The results may indicate that TBTO exposure disrupts hemoglobin synthesis by interfering with iron uptake or by promoting iron loss **[13]**.

[14] reported that tin appears to interact with the process of iron uptake on the surface within the mucosal cells as well as with iron releasing process at the contraluminal side . Tin also appears to interfere with the absorption and metabolism of essential elements like Zn, Cu as well as iron, and therefore inhibits hematopoiesis [8]. In4- or 13 - week feeding studies with rats given various tin salts (including tin(II) chloride) or tin oxides at dose levels of 50- 10 000 mg/ kg of food, doses above 3000 mg/ kg caused anemia [15]. A dose - related in hemoglobin concentration was noted in rats after oral treatment with inorganic tin **[7]**. A minolevulinic acid dehydatase activity was decreased in rats given stannous chloride [9]. Therapeutic plants are plants that by and large contain constituents that have been found valuable for the treatment and administration of both creature and human maladies. The ancient Chinese have identified 11,146 medicinal species from 383 families, and more than 400 of which are widely used throughout the world[16,17]. Panax ginseng (Ginseng) is a well-known herb in traditional Chinese medicine (TCM) [18].Ginseng, called the king of all herbs, has been used as a traditional medicine for the treatment of diseases for thousands of years in East Asian countries. In the last three decades, it has become one of the most popular herbs worldwide [19]. Panax means cure for all disease, as it combines the Greek words pan meaning all and zxos meaning medicine [20]**.** In TCM, food and medicine are understood to share similar origin but with diverse applications and uses [21]. In this way, the Chinese commonly joins assortment of TCM herbs into their eat less to form a number of solid nourishment formulas that are more engaging of superior taste, moved forward surface,improved texture, and will most importantly improve one's health [22]. Ginseng, a traditional medicinal plant, embodies an important position in the oriental pharmacopeia. Traditionally it is used primarily for treating illness, restoring homeostasis, and promoting longevity [23], but more recently is has been identified as the most commonly used herbal for controlling CVD risk factors [24].

MATERIALS AND METHODS

Tested compounds

In this study stannous chloride ($Sncl_2$) and ginseng were used. $Sncl_2$ (purity 400g/L) was purchased from B &W agrochemichals (China) and ginseng was obtained from Superior Nutrition and Formulation by Jarrow Formulas, Los Angeles, USA. All other chemicals utilized within the test were of expository review.

Experimental animals

Mature male New Zealand White rabbits age of 6 months and initial weight of (1.891 ± 27.6 Kg) were used. Animals were separately housed in cages and weighed week by week all through 3-months test period. Feed and water were provided ad libtum. Rabbits fed pellets which consisted of 30 % berseem (Trifolium alexandrinum) hay, 25 % yellow corn, 26.2% wheat bran, 14 % soybean meal, 3 % molasses, 1 % $CaCl_2$, 0.4 % NaCl, 0.3 % mixture of minerals and vitamins, and 0.1 % methionine. The vitamin and mineral premix per kg contained the following IU/gm for vitamins or minerals: vit A-4000,000, vit D3-5000, 000, vit E-16,7 g, K-0.67 g, vit B1-0.67 g, vit B2-2 g, B6-0.67 g, B12-0.004 g, B5-16.7 g, Pantothinc acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co.). The

chemical analysis of the pellets (AOAC, 1990) showed that they contained 15.8 % crude protein, 11.3 % crude fiber, 3.7 % ether extract, 7.2 % ash, 92.9 % organic matter and 62.4 % nitrogen free extract % as DM basis.

Twenty mature male rabbits were randomly divided into four equal groups (each five rabbits) as follows: - Group I: Rabbits were used as control daily for 12 successive weeks. Group II: Rabbits were treated with ginseng. Ginseng was given ginseng daily by gavage at a dose of 100 mg/kg B.W, [25] which dissolved in corn oil for 12 successive weeks. Group III: Rabbits were treated daily with $SnCl_2$ by gavage at a dose of 43.2 mg/kg B.W/day (1/50 of $SnCl_2$) lethal dose [26]. Group IV: Rabbits were given with $Sncl_2$ daily at a dose of 43.2 mg/kg B.W./day by gavage like group III and given the ginseng concurrently daily at a dose of 100 mg/kg B.W./day by gavage like group II for 12 successive weeks. he doses of the $Sncl_2$ and ginseng were calculated according to the animal's body weight on the week before dosing. The tested doses of $SnCl_2$ and ginseng were given daily for 12 weeks. Body weight of each animal was recorded weekly throughout the 12-week of the experimental period. The weight measurements were carried out in the morning before access to feed and water. At the end of treatment period, all animals of each group were slaughtered. Weights of liver, lung, heart, kidney, spleen and testis were also recorded. These organs were individually identified and kept frozen (–20°C) until assays performed. Blood tests were collected from the ear vein of all creatures each week all through the 6-weeks test period. Heparin was used as anticoagulant. Plasma was obtained by centrifugation of samples at 860*g for 20 min, and was stored at -20C until used for analysis. Hematological parameters including White blood cells (WBC), red blood corpuscles (RBC), hematocrit (HCT), Hemoglobin (HG), and platelets were evaluated using an automatic blood cell analyzer (XP-300 Automated HematologyAnalyzer, Sysmex American, Inc).

Statistical analysis

Data were analyzed as a completely randomized design [27] using the General Linear Model procedure of [28]. Means were statistically compared using Least Significant Difference (LSD) test at 0.05 significant levels[27].

RESULTS

The changes in body weight (BW) and the relative weights of liver, kidney, lung, spleen, testes, and heart of male rabbits. The relative organ weights (%) were calculated as g/100 g body weight throughout the 12-week experimental period of rabbits treated with ginseng (G_S), Stannous chloride ($Sncl_2$) and their combination were summarized in (Table 1 and Figurers 1 to 3). Overall means indicated that treatment with $Sncl_2$ caused significant ($P<0.05$) decrease in BW and relative weight of liver, kidney, spleen, testes, and heart compared to control animals. On the other hand the BW and relative weight of liver, kidney, lung, spleen, testes, and heart were significantly ($P<0.05$) increased in rabbits treated with ginseng alone as compared to control animals. The combination between ginseng and $SnCl_2$ caused significant increase in the reduction of BW and improvement in relative organ weights due to treatment with $SnCl_2$, and this means that ginseng alleviated its toxicity.

Table 1. Body weight (BW) and relative weight of kidney, liver, lung, brain, heart, testes and spleen of male rabbits treated with ginseng, stannous chloride ($SnCl_2$) and their combination

Parameter	Experimental groups			
	C	G_S	$SnCl_2$	G_S+$Sncl_2$
BW (gm)	1.891± 35.64	2.006 ± 37.13	1.756 ± 58.65	1.928 ± 24.92
Kidney (g/100gm)	11.84± 1.275^{a}	12.90± 0.640^{a}	10.34± 0.382^{a}	12.46± 0.936^{a}
Liver (g/100gm)	42.78 ± 0.697^{a}	44.26 ± 3.811^{a}	39.10 ± 1.453^{a}	45.00 ± 2.302^{a}
Lung (g/100 gm)	8.520 ± 0.736^{ab}	10.82 ± 1.115^{a}	7.600 ± 0.510^{b}	8.300 ± 0.186^{ab}
Heart (g/100 gm)	6.400 ± 0.872^{a}	7.880 ± 0.845^{a}	5.900 ± 0.332^{a}	6.400 ± 1.134^{a}
Testes (g/100 gm)	4.080 ± 0.972^{a}	6.380 ± 1.00^{a}	3.604 ± 0.713^{a}	4.560 ± 0.509^{a}
Spleen (g/100 gm)	4.080 ± 0.972^{a}	6.380 ± 1.00^{a}	3.604 ± 0.713^{a}	4.560 ± 0.509^{a}

Values are expressed as means ± SE; n = 5 for each treatment group. Mean values within a row not sharing a common superscript letters (a, b, c) were significantly different,$p<0.05$.

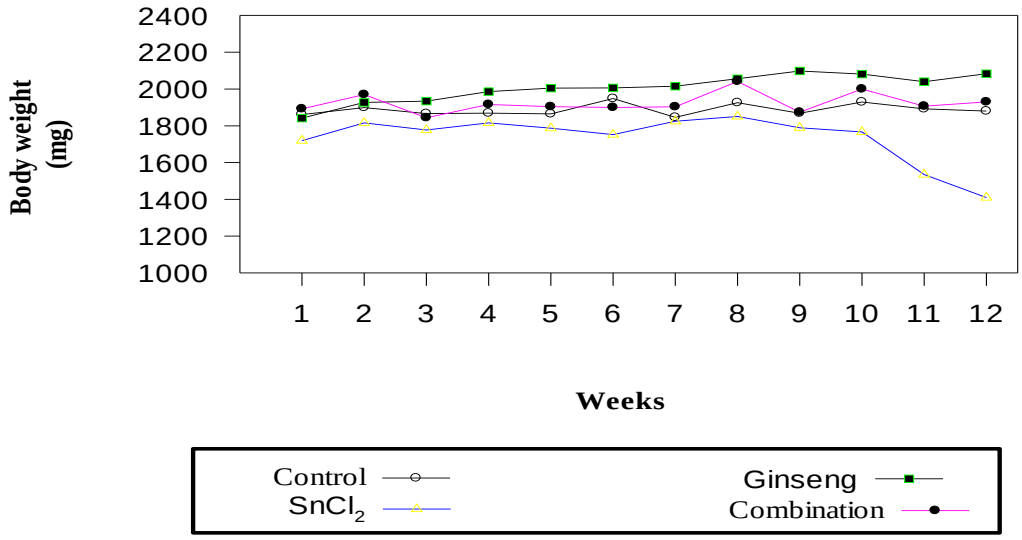

Figure 1. Changes in bady weight treatment of male rabbits with ginseng, stannous chloride($SnCl_2$) and/or combination.

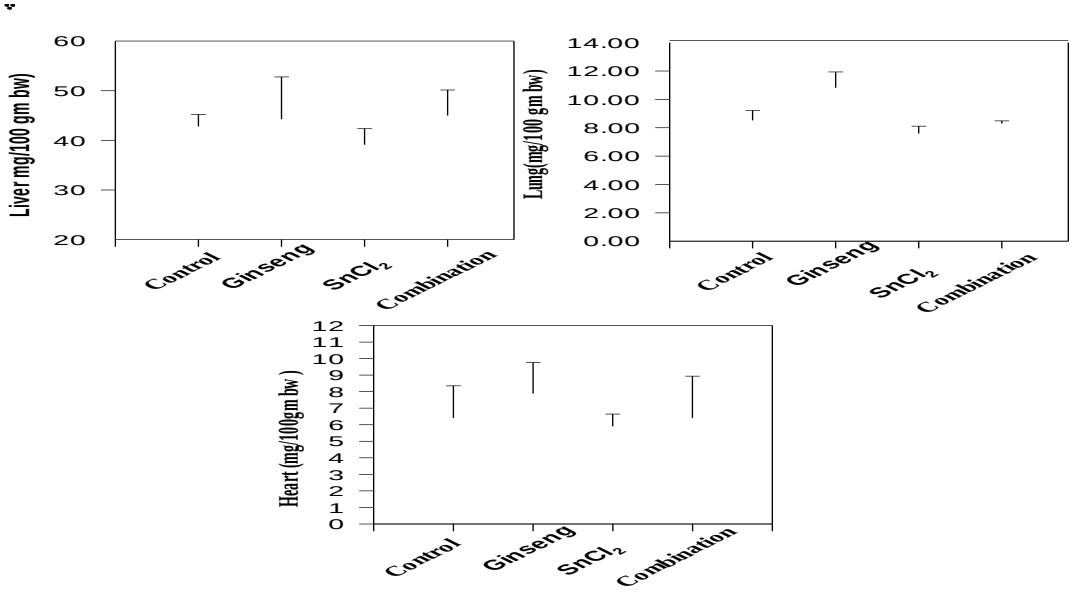

Figure 2. Changes in relative weight for Liver, Lung and Heart during treatment of male rabbits with ginseng, stannous chloride($SnCl_2$) and/or combination.

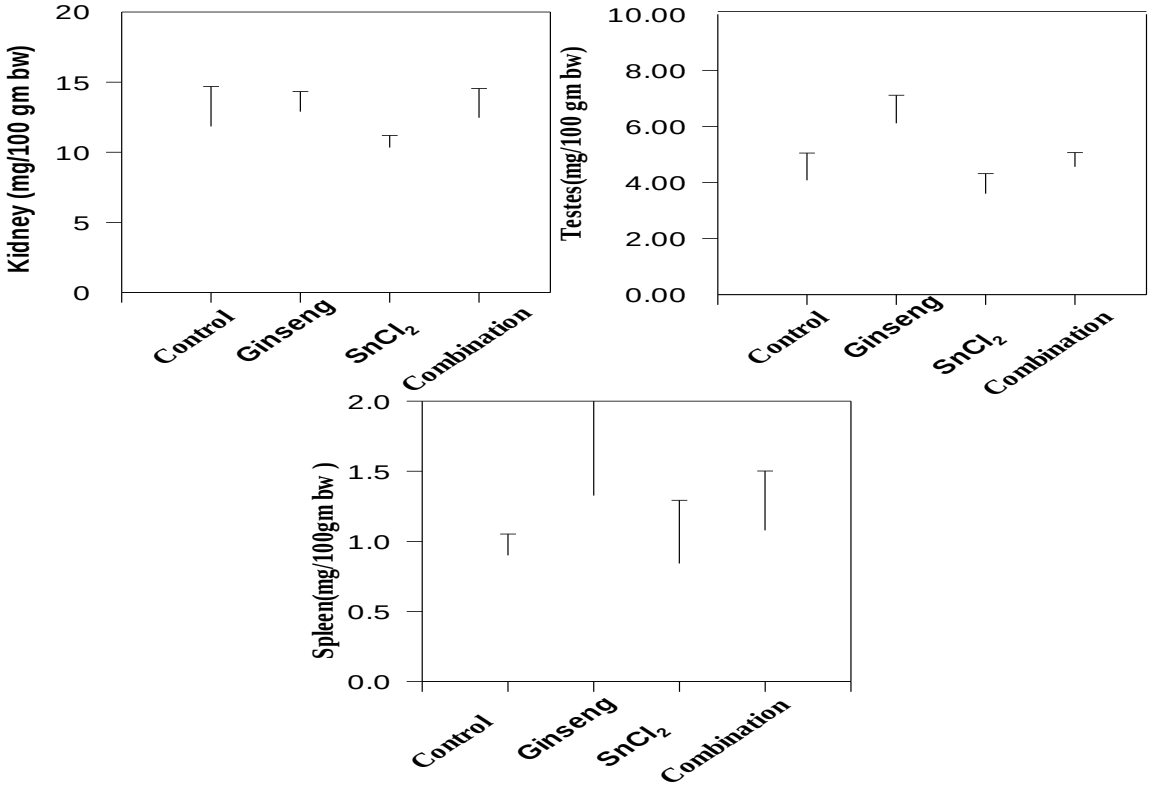

Figure 3. Changes in relative weight for Kidney, Testes and spleen during treatment of male rabbits with ginseng, stannous chloride($SnCl_2$) and/or combination.

Table2. and Figures 4 to 6 presents the hematological parameters of male rabbits treated with ginseng, $SnCl_2$ and their combination. Results indicated that treatment with ginseng caused significant increase in WBCs and significant increase in Hb, RBCs, PCV and platelets compared to control. On the other hand stannous chloride ($SnCl_2$) caused significant increase in white blood cells (WBCs) and insignificant decrease in haemoglobin (Hb), red blood cells (RBCs), packed cell volume (PCV) and platelets. The presence of ginseng, $SnCl_2$ returned the values of the previous parameters to near to the control values.

Table 2. Changes Complete blood counts Red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), platelets count (PLT), hemoglobin (Hb), of male rabbits treated with ginseng, stannous chloride ($SnCl_2$) and their combination.

Parameter	**Experimental groups**			
	C	**GS**	**$SnCl_2$**	**GS + $SnCl_2$**
RBC ×106 (µl)	6.04 ± 0.110a	6.26 ± 0.084a	5.46 ± 0.171b	6.08 ± 0.100a
WBC ×103(µl)	8.5 ± 0.18b	9 ± 0.22b	9.2 ± 0.15b	10.4 ± 0.24a
PCV×103(µl)	40.01±0.428a	40.48 ± 0.465a	32.83 ± 0.395b	38.96 ± 0.711a
PLT ×103(µl)	288.03±6.35b	441.81 ± 25.66a	162.03 ± 8.80c	310.30±6.417b
Hb (g/dl)	12.44 ± 0.14a	13.31 ± 0.19a	10.80 ± 0.19b	12.62 ± 0.19a

Values are expressed as means ± SE; n = 5 for each treatment group. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$.

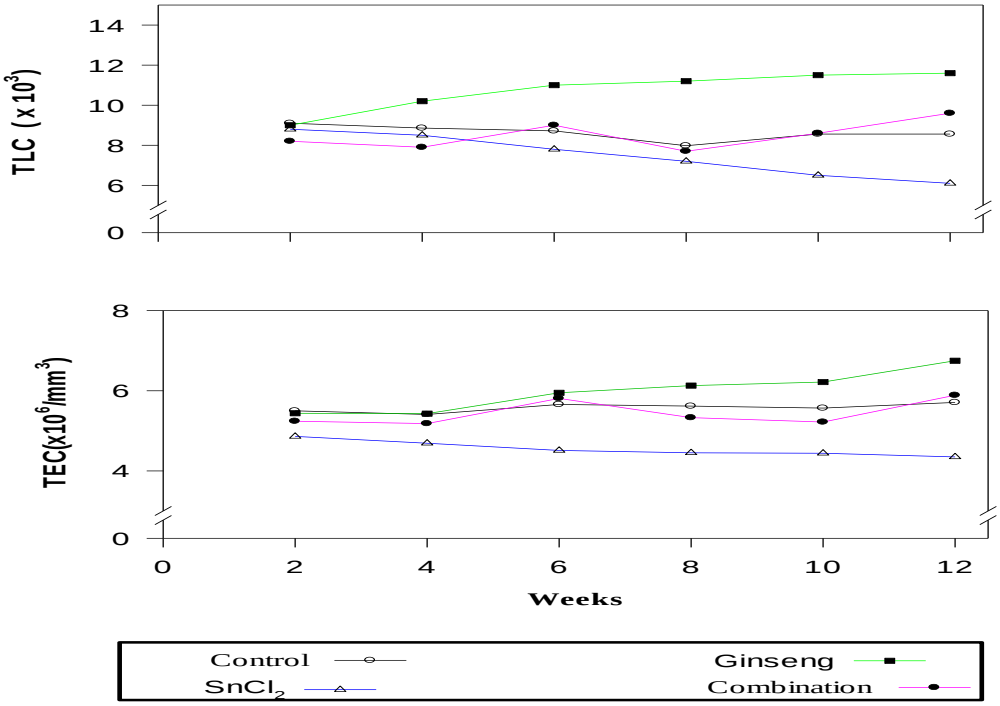

Figure Error! No text of specified style in document..Changes in Total leucocyte counts (TLC) and Total erythrocyte counts (TEC) during treatment of male rabbits with ginseng, stannous chloride($SnCl_2$) and/or combination.

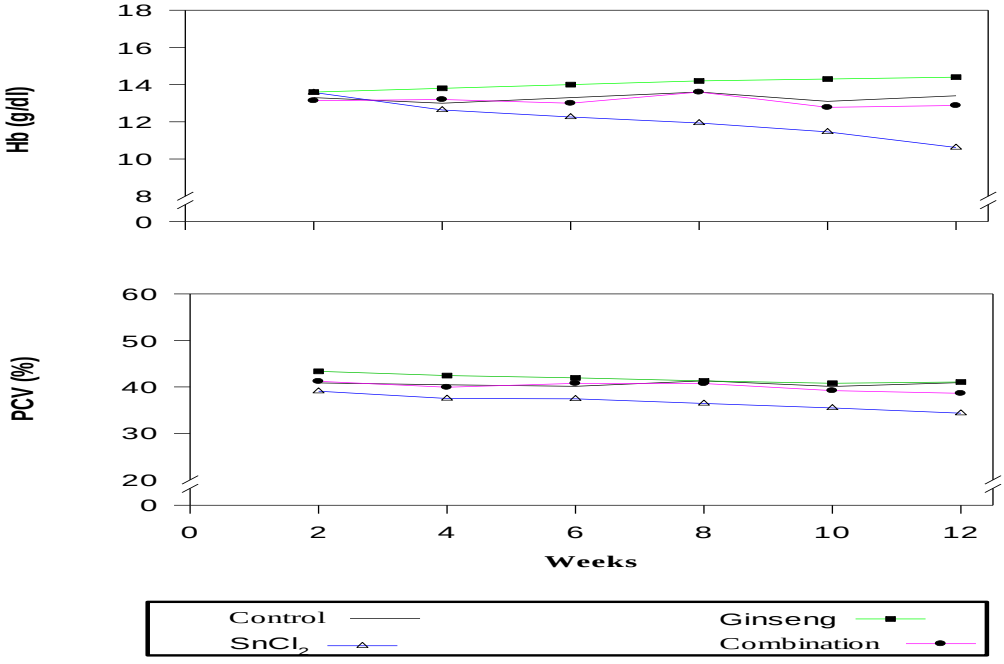

Figure 5. Changes in hemoglobin (Hb) and pack cell volume during treatment of male rabbits with ginseng, stannous chloride($SnCl_2$) and/or combination

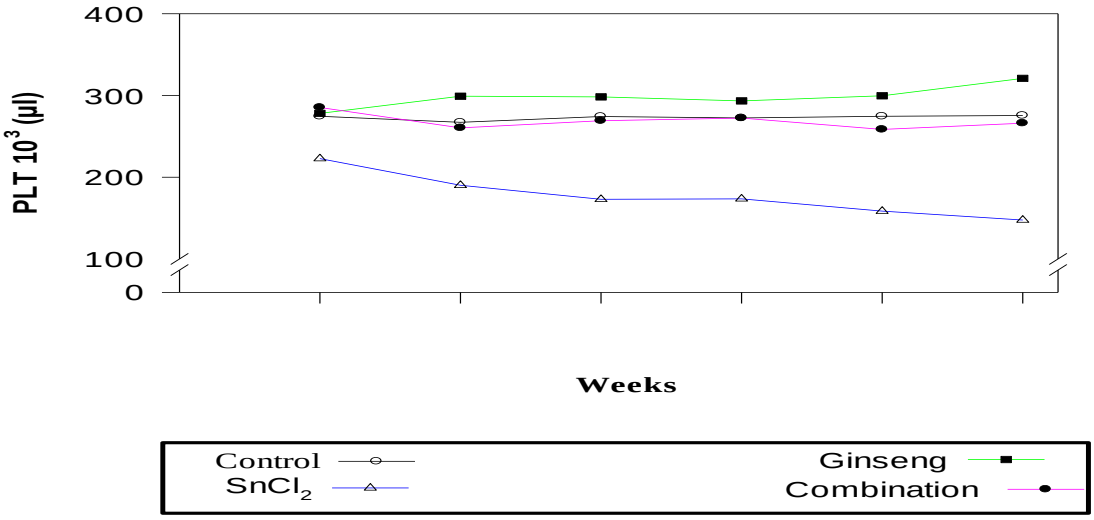

Figure 6. Changes in Platelets count (PLT) during treatment of male rabbits with ginseng, stannous chloride ($SnCl_2$) and/or combination

DISCUSSION

The present results indicate that treatment with $SnCl_2$ caused significant reductions in body weight (BW)and relative organs weight (ROW) (Table 1 and Figures 1 to 3). The reduction in BW and ROW of the SnCl2 treated rabbits is in agreement with the finding of [29]. Relative organs weight (R0W) were reduced by $SnCl_2$ treatment. Similar results were obtained by [30 -32] in rats. Also, testicular degeneration was observed in rats receiving 10 mg of tin(II) chloride per kg in the feed for 12 weeks[33]. The increase body weight and relative weight observed in the present study due to treatment with ginsing is agreements with **[34-37]**. Some investigators reported that using medicinal and aromatic plants in rabbit diets improved body weight, body weight gain and performance index,[38,39]**.** Also ginseng (Araliacease), also called Asian ginseng, is one of the most renowned herbal plants worldwide, but particularly in Asian countries and has been used for thousands of years to maintain homeostasis of the body and enhance vital energy [40]. The results of the present study showed that ginseng has increased relative organs weight in rabbits. Good development of these organs is also considered to be crucial for optimal site of immunoglobulin synthesis**[41]**. In this study, the inclusion of dietary ginseng increased the relative weight of the relative organ weight compared with control treatment, which accords with **[42]** who suggested polysavone (main saponin and polysaccharides) supplementation increased the relative spleen weight and bursa weight in comparison with control treatment. The present study showed that $SnCl_2$ caused decrease RBCS , Hb ,PCV and PLT (Tables 2 and Figures 4 to 6) agreement with [43] who found that iron status (press, hemoglobin, hematocrit, ruddy blood cell check, plasma press, add up to press official capacity and transferrin immersion) in rabbits was not impacted by dietary tin concentrations < 100

mg Sn/kg count calories as $SnCl_2$ for 28 days. Higher dietary admissions of tin caused a diminish in these parameters. Food intake and body weights were not reported . A study in Wistar rats fed on diets containing various concentrations of tin (1, 10, 50, 100 and 200 mg Sn/kg as $SnCl_2$) for 28 days showed that iron, copper and zink tissue and plasma concentrations were seemingly unaffected at 1 mg and slightly decreased at 10 mg Sn/kg diet (~ 0.7 mg Sn/kg body weight/day). Greater effects were seen at 50 mg/kg diet (~ 3.5 mg Sn/kg body weight/day). The blood haemoglobin concentration and percentage transferrin saturation decreased in a linear manner as the level of dietary Sn increased. [7] investigated the effects of 0, 250 or 500 mg Sn/kg diet (as SnC_{l2}) in a 4-week study on weanling Wistar rats. Haemoglobin was decreased and body weights reduced in a dose-related way in the tin-fed groups. Crypt depth, villus length and cell turnover were increased in parts of the intestine. In week 4, the estimated doses of tin were about 25 and 50 mg Sn/kg body weight/day, respectively . Increase in hematological parameters (Tables 2 and Figures 4 to 6) of rabbits treated with ginseng (100 mg/kg) are in agreement with the finding of[44] who reported that gensing improved the haematological parameters of rats. However, other research indicates that gensing enhances phagocytosis, natural killer cell activity (NK), and the production of interferon while improving the physical and mental performance of mice and rats[45]. **[46,47]** report that ginseng increases both humoral and cell-mediated immune responses. In addition, [48]Kenarova et al. (1990) observed that ginseng increased the number of antigenreactive T helper cells, T lymphocytes, and NK cells. In addition, although increases in leucocytes, lymphocytes, and alveolar macrophages in gensingtreated animals have been reported ,[49,50] such findings are disputed by [51]. Overall, these various findings lead us to conclude that the antioxidant properties of ginseng and its stimulatory effects on erythropoiesis. From the results of this study and others, we suggest that Panax ginseng may stimulate the activity of the bone marrow stem cells and that nutritional supplementation with it may help overcome fluorotic anemia in humans as well as in animals. However, further research is required to investigate its effects on the regulation of the immune system and erythrocyte production in bone marrow.

Conclusion

the results of the present study convincingly demonstrated that stannous chloride exposure resulted in varying degrees of changes in hematological parameters in the plasma of rabbits. ginseng is broadly utilized in conventional medication to treat sickness. Using ginseng capability to alleviate the harmful effect of stannous chloride.

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