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Angelica Archangelica Roots Water extraction as a Natural Antioxidant Tolerating ROS Production in Lead Poisoning

Bassem M Raafat [1]*, Saad M Zahrany [2], Ali S Al-Zahrani [2], Emad Tawifiek [2], Anas M Al-Omery [2]

[1]Genetic Engineering and Biotechnology Division, Cairo, NRC, EGYPT
[2]Faculty of Applied Medical Sciences, Taif University, KSA

ABSTRACT

Lead intoxication is one of the most common environmental pollutants especially in the third world. Recent researches showed that even very little concentrations revealed with health risks. This work aimed to find out to what extend application of natural chelators can compensate the oxidative stress risk of lead intoxication. Sixty Orycytolagus Cuniculus male rabbits were conducted to this study. These animals were subdivided into four groups representing animals did not receive neither lead acetate nor treatment for 21 days, positive control and animals received Angelica Archangelica roots water extraction as a natural chelator either with and without further exposure to lead for another 21 days after stoppage the intoxication process for 21 days. Super Oxide Dismutase (SOD) and glutathione peroxidase activities were measured as well as hemoglobin auto-oxidation rate in all groups. Results showed elevated auto-oxidation rate in hemoglobin of animals received lead acetate in drinking water with high level of compensation after application of the herbal chelator. Both antioxidants showed significant elevation in animals received lead poisoning and also significant reduction of these activities was shown after chelation therapy. All results showed highly significant enhancement in animals started the chelation therapy after complete isolation of lead intoxication source. In conclusion : administration of natural chelator is significantly recommended as a compensating method avoiding the oxidative stress of lead poisoning especially after removal of lead poisoning source.

Keywords: lead – antioxidant – oxidative stress – SOD – glutathione – Angelica archangelica.

Corresponding author
Email: bassemraafat@hotmail.com

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INTRODUCTION

Lead is one of industrial pollutant that has been detected in almost all phases of environmental and biological systems. The quantity of lead used in the 20th century far exceeds the total consumed in all previous eras [1]. This heavy metal use has caused local and global contamination of air, food, dust, and soil [2].

Gastrointestinal absorption of soluble lead salts in adult humans can be high during fasting (40–50%), but is about 3–15% when ingested with food [3]. Studies in animals provide evidence that gastrointestinal absorption of lead is much higher in younger organisms [4]. Lead is distributed to the blood plasma and soft tissues, but under steady state conditions 99% of the lead in blood is found in the erythrocyte [5], where much of it is bound to hemoglobin. Lead stored bone, has half life 25 years, can mobilized into blood and subsequently to other tissues [6].

Lead interferes with the synthesis of heme resulting in a reduction in blood hemoglobin and in a hypochromic, normocytic anemia also lead produces cardiac lesions and electrocardiographic abnormalities, at higher levels of exposure [7]. Many epidemiological studies have found increases in blood pressure to be associated with increases in blood lead level [8]. Oral exposure of animals to lead causes renal damage; histopathology is similar in humans and animals and includes intranuclear inclusion bodies, swollen mitochondria, and tubular damage [9]. Adverse effects on the testes and sperm counts and morphology have been seen in occupationally exposed men with blood lead levels of 40–50: g/dL [10].

Lead can affect virtually every organ or system in the body through mechanisms that involve fundamental biochemical processes [11]. These mechanisms include the ability of lead to inhibit or mimic the action of calcium and to interact with proteins. In the interaction with proteins, lead binds with virtually every available functional group, including sulfhydryl, amine, phosphate, and carboxyl groups, with sulfhydryl having the highest affinity. In its binding with sulfhydryl groups, lead may interfere with the activity of zinc metalloenzymes, as zinc binds to a sulfhydryl group at the active site. Lead also binds to metallothionein, a sulfhydryl-rich protein, but does not appear to displace cadmium or zinc. Metallothionein is induced by cadmium, zinc, and arsenic, but apparently not by lead, although metallothionein sequesters lead in the cell. Another lead-binding protein is an acidic, carboxyl-rich protein found in the kidney and brain [12].

One current theory as to how lead exerts its toxic effects suggests that lead-induced oxidative stress contributes to the pathogenesis of lead poisoning by disrupting the delicate prooxidant / antioxidant balance that exists within mammalian cells [13]. The mechanisms for lead-induced oxidative stress include the effect of lead on membrane, DNA, and antioxidant defense systems of cells. On cell membrane, the presence of double bonds in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the double bonds and makes hydrogen

removal easier [14]. Therefore, fatty acids containing zero to two double bonds are more resistant to oxidative stress than are the polyunsaturated fatty acids with more than two double bonds [15]. After incubation of linoic, linolenic, and arachidonic acid with lead, the concentration of a final product of oxidative stress, malondialdehyde "MDA" was increased with the number of double bonds of fatty acid [16].

One of lead's primary effects is hematotoxicity, specifically inhibition of heme synthesis. Lead inhibits three enzymes in heme biosynthesis pathway, co-porphyrinogen oxidase, ferrochelatase and delta-aminolevulinate dehydratase "ALAD". These enzymes are responsible for the second step in the production of heme, the iron-containing part of hemoglobin [17]. All of these hazardous effects of lead poisoning is based on the negative role of ROS produced by lead ions [18].

Chelation therapy is the administration of chelating agents to remove heavy metals from the body [19]. For the most common forms of heavy metal intoxication—those involving lead, arsenic or mercury—the standard of care in the United States dictates the use of dimercaptosuccinic acid (DMSA). Other chelating agents, such as 2,3-dimercapto-1-propanesulfonic acid (DMPS) and alpha lipoic acid (ALA), are used in conventional and alternative medicine. Chelation therapy is used as a treatment for acute mercury, iron (including in cases of thalassemia), arsenic, lead, uranium, plutonium and other forms of toxic metal poisoning [20]. The chelating agent may be administered intravenously, intramuscularly, or orally, depending on the agent and the type of poisoning [21].

A novel natural chelating agent that proved a very highly significant chelation potency of lead ions is that derived from the Angelica Archangelica roots water extraction. It is safe to be administered orally in a previous experimental animal test [22].

Angelica Archangelica, commonly known as Garden Angelica, Holy Ghost, Wild Celery, and Norwegian angelica, is a biennial plant from the Apiaceae family Apiaceae family, formerly known as Umbelleferae. Synonyms include Archangelica officinalis Hoffm., and Archangelica officinalis var. himalaica C.B.Clarke) [23].

During its first year it only grows leaves, but during its second year its fluted stem can reach a height of two meters (or six feet). Its leaves are composed of numerous small leaflets, divided into three principal groups, each of which is again subdivided into three lesser groups. The edges of the leaflets are finely toothed or serrated. The flowers, which blossom in July, are small and numerous, yellowish or greenish in color, are grouped into large, globular umbels, which bear pale yellow, oblong fruits. Angelica only grows in damp soil, preferably near rivers or deposits of water. Not to be confused with the edible Pastinaca sativa, or Wild Parsnip. [23].

Angelica Archangelica grows wild in Finland, Sweden, Norway, Denmark, Greenland, the Faroe Islands and Iceland, mostly in the northern parts of the countries. It is cultivated in

France, mainly in the Marais Poitevin, a marsh region close to Niort in the département Deux-Sèvres. It also grows in certain regions in Germany like the Harz mountains, and in certain regions of Romania, like the Rodna mountains [24].

From the 10th century on, angelica was cultivated as a vegetable and medicinal plant, and achieved great popularity in Scandinavia in the 12th century and is still used today, especially in Sami culture. A flute-like instrument with a clarinet-like sound can be made of its hollow stem, probably as a toy for children. Linnaeus reported that Sami peoples used it in reindeer milk, as it is often used as a flavoring agent [23].

In 1602, angelica was introduced in Niort, which had just been ravaged by the plague, and it has been popular there ever since. It is used to flavour liqueurs or aquavits (e.g. Chartreuse, Bénédictine, Vermouth and Dubonnet), omelettes and trout, and as jam. The long bright green stems are also candied and used as decoration [24].

Angelica is unique amongst the Umbelliferae for its pervading aromatic odour, a pleasant perfume entirely different from Fennel, Parsley, Anise, Caraway or Chervil. One old writer compares it to Musk, others liken it to Juniper. Even the roots are fragrant, and form one of the principal aromatics of European growth - the other parts of the plant have the same flavour, but their active principles are considered more perishable. Angelica contains a variety of chemicals which have been shown to have medicinal properties, and the plant is used as a digestive aid [24].

The aim of this work is to study to what extend application of natural heavy metal chelators specially those also have antioxidant potency can overcome the hazardous effects of lead ions elevated levels by tolerating the ROS concentration.

MATERIALS AND MAETHODS

The study was conducted to 60 male Orycytolagus Cuniculus rabbits. They were individually housed in stainless steel cages in an air conditioned room with temperature maintained at 25 ± 2^{o}C under a 12 hr light/dark cycle from 6 am to 6 pm and free access to foods was allowed. The food is checked not to contain any potential source of contamination that could result in results interference. The approximate animal weight was 1.25 kg (± 0.1kg). Animal design was approved by the Ethical Committee of the National Research Center (NRC). Before dosing of rabbits they were acclimatized for 7 days then randomly subdivided into six groups (7 animals each) as follow:

First group (G1) represents the control group in which animals did not receive neither lead in drinking water nor treatment (n = 15).

Second group (G2) represents positive control group in which animals received lead acetate (Pb $(CH_3COO)_2$. 3H2O El Nasr Pharmaceutical Chemicals Co.; 1000 ppm) in drinking water for twenty one day (n = 16).

Third group (G3) represents animals exposed to lead acetate in drinking water for twenty one day with the same dose with application of 0.11 g/kg body weight of Angelica Archangelica roots water extraction from the beginning of lead poisoning process (n = 15).

Fourth group (G4) represents animals exposed to lead in drinking water for twenty one day with the same dose with application of 0.11 g/kg body weight of Angelica Archangelica roots water extraction after stoppage of lead poisoning process (n = 14).

Drinking water was prepared as 1000 ppm lead acetate solution. Acidification of water is essential for dissolving lead acetate in water so 1 ml of conc. hydrochloric acid was added per liter of deionized water 1000 ppm lead acetate dose is chosen to give a desired level of toxicity in blood and other soft tissue pools.

Sample preparation attention was paid to avoid contamination, therefore every item from the moment of sampling until analysis was regarded as potential source of contamination and was checked not to contain or leach detectable amount of any contaminant. Sample collection was performed after finishing the 21 days for the first group and 42 days for the rest.

The roots of Angelica Archangelica (whole root, first grade) were provided and identified by Mr. Haraz (Famous and Oldest Egyptian Herbs & folk medicine manufactures and suppliers, Cairo, Egypt) in March 2010. The samples of Angelica were harvested from Syria in August 2008. All chemicals were used without further purification.

Determination of blood lead level was carried out according to the method described in the Pye-unicum instruction manual (1980) using a Pye-unicum SP 90 series Atomic absorption spectrophotometer. Ashing technique was done by taking 2 ml blood added to 2 ml of HNO_3 of specific gravity 1.3 plus one milliliter of HCl in a 100 ml beaker to be placed on hot plate till dryness. When the contents become almost dry, the side of the beaker was flushed with few drops of water, then 3 ml of HNO_3, was added and the contents will be digested again to dryness. The deporteinized blood was dissolved, centrifuged and transferred quantitatively to the original volume of the sample is 2 ml. Then the sample was introduced into the apparatus for reading, the point of intersection between the sample reading and the standard curve indicates the contents of lead in the blood sample expressed in mg/dl.

Measurement of auto-oxidation rate was carried out spectrophotometrically as described by Wallace et al., and Guillochon et al., [25, 26]in air saturated 0.1 M phosphate buffer of pH 7.05 with 2 mg/ml HbO_2. The pH was checked before and after each experiment

and spectra of hemoglobin was recorded in order to confirm the absence of hemichrome during auto-oxidation rate measurements.

Determination of superoxide dismutase (SOD) activity was carried out by a RANDOX kit package, cat. No. SD 125 [27].

Determination of glutathione peroxidase activity was carried out by a RANDOX kit package, cat. No. RS 504 according to.

RESULTS

Table (1) : Blood lead ions concentration in rabbits exposed to lead poisoning in drinking water as compared to those exposed to lead poisoning with application of Angelica Archangelica roots water extraction with and without further exposure of lead (Mean ± SD)

Group	Lead ions concentration µg/dL
G1 (n = 15)	4.25 ± 0.26
G2 (n = 16)	67.48 ± 4.25**
G3 (n = 15)	18.55 ± 1.67*
G4 (n = 14)	11.25 ± 1.25***

* P < 0.01 , ** P < 0.5 *** P < 0.001 compare between each group and control

Lead acetate in drinking water for twenty one day resulted in a significant toxicity level on blood (67.48 ± 4.25 µg/dL). After administration of Angelica Archangelica roots water extraction for another twenty one day concomitant with lead exposure from the beginning of lead intoxication process resulted in blood lead concentration (18.55 ± 1.67 µg/dL). A better chelation potency of the herbal extraction is appeared in animals treated with the extraction after stoppage of lead poisoning.

Table (2) : Super Oxide Dismutase (SOD) and Glutathione peroxidase (GPx) of rabbits exposed to lead poisoning in drinking water as compared to those exposed to lead poisoning with application of Angelica Archangelica roots water extraction with and without further exposure of lead (Mean ± SD)

Group	SOD (U/ml)	GPx (U/ml)
G1 (n = 15)	62.66 ± 4.25	5781.25 ± 325.85
G2 (n = 16)	152.45 ± 12.58**	9523.48 ± 825.14*
G3 (n = 15)	97.25 ± 5.27***	7452.85 ± 523.25**
G4 (n = 14)	66.08 ± 4.44**	6215.84 ± 412.58**

* P < 0.01 , ** P < 0.5 *** P < 0.001 compare between each group and control

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In table (2) antioxidant activity levels were showed in animals received lead acetate in drinking water as those received Angelica Archangelica roots water extraction for another twenty one day either with or without continuous exposure to lead. It is very noticeable that in animals received lead ions with no treatment administration (G2), both antioxidants activity showed significant elevated levels. The best results regarding the both antioxidants were recorded in animals received treatment after stoppage of lead ions exposure (G4).

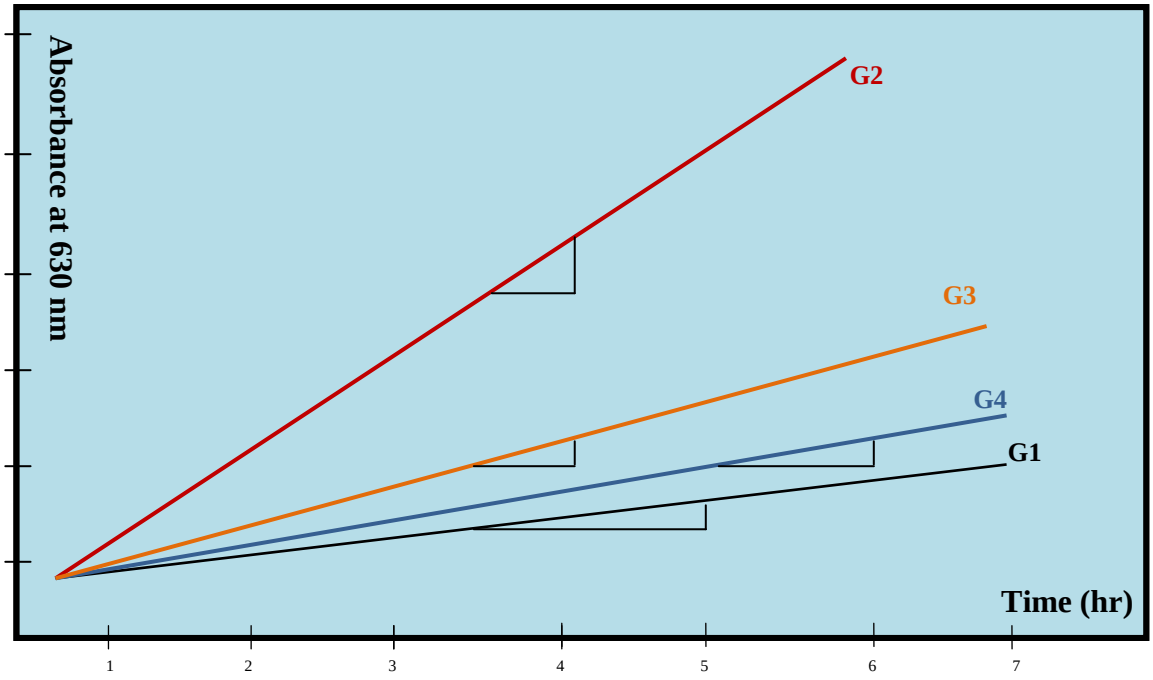

Fig. (1) : Hemoglobin auto-oxidation rate of animals received lead ions in drinking water for twenty one day as compared to those received Angelica Archangelica roots water extraction for another twenty one day either with or without further exposure to lead.

Auto-oxidation rate of hemoglobin in animals received lead acetate in drinking water for twenty one day and then treatment started after complete isolation of lead ions source showed the lowest auto-oxidation rate (G4). Those animals nearly showed a rate very close to those did receive neither lead acetate nor Angelica Archangelica roots water extraction.

DISCUSSION

Lead poisoning (also known as plumbism, colica Pictonum, saturnism, Devon colic, or painter's colic) is a medical condition caused by increased levels of the heavy metal lead in the body [29]. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders. Symptoms include

abdominal pain, confusion, headache, anemia, irritability, and in severe cases seizures, coma, and death [30].

Routes of exposure to lead include contaminated air, water, soil, food, and consumer products. Occupational exposure is a common cause of lead poisoning in adults [31]. One of the largest threats to children is lead paint that exists in many homes, especially older ones; thus children in older housing with chipping paint are at greater risk. Prevention of lead exposure can range from individual efforts (e.g. removing lead-containing items such as piping or blinds from the home) to nationwide policies (e.g. laws that ban lead in products or reduce allowable levels in water or soil) [32].

Elevated lead in the body can be detected by the presence of changes in blood cells visible with a microscope and dense lines in the bones of children seen on X-ray. However, the main tool for diagnosis is measurement of the blood lead level; different treatments are used depending on this level [33].

Humans have been mining and using this heavy metal for thousands of years, poisoning themselves in the process. Although lead poisoning is one of the oldest known work and environmental hazards, the modern understanding of the small amount of lead necessary to cause harm did not come about until the latter half of the 20th century. No safe threshold for lead exposure has been discovered—that is, there is no known amount of lead that is too small to cause the body harm [34].

Lead poisoning can cause a variety of symptoms and signs which vary depending on the individual and the duration of lead exposure [35]. Symptoms are nonspecific and may be subtle, and someone with elevated lead levels may have no symptoms. Symptoms usually develop over weeks to months as lead builds up in the body during a chronic exposure, but acute symptoms from brief, intense exposures also occur. Symptoms from exposure to organic lead, which is probably more toxic than inorganic lead due to its lipid solubility, occur rapidly. Poisoning by organic lead compounds has symptoms predominantly in the central nervous system, such as insomnia, delirium, cognitive deficits, tremor, hallucinations, and convulsions [36].

Symptoms may be different in adults and children; the main symptoms in adults are headache, abdominal pain, memory loss, kidney failure, male reproductive problems, and weakness, pain, or tingling in the extremities. The classic signs and symptoms in children are loss of appetite, abdominal pain, vomiting, weight loss, constipation, anemia, kidney failure, irritability, lethargy, learning disabilities, and behavior problems. Children may also experience hearing loss, delayed growth, drowsiness, clumsiness, or loss of new abilities, especially speech skills. Symptoms may appear in children at lower blood lead levels than in adults [3].

Several mechanisms have been proposed for lead induced various abnormalities, but none have yet been defined explicitly [37]. Oxidative stress has recently been reported as one of the important mechanism of toxic effect of lead [38,39]. It is suggested that the changes in glutathione as well as antioxidant enzyme activities implicate oxidative stress in the toxicity of lead. Few earlier studies indicate that the disruption of reducing status of tissue leads to the formation of reactive oxygen species (ROS), which may damage essential bio-molecules such as protein, lipids and DNA (17,40). Studies also demonstrated that following an oxidant injury, disulfide bonding between lens proteins and cellular thiols such as GSH and cystein occur prior to cataract formation. A report by Ercal et al. suggested that in vivo generation of highly reactive oxygen species like hydroxyl radical (OH^{o}), hydrogen peroxide (H_2O_2), superoxide radical (O_2^{o}) and lipid peroxide (LPO), in the aftermath of lead exposure, may result in systematic mobilization and depletion of the cells intrinsic antioxidant defenses [2]. At high levels, these reactive oxygen species can be toxic to cells and may contribute to cellular dysfunction and poisoning. We recently reported beneficial role of antioxidants in the prevention and treatment of acute and chronic lead poisoning [34,40]. The proposed mechanism of action of these antioxidants is still unclear. It is however believed that antioxidants shields the cells from the influence of oxidative stress by scavenging the free radicals generation and halting lipid peroxidation chain reactions that may cause damage to DNA. Several anti-oxidative strategies may be therapeutically useful including supplementation with antioxidant and up-regulation of endogenous anti-oxidative defense system [41]. Recent reports strongly suggest the beneficial effects of melatonin as an antioxidant moiety in protecting cells from the toxic effects of the free radical species and oxidative stress [42]. Melatonin not only prevents neurotoxicity induced by Kainate but also proved beneficial in preventing traumatic brain injury resulting from the generation of oxidative stress [43]. The therapeutic efficacy of melatonin has been reported to be better than other antioxidants like glutathione and vitamin E [42]. N-acetylcysteine (NAC), a thiol containing antioxidant has been utilized to mitigate various conditions of oxidative stress. Its antioxidant action is believed to originate from its ability to stimulate glutathione synthesis, thus, maintaining intracellular GSH levels [44] and scavenging ROS [45]. Recent studies suggest that NAC has a moderate chelating ability too, against heavy metal poisoning [46]. Chelation is the treatment of choice in lead poisoning. Meso 2,3-dimercaptosuccinic acid (DMSA) is an orally effective chelating agent that was approved by U.S. Food and Drug Administration in 1991 for the treatment of lead poisoning in children. It has two sulfhydryl groups in its structure, which may be useful in complexing lead, in scavenging free radicals and eliciting antioxidant properties. Ercal et al. (1997) reported that DMSA might confer only moderate degree of protection against lead induced oxidative stress. They however, suggested possible therapeutic usefulness of NAC when administered in combination with DMSA in low-level lead exposure [35].

In our previous researches we introduced a new chelating agent derived from Angelica Archangelica roots water extraction. It is mentioned in the traditional alternative Arabic medicine as a heavy metals removal. This extraction revealed a very significant chelating potency of lead regarding lead ions concentration in blood, liver, bone and brain. No side

effects were recorded through the extraction oral administration. Doses were compared with those used from DMSA as a body weight base.

Angelica Archangelica roots water extraction also revealed a significant antioxidant activity regarding the ROS produced with elevated lead ions concentration. So, this extraction approved a very important role in both chelation and antioxidant activity that is way we strongly recommend it as a double impact natural chelator.

Results of this work also confirmed the previous finding by measuring the antioxidant activities after administration of dominant lead poisoning levels. Significant enhancement in both super oxide dismutase and glutathione peroxidase was dramatically reduced after been elevated due to ROS production in lead intoxicated animals.

Another evidence of free radicals reduction with administration of Angelica Archangelica roots water extraction is the reduction in met-Hemoglobin concentration. That is the non-functional hemoglobin form produced by the hemoglobin oxidation process carried out by free radicals. It is spectrophotometrically characteristic by a new peal at 630 nm in the hemoglobin spectrum. A low slope value was calculated in animals those treated with the herbal extraction after complete stoppage of lead acetate in drinking water for the same time period of the poisoning process. The most aggressive auto-oxidation rate was recorded to hemoglobin of animals received lead acetate with no treatment. Application of the treatment concomitant with administration of lead acetate in drinking water revealed a moderate enhancement in both antioxidant activities and auto-oxidation rate while the best results showed in animals received the treatment with no continuous of lead poisoning.

In conclusion, Angelica Archangelica roots water extraction revealed a significant antioxidant role beside its chelation potency of lead ions. This extraction revealed a significant reduction of Met-Hb derivative. It is highly recommended for further work to be identified and specify dose calculation.

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