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Investigation on Oral Toxicity of Diallyl Sulfide: A Principle Organosulfur Compound Derived from *Allium Sativum* (Garlic) in Mice

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ABSTRACT

Allium sativum (garlic) is used as food additives and medicines. Its health benefits are well known, which are mainly contributed by the active organosulfur compounds present in it. Though garlic widely used, but limited is known about preclinical acute toxicity of its organosulfur compounds in mice. The present study aimed at toxicity evaluation of diallyl sulfide (DAS) in C57BL/6 mice following oral administration at range of concentrations (40 mg/kg, 400 mg/kg, 800 mg/kg and 1600 mg/kg). Survival, hematological, organ coefficients, and histopathology studies were performed to establish the DAS toxicity in mice**.** Stability studies performed *in vitro* by HPLC showed rapid and time dependent changes in DAS area**.** A single oral dose upto 1600 mg/kg was well tolerated in mice without any significant changes in standard toxicological parameters. No death was recorded at the tested concentrations. Also no significant changes in the organ coefficient were observed when compared to vehicle treated and sham control. Mild alterations in liver pathology and hematological changes were observed post 1600 mg/kg DAS administration orally. This study demonstrates that single oral administration (upto 1600 mg/kg) of DAS is within the safe limits with no observable adverse effects in mice. Based on the safety profile of DAS, we conclude that DAS can be further explored for use in humans as a potential radiomitigator.

Keywords: Acute toxicity; Diallyl sulfide; Garlic; Oral

NOMENCLATURE

CDSCO	Central Drugs Standard Control Organization
DADS	Diallyl Disulfide
DAS	Diallyl Sulfide
DASO	Di Allyl Sufoxide
DASO2	Di Allyl Sulfone
DATS	Diallyl Tri Sulfide
GRA	Granulocyte
Gy	Gray
HCT	Hematocrit
IAEC	Institute Animal Ethical Committee
LD	Lethal Dose
LYM	Lymphocytes
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
MTD	Maximum Tolerable Dose
NOAEL	No Observed Adverse Effect Levels
PLT	Platelets
RBC	Red Blood Cell
RDW	Red Cell Distribution Width
RP-HPLC	Reverse Phase-High Performance Liquid Chromatography
RT	Room Temperature
WBC	White Blood Cells

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1. INTRODUCTION

The preventive and therapeutic benefits of natural products or plant derived molecules have been widely known in Ayurveda and other ancient systems of medicines. Owing to the diverse pharmacological properties alongwith better safety and efficacy window, natural products have always been reliably used as nutritional supplements or in treatment of many ailments in comparison to synthetic products. Moreover, plants are also rich source of bioactive molecules for drug development. One such class of compounds is lipid soluble allyl sulfides, which are majorly found in *Allium sativum* or garlic. Garlic is widely used for flavoring in cooking as well as medicine in modern and ancient times to treat many ailments[1,2]. It is believed that the health benefits of garlic is primarily contributed by the volatile organo sulfur compound, diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl tri sulfide (DATS) present in it[2]. Among these metabolites, DAS is the principle oil-soluble organosulfur compound, which is well known for its pharmacological role in prevention and treatment of cancer and other disease conditions [1,3,4,5].

In animal models, DAS administration was found to effectively attenuate N-nitrosodiethylamine (NDEA)-induced liver tumorigenesis[6] and gentamicin induced-nephrotoxicity[7]. Involvement of Nrf2 pathway in DAS-mediated protection against HIV protein-induced toxicity[8] and attenuation of hyper-glycemia mediated cellular toxicity were reported in VL-17A cells[9]. Investigations have also showed the protective

effects of DAS and other organosulfur compounds against acetaminophen-induced toxicity[10-11] and against thioacetamide-induced toxicity in rats[12].

We are developing DAS as radiation countermeasure agent that can mitigate the damaging effects of radiation in humans. Previous studies performed in our laboratory have shown that single intraperitoneal (i.p) administration of DAS at a dose of 160 mg/kg body weight extends survival benefit in mice (upto 37%) as opposed to 100% mortality in 9Gy total body irradiated group[13]. Also, oral administration of DAS (40 mg/kg) has extended considerable survival benefits in mice (unpublished data). Henceforth, to develop DAS into clinically accepted drug, it is important to demonstrate preclinical toxicity. Limited published toxicity data currently are available for oral DAS in small animals with no available information of DAS toxicity in mice.

In the present study, we have characterised the acute toxicity following a single administration of DAS to male mice at variable concentrations. The studies were performed partly as per the animal toxicology (non-clinical toxicity studies) mentioned in Schedule Y guidelines (appendix III) of Central Drugs Standard Control Organisation (CDSCO), Govt. of India[14]. Stability studies were performed using HPLC, while common laboratory assessments included body weight recording, food consumption monitoring, organ pathologies and hematological examinations. The results from these studies collectively demonstrate DAS has considerable safe profile within 1600 mg/kg dose range when administered via oral route in mice.

2. MATERIALS AND METHOD

2.1 Test Molecule

Purified DAS from *Allium sativum* was commercially procured from Sigma Aldrich, St. Louis, MO (CAS Number 592-88-1; 97 % purity). Before administration to the mice, *in vitro* stability of DAS was characterised using HPLC method.

2.2 *In Vitro* Stability

100 mg/ml DAS was prepared in canola oil and incubated separately at RT and 2-8 ºC in dark condition. The time points taken for stability study were 0, 2, 6, 48, 72 h, 7th day, 20th day & 65th day. Sample at different time points were drawn and was diluted to 0.5 mg/ml with mobile phase (acetonitrile and water). RP-HPLC analyses of samples were done by Sunfire C18 column (4.6 X 250mm, 5µm). The mobile phase was acetonitrile and water in ratio of 70:30, v/v. Before sample injection, the column was first equilibrated for 60 min. with mobile phase. 20 µl sample was injected at the flow rate of 1ml/min and the peak was detected at 240 nm.

2.3 Study Design

Eight-ten week old C57BL/6 male mice maintained at the institute's animal experimental facility were used. Mice maintained at 24±2 ºC with a 12 h dark- light cycle were given a standard diet of rodent pellets (Golden Feed Pvt. Ltd., Delhi, India) and water, *ad libitum*. Mice (n=5/group) were administered with a single dose of DAS orally using canula. All animal experiments were conducted strictly according to Institute's animal ethical committee guidelines (Approval No.; INM/IAEC/2019/03).

Four doses of DAS were used for oral toxicity (Gps. III-VI) studies (Table 1). The mice from different treatment groups that received oral dosing were compared with corresponding control (Gp.I) and vehicle control treated with 200 µl canola oil (Gp. II). The starting dose levels for oral (Gp. III) were selected based upon the established efficacy study of DAS in C57BL/6 mice in our laboratory (unpublished data). Detailed group design and dose levels are listed in the following Table1.

Table 1. Group designation and dose levels

Group level	No. of animals Male	Dose Concentration mg/kg BW DAS	Dose levels*
Group I	05	-	Untreated
Group II	05	-	200µl canola oil
Group III	05	40	X (ED)
Group IV	05	400 (low)	10X
Group V	05	800 (middle)	20X
Group VI	05	1600 (high)	40X

* Dose levels were determined based on the escalation of the effective dose (ED; 40mg/kg) used orally for radiomitigation studies in C57BL/6 mice.

2.4 Scheduled Sacrifice of Mice

Animals were monitored for body weight, food consumption and fluid intake, morbidity and mortality till 14 days study period. On Day 15, all surviving animals were fasted for ~4 h before termination. On day 15, mice from all the groups were euthanised and investigated for hematology, organ to body weight ratio, gross and pathological observations

2.5 Body Weight Changes, Food Consumption and Fluid Intake Monitoring

Per cent loss or gain in the body weight was calculated by the formula:

$$\frac{\text{Mice body weight - initial weight}}{\text{Initial body weight}} \times 100$$

Food consumption and water intake were calculated by weighing the food quantity and measuring the drinking water volume.

2.6 Blood Hematology Analysis

Hematology was performed at all the dose levels. The blood collected in heparanised vacutainers was analysed for changes in the blood cell composition in a fully automated three-part hematology analyser (Celltac-α; Nihon Kohden, Germany). Data are represented as mean of values retrieved from five mice individually.

2.7 Organ Indexes (Organ to Body Weight Ratio)

Organs were isolated from animals sacrificed at schedule interval (15 day) and weighed. Organ indexes were calculated as organ weight to body weight ratio.

2.8 Histopathology Evaluation

All the organs isolated after scheduled termination were fixed in 10% buffered formalin, dehydrated through graded series of ethanol, cleared in xylene and infiltered with melted paraffin wax. Paraffin blocks were cut into 5 µm serial sections and stained with hematoxylin and eosin.

2.9 Spleen and Bone Marrow Cell Suspension Preparation

Spleen tissue and femur bones were aseptically isolated after schedule termination (15th day) and rinsed with ice-cold phosphate-buffered saline (PBS). Initially, spleen size and weight were measured. For single-cell suspension preparation from spleen tissues, the complete spleen was gently minced with PBS between the two chilled frosted glass slides. Bone marrow cells from the femur bones were isolated by flushing the bone with the 24 gauge needle using PBS. Isolated bone marrow cells and splenocytes were centrifuged at 2,000rpm for 10 min at RT. The cells were incubated with RBC lysis buffer to remove the RBCs, cells were washed twice with the ice-cold PBS. Cells were then counted in the Neubeaur's chamber to record changes in the cell counts in response to different treatments. Spleen and bone marrow cell counts were represented as million cells/ml.

Table 2. *In vitro* stability of DAS as observed by changes in % DAS area at different time intervals

Time point (hr)	Area % of DAS	
	4 °C	RT
0	97.49	97.49
2	92.29	95.08
6	71.05[a**]	69.09[b**]
48	68.22[a**]	68.77[b**]
72	70.09[a**]	68.21[b**]
168 (7th day)	67.33[a**]	66.15[b**]
480 (20th day)	38.37[a***]	36.91[b***]
1560 (65th day)	58.38[a**]	51.98[b**]

The values at each time points in each treatment group were compared with the DAS area of 0h. [a]: % DAS area at each time point vs. 0h at 4°C; [b]: % DAS area at each time point vs. 0h at RT. A $p<0.05$ was considered significant. **= $p< 0.01$; ***= $p< 0.001$.

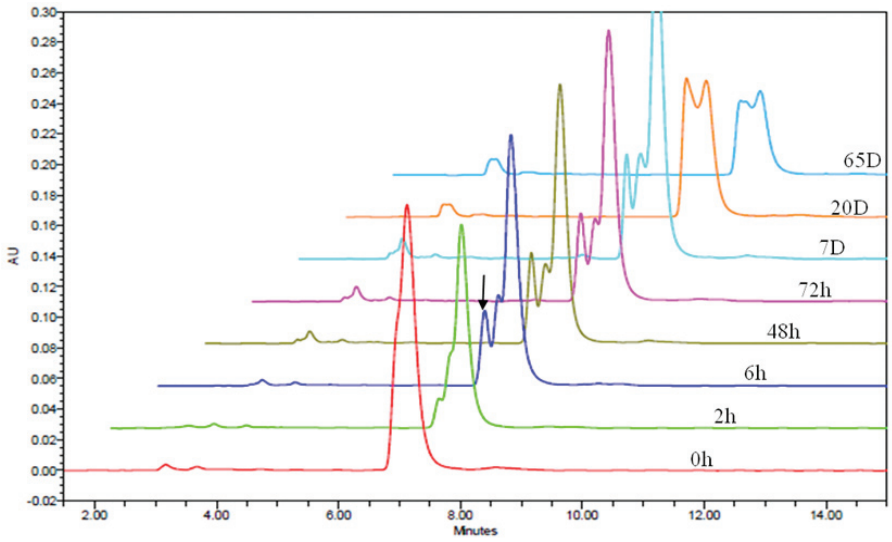

Figure 1. Representative chromatograms showing % change in DAS area at different time intervals when incubated at 4 °C. The arrow indicates appearance of a new peak at all time points from 6h onwards.

2.10 Statistical Analysis and Data Evaluation

Data are presented as the means ± SEM. The differences in body weight, heamatological, and histopathological endpoints between each treatment groups were compared with corresponding control and vehicle control. Statistical analysis were performed using GraphPad Prism version 6.01. (San Diego, CA). A value of $p\leq0.05$ was considered significant. The *, ** and ***signifies $p<0.05$, $p<0.01$, and $p<0.001$values, respectively, whereas ns signify nonsignificant value.

3. RESULTS

In the present investigation, commercially available DAS was diluted in canola oil (vehicle) at desired concentrations, was initially checked for stability followed by oral toxicity evaluation in mice.

3.1 *In Vitro* Stability of DAS

The stability analysis of DAS monitored at RT and 4 °C at different time points (2, 6, 24, 72h, 7, 20 and 65 days) by the given HPLC method and compared with DAS area at 0h. Comparison of the chromatograms revealed decline in % DAS area from 6h of incubation. At 6 h, nearly 30 % degradation in both conditions were observed in contrast to the 0h. As shown in Table 2 and Fig. 1, the area percent of DAS peak decreased and this was accompanied by the co-occurrence of two new peaks. The degradation was found to be time dependent and a steady state decrease in % DAS area upto 20th day was observed. Till 7th day, the degradation of DAS kept at 4°C and RT was 32.67% and 33.85% respectively. On 20th day, increased degradation (65-70%) of the DAS peak was re-observed and it was found that only 38.37% and 36.91% of DAS remained in sample kept at 4 °C and RT respectively. Surprisingly, on 65th day the area of the DAS peak increased by 20 % and 15% at 4 °C and RT respectively.

3.2 Effects of DAS on Signs, Symptoms, Body Weights and Survival

DAS mediated death, mean body weight changes and adverse effects through oral route were recorded during the 14 days observation period. No observed adverse effect levels (NOAEL) were found in mice administered with DAS at all the tested concentrations. Mild breathing abnormalities and decreased activity were observed immediately after DAS administration, which were temporary and lasted for about 1h-2h of DAS administration. These mice ate, behaved normally and had usual motor

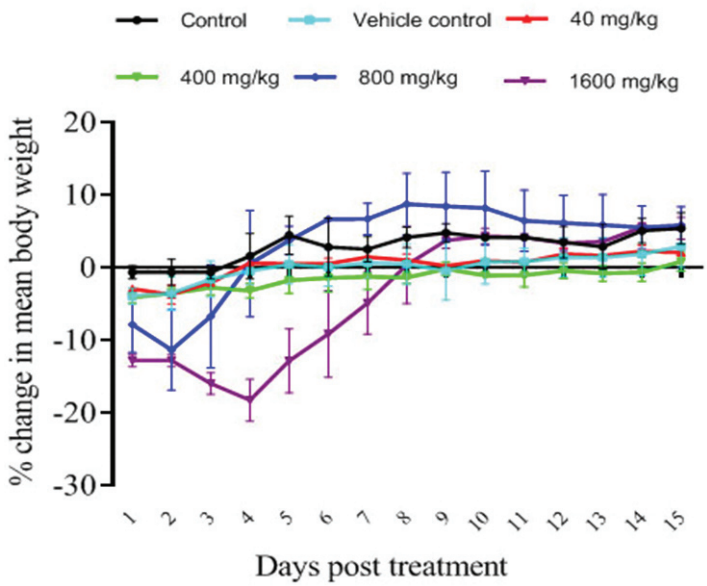

Figure 2. Mean body weight changes in mice administered with DAS orally at different concentration. In vehicle control group, mice were administered only with canola oil. The changes in body weight were monitored for 15 days post DAS administration.

activity. However, mice that received 1600 mg/kg orally showed comparatively more severe symptoms and respiratory distress. The discomfort lasted for 3-4 days in these mice before they reverted back to normal activities.

Figure 2 shows DAS mediated mean body weight changes in mice. An increasing trend in mean body weights of control and vehicle treated mice was recorded till the end of observation period. After initial body weight fall for 4-5 days, an increasing trend was observed overtime during 14 days study period in mice treated with low (400 mg/kg) DAS concentration (Fig. 2). The mice that received 800 mg/kg and 1600 mg/kg doses displayed significantly lower mean body weight than 400 mg/kg (Fig. 2). The % change in the mean body weight was maximum at 1600 mg/kg dose level, though mice in this group showed considerable increase in body weight post 8 days. Interestingly, mice from these groups revealed faster weight gain, in contrast to the low dose level group. We could not find any correlation between food consumption and mean body weight changes. Mice from all the treatment groups were comparable with the control group in terms of food consumption and the pattern appeared not to be dose dependent. No lethality was observed at any of the doses tested through oral route.

3.3 Effects of DAS on Organ Indexes

Gross examination of organs of animals after scheduled necropsy (15th day) revealed no distinct changes in the organs, except for appearance of pale liver in mice administered with the highest dose level (1600 mg/kg). Also, there were no indications of drug depositions in the abdominal cavity in any of the DAS treated groups. As illustrated in Fig. 3, no marked differences in the weight indices (organ to body weight ratio) was observed for spleen, brain, lungs, kidney, testis, heart, liver and thymus. Interestingly, the stomach index showed mild, and significant decline at 400 ($p<0.05$) and 800 mg/kg ($p<0.05$). At

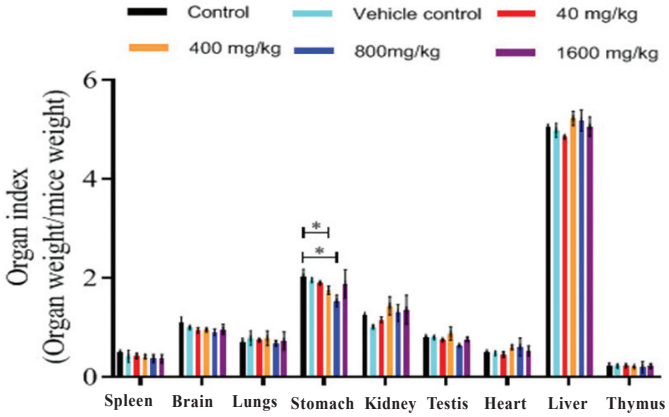

Figure 3. Effect of DAS on organ indices. C57BL/6 mice. Following scheduled sacrifice on 15th day, the organs were dissected and weighed. The organ indices are represented as ratio of organ to mice body weight.

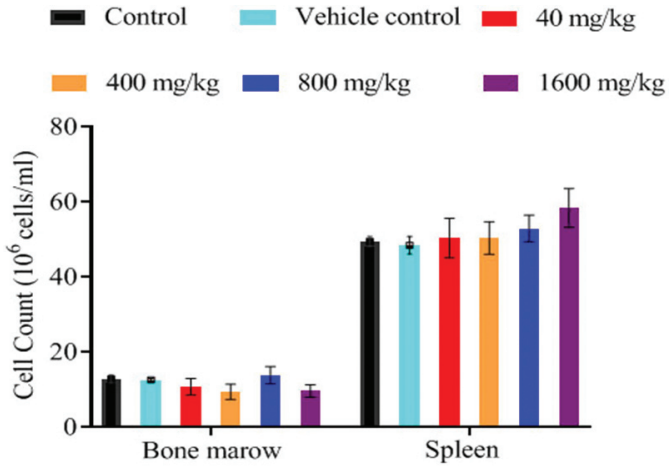

Figure 4. Effect of DAS on spleen and bone marrow counts. Spleen and bone marrow from C57BL/6 mice on 15th day after scheduled sacrifice were processed for single cell suspension preparation and cell counting.

1600 mg/kg, the stomach index was higher than 400 mg/kg and 800 mg/kg dose levels.

3.4 Effects of DAS on Spleen and Bone Marrow Counts

Impact of DAS treatment was also checked on spleen and bone marrow cell counts in control, and all DAS treated dose groups (Fig. 4). Only a slightly lower bone marrow cell counts were observed at 400 mg/kg and 1600 mg/kg doses, but the counts were well within the normal range and comparable to the corresponding non-treated and vehicle controls (Fig. 5). We did not find any significant changes in spleen cell count in all DAS treated male mice. The spleenocyte counts were close to the control (Fig. 4). Overall observations from this study suggest that DAS did not cause any organ injuries when administered orally upto concentration of 1600 mg/kg.

3.5 Effects of DAS on the Organ Toxicology

In line with the organ indexes, histopathological assessment of the vital organs was performed for control and all other treatment groups. H & E stained organ cross sections

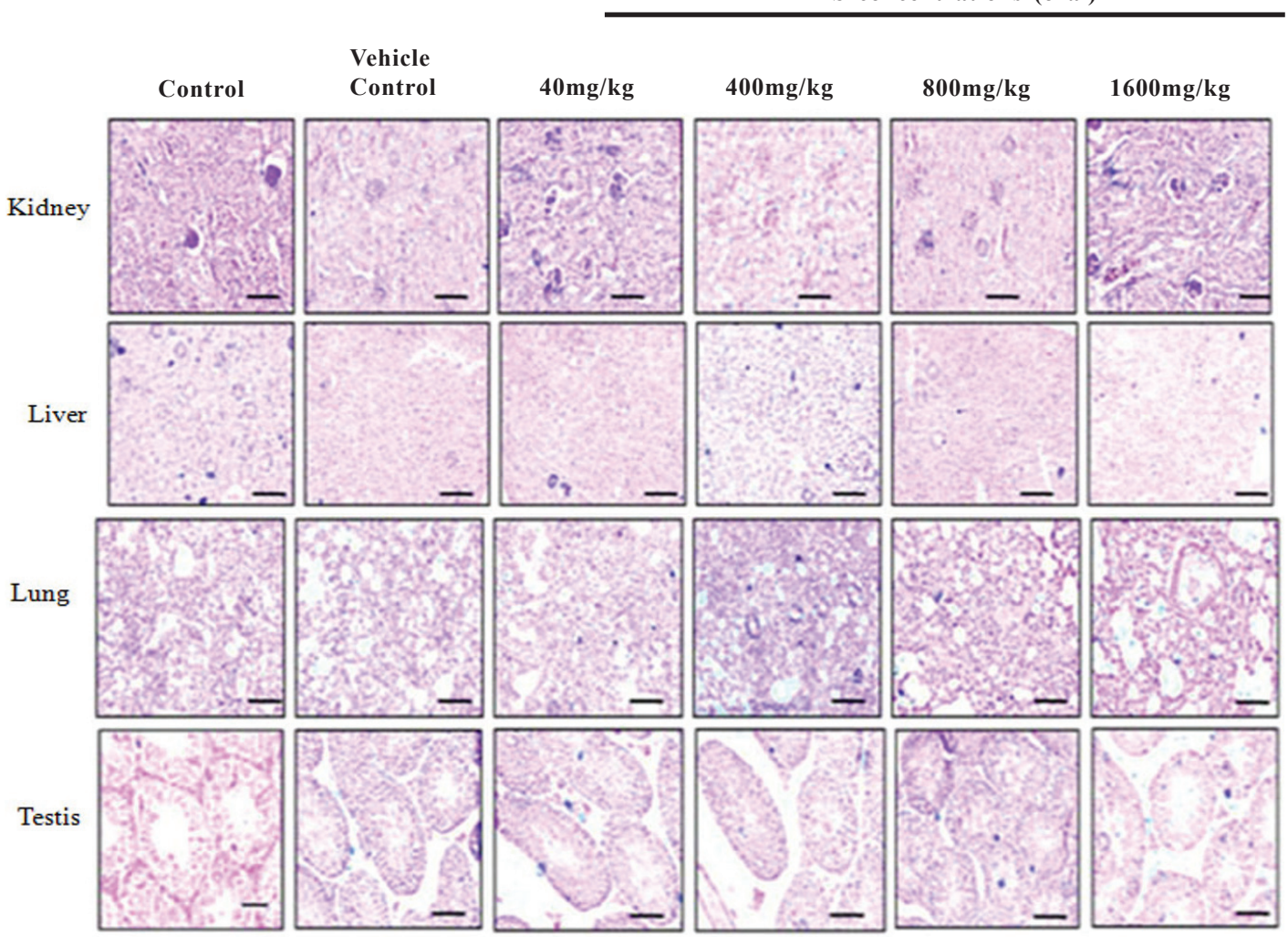

Figure 5. Representative photomicrogram of haematoxylin and eosin (H&E) stained kidney, liver, lungs and testes cross sections. The structural details of each organs for control, vehicle control and different concentration of DAS are presented. Magnification, 100x for all H&E stained tissue cross sections. Scale bar-200 µm.

showed no abnormal morphological changes in kidney, lungs, and testis at all the tested DAS concentrations. The organ architectures were more or less similar to their corresponding controls (Fig. 5).

The kidney sections from control and all DAS treated mice showed well defined tubular epithelium, glomeruli architecture with cells in perfect shape and size. The renal cortex, stroma and medular regions were found unaltered even at 1600 mg/kg DAS concentrations. DAS treated lung cross sections also revealed clearly visible terminal bronchioles, veins, alveoli ducts and alveoli sacs. No signs of accumulation of edematous fluid, deformation of bronchial epithelium and alveoli were visible in any of the cross sections. Histopathological observations of testis cross section demonstrated normal seminiferous tubule and stereocilia count in all DAS treated groups.

Though most of the organs showed normal architecture upon histopathological investigation, but we have noted some abnormalities in liver cross-sections from mice administered with 1600 mg/kg DAS. Presence of normal portal triad and central vein surrounded by hepatocytes with well defined nuclei and cytoplasm was seen in control and DAS concentrations upto 800 mg/kg. Occurrence of mild fatty liver changes and hepatocyte vacuolation was observed only in liver of 1600 mg/kg DAS animal group (Fig. 5).

3.6 Effects of DAS on Hematology

Hematology profiles for male mice exposed to different concentrations of DAS orally and sacrificed on 15th day are shown in Table 2. No significant changes in the blood profiles were observed in DAS treated groups (upto 400 mg/kg) compared with vehicle treated and control groups. However, we did observe fall in total blood cell at 1600 mg/kg DAS concentration. The RBCs were also significantly less in both 800 ($p<0.001$) and 1600 mg/kg ($p<0.001$) treated DAS. Also significant increase in MCH ($p<0.01$) and MCHC ($p<0.001$) levels were seen at 800 and 1600 mg/kg dose levels respectively (Table 3).

4. DISCUSSION

We are exploring the potential of DAS for mitigating the damaging effects of radiation. Preclinical efficacy studies conducted previously in our laboratory showed 33 % irradiated

Table 3. Hematology in male mice treated with DAS at different concentrations

Parameters	Normal ranges	Control	Vehicle control	40 mg/kg	400 mg/kg	800 mg/kg	1600 mg/kg
WBC ($x10^3$ cells/µl)	4.7-9.2	5.935±1.3	4.653±0.93	5.37±2.2	6.5±2.9	6.54±3.4	4.4±0.39
RBC ($x10^3$ cells/µl)	7.09-8.7	9.445±0.7	8.65±0.542	7.65±0.365	9.5±0.59	4.655±0.50^{a***}	4.68±0.54^{b***}
HGB (g/dl)	13.2-17.1	15.78±0.6	14.65±1.68	15.15±0.65	14.56±1.03	15.15±0.07	15.03±1.42
HCT (%)	40.4-49.6	48.3±1.5	48.5±1.25	40.55±1.05	41.26±2.24	40.85±1.2	50.03±1.58
MCV(fL)	42.2- 68.8	46.3±1.2	45.2±2.05	42.33±1.52	43.5±0.85	45.1±2.26	43±1.73
MCH (pg)	17.8-20.2	15.3±0.9	16.50±0.65	15.52±0.35	15.34±0.30	32.7±3.67^{a**}	32.13±0.85^{b**}
MCHC (g/dl)	32.0-35.2	32.7±0.9	30.62±0.32	31.6±0.55	35.24±0.59	72.75±4.59^{a***}	74.93±1.46^{b***}
RDW (%)	0-50.0	13.11±1	12.52±0.55	32.52±0.65	15.32±0.43	23.75±1.2	22±1.32
PlT ($x10^3$ cells/µl)	597-1490	1109±136	890±45	803±120	953±93.4	496±122	501±132
MPV (fL)	0- 0-20	8.03±1.4	9.05±1.4	5.87±0.21	5.5±0.29	6.45±1.34	5.73±0.53

Values are expressed as mean± SEM. [a]: Control vs 800mg/kg group; [b]: Control vs 1600mg/kg group. A $p<0.05$ is considered statistically significant. *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$.

mice survived against 100 % lethality[13]. Since we intend to use DAS for single administration to radiation exposed victims, evaluation of acute toxicity via oral route were planned in mice model. Considering that this is one of the most preferred routes for drug administration in humans, henceforth the present study was considered essential. Three escalated doses, i.e. 400 (low), 800 (mid) and 1600 mg/kg (high) were selected for this study, which was 10, 20 and 40 times more than that of the effective dose (40 mg/kg) of DAS for radiomitigation studies through oral route. We were interested to see the morbidity and mortality during the 14 day study period followed by hematology and organ pathologies of all the surviving mice after administration of DAS through this route.

Monitoring of hematological and histopathological parameters are important for assessing drug mediated tissue toxicities and organ functionality. Some degree of heamatopoietic toxicity was found at doses 800 mg/kg and 1600 mg/kg, and these alterations were more prominent at 1600 mg/kg (Table 3). It has also been reported that the intake of garlic oil at high dose significantly affected the levels of several hematological parameters such as erythrocyte count, hemoglobin and platelets ($p<0.05$), thereby influencing hemostatic balance[15]. Observations like alterations in TLCs, MCH, MCHC were also noted with oral administration of DAS at mid and high dose levels in the present study. Except for some structural changes in liver, no prominent histopathological changes were observed in vital organs even after administering DAS at high (1600 mg/kg) concentration (Fig. 5). The pathological alterations in the liver can be correlated with pale liver on gross observations. In our previous studies on acute toxicity via intraperitoneal route[18] demonstrated mild fatty liver changes and significantly increased hepatic enzyme activities in serum at 1600 mg/kg and 1920 mg/kg dose levels. Our data supports previous findings that have also reported that a high garlic dose can induce liver toxicity[16-17]. DAS is the principle oil soluble compound present in garlic. Henceforth, similar changes in the liver were seen following DAS treatment also via oral route in the present study and intraperitoneally from our previous findings[18]. Overall these findings strongly indicates that DAS at higher dose (>1600 mg/kg) can induce liver dysfunctions, thus suggesting that liver is one of the target organ for DAS mediated toxicity.

Taken together, these results show that the DAS upto 1600 mg/kg through oral route is very safe in mice based on clinical signs, mortality and histopathological parameters. Though we did observe some significant hematological alterations and histopathological changes in liver mice administered with 1600 mg/kg DAS orally. But these changes did not cause any observed lethality in mice at any dose levels. A dose of 40 mg/kg found effective for radiomitigator studies was also non toxic based on the present findings. However, we did not test beyond 1600 mg/kg because as per the schedule 'Y' guidelines a limit of 2 g/kg is recommended for oral dosing. Henceforth the MTD or lethal doses of DAS when administered orally could not be established in the present study.

Lipid soluble allyl sulfides, are main constituents found in food supplement, garlic. Its main volatile organo sulfur compound, allicin rapidly undergo metabolism to form diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl tri sulfide (DATS)[1-2]. Garlic essential oil comprising of these organosulfur compounds (DAS, DADS and DATS) is also used for control of pests at very low concentration 3.5 µl/L in fumigation assay[19-20]. Among these metabolites, DAS is the principle oil-soluble organosulfur compound, which is well known for its pharmacological role in prevention and treatment of cancer[1, 3-5]. Diallyl sulfide has been well documented to have several beneficial properties against cancer, several diseases, alcohol, analgesic drugs and xenobiotics induced toxicities[1], which can be attributed to garlic consumption. Garlic is widely consumed as food supplement and its used extensively in Ayurvedic and modern medicines also. Hence, it is important to evaluate the toxic effects along with its beneficial properties.

DAS is the principal organosulfur compound present in garlic, hence we intended to evaluate DAS toxicity orally. Previous studies strongly suggest that usage of DAS at very high concentrations can induce significant toxic effects[21-22]. Other studies have also demonstrated considerable toxicities of garlic at higher doses. Previous reports have reported LD50 for garlic extracts at a dose range from 0.5 ml/kg to 30 ml/kg given by various routes to rats and mice. Garlic oil/extract has also shown to induce anemia in dogs in chronic toxicity studies[23]. Some studies have reported toxicity of DAS by oral, i.v and dermal routes in different species. The LD50 of 2980 mg/kg in rat following oral administration has been reported[24]. In rabbits, dose of 330 mg/kg was found as LD10 dose on i.v administration[25] and a dose of >5 gm/kg as LD50 upon dermal application[24]. Based on the observed toxicity of garlic extract and DAS, the daily intake of DAS was recommended to be 0.55 mg or 7 µg/kg/day[26]. But no reports are presently available for toxicity of DAS in mice via oral mode of administration. Though, some reports are available for garlic and DAS mediated toxicity, but these studies were carried out much earlier and none of these studies reports DAS toxicity in mice when given orally.

5. CONCLUSION

The findings from the present study indicate that DAS upto 1600 mg/kg prepared in canola oil as vehicle is safe by oral route in mice. This claim is based on no observable changes in mean body weight, food consumption, gross/microscopic parameters. To develop this molecule further into clinically accepted drug will depend on its toxicological evaluation in large animals (non human primates).

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