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Research Article

Effects of aqueous garlic (*Allium sativum*) extract against di-(2-ethylhexyl) phthalate induced cytotoxicity in peripheral blood and liver of adult female mice

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ABSTRACT

This study aimed to investigate the toxic effects of most commonly used plasticizer Di-(2-ethylhexyl) phthalate (DEHP) in the peripheral blood and liver of adult female mice, and protective effect of a commonly used spice (*Allium sativum*) against DEHP induced pathology. Animals were treated with aqueous garlic extract, DEHP, and DEHP+Garlic extract (aqueous) at dosage of 500 mg/kg body weight each (intra-gastric treatment) (n=10) for 28 days. DEHP treatment resulted in a significant decline in mean body weight while a significant increase in the mean liver weight was noticed as compared to the control group. Results indicated various liver histopathologies in DEHP exposed animals including sinusoid dilation, deshaped hepatic parenchyma cells with nuclear anomalies, and increased number of inflammatory cells. A significant increase in average cross-sectional area (ACSA) of the central vein and number of mononucleated, binucleated, and oval cells was noticed in the DEHP group as compared to the garlic group. A significant increase in cellular diameter of hepatocytes in DEHP and DEHP+Garlic group was also observed. Aqueous garlic extract treatment significantly ameliorated the DEHP-induced histopathological and micrometric alterations in the DEHP+Garlic group. Serum ALT, AST, and ALP levels were significantly decreased in the DEHP+Garlic group as compared to the DEHP group. DEHP treatment caused different nuclear anomalies in the white blood cells of female mice, however, no significant signs of recovery were observed by the aqueous garlic extract treatment. The results showed that DEHP is highly toxic to the female mice's liver, and garlic extract could potentially protect and rescue DEHP-induced liver damage in the female mice.

Keywords:
DEHP, Phthalates, Hepatotoxic, Genotoxic, Garlic, Amelioration

1. INTRODUCTION

DEHP is a major representative of phthalates that are the most used plasticizers to improve the elasticity and workability of the polymers. The global production of DEHP is 2 million tons per year[1]. It is widely used in several industries making walls and floor coverings, wires, and tubes, automobile interiors, vinyl gloves, wall-to-wall tapestries, toys, medical equipment, food contact items solvents, detergents, and oils for lubrication, such as paints or adhesives[2]. Because of weak bonding to the polymer, it leaches and migrates into the atmosphere, into food, or directly into body fluids, thereby exposing the environment and individuals[3-4]. The most common routes of exposure are oral and dermal. Studies have reported that these phthalates can be easily absorbed from the gastro-intestinal tract e.g., the absorption for DEHP was more than 50%. In addition, many reports from Europe and Asia have found heavy amounts of phthalate's metabolites in the urine, blood, and semen samples of plastic industry workers as compared to the general population[5-7].

In rats and rodents, the liver is most sensitive to DEHP exposure. The most significant gross disease shift in rodents due to DEHP is an increase in a liver mass

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known as hepatomegaly[8]. DEHP caused hepato-cellular carcinomas or liver neoplastic nodules in both sexes of mice (B6C3F1) and rats (F344)[9]. In hepatocytes, DEHP causes the proliferation of peroxisomes and induces peroxisomal proteins, which induce proteins from non-peroxisomal metabolism. It also acts by inducing hepatocytes proliferation, suppressing apoptosis, generating ROS, destroying DNA, and inhibiting contact between gap junctional intercellular contacts. DEHP is genotoxic and is toxic for chromosomal, spindle, and mitosis results[10-11]. DNA damage was observed by *in vitro* di-(2-ethylhexyl) phthalate exposure to human cells or tissues that altered mitotic rate, apoptosis, cell proliferation, tumor mobility, and tumor cell line spread; and activation of different nuclear receptors[11]. Human and rodents' data indicated that through multiple molecular signals, including DNA damage, DEHP induces cancer[12].

Garlic (*Allium sativum*) is a plant in the genus Allium[13]. It is used as an antioxidant[14], anticancer[15], hypolipidemic[16], hypocholesterolemic[17], and immune modulator[18]. Low oral doses of garlic are not toxic, but high doses can be toxic to rats[19]. Specifically, there is an acute dearth of information regarding the effects of phthalates on the health of the general population. All of us are exposed to large amounts of phthalates while performing different tasks, and garlic as a regular spice. Therefore, the present study was conducted to evaluate the protective effect of garlic against DEHP-induced hepatic and DNA damage in the female mice.

2. Materials and Methods

2.1. Animal care and dose groups

Lab-raised female albino mice (*BALB/c)* of 24-30 g weight and age 5-6 weeks were used as experimental models in this study. As effects of DEHP on male mice have been published in another study,[20] this study was designed to investigate the effects of DEHP specifically in female mice. This study was approved by the Biosafety and Ethical Review Committee of the University of Sargodha, 40100 Pakistan (Ref: SU/Acad/1167/2, Date: November 06, 2019). Animals were placed in a controlled environment at a temperature of 25±2ºC, 12-hour day and night cycle and humidness 45%, regular drinking water and feed provided *ad-labitum* in the animal house of University of Sargodha, Sargodha. The body weight of all animals used in this experiment was recorded daily by digital balance for further analyses. Forty animals were divided into 4 groups (n=10). Control group animals were treated with 0.2 mL corn oil daily throughout the experimental period. Garlic group was administered with 500 mg/kg garlic in water (0.2 mL)[21-22]. DEHP group received 500 mg/kg of body weight DEHP using corn oil (0.2 mL) as vehicle. DEHP+Garlic group received 500 mg/kg of body weight DEHP dissolved in corn oil (0.2 mL) and 500 mg/kg garlic extract in water (0.2 mL). Doses were provided once daily for 28 days through gavages.

2.2. Dose preparation

Moderate doses were selected by looking at the Ld50 values and also by reading literature where these doses were reported in several studies[23-25]. Desired dose (500 mg/kg of body weight) of DEHP (provided by Sigma-Aldrich (99%)) was prepared in corn oil. Single dose volume (0.2 mL) remains constant while the DEHP concentration was adjusted by appropriate dilution of standard solution according to the body weight. Garlic (NARC G-1) was purchased from local market. Fresh garlic extract was made by crushing garlic cloves (30 g) in a marble mortar-pestle along with 10 mL distilled water and squeezed through double cheesecloth. Fresh garlic extract was prepared daily and diluted accordingly.

2.3. Organ's recovery

On the 28th day, after cervical dislocation animals were dissected to collect blood and liver samples. Blood was drawn with the help of syringes from the left ventricle of the heart. The liver was weighed and fixed in Conroy's fixative for histological preparation, while blood samples were kept in EDTA tubes for further processing to study DNA damage and tubes coated with clot activator were used to keep blood for serum hepatic enzymes markers analyses of liver functioning.

2.4. Biochemical tests

The blood samples were centrifuged at (548 ×g /15 min) to separate the serum and kept at -20EC for further biochemical analyses. The serum concentration of AST, ALP, ALT and total protein were estimated by kits provided by Biolab and biomerieux companies. All procedures were performed according to the instruction of each kit[26-28].

2.5. Histological processing

After gradual alcohol dehydration, liver tissues were processed in xylene for transparency and embedded in paraffin wax. Using ERMA TOKYO 42 rotatory microtome, serial sections (5 microns thickness) were obtained. Photomicrographs of Hematoxylin and Eosin-stained sections[29] were taken by the advanced camera of the Huawei (Model no DSC-W35-13 megapixel) connected to the trinocular microscope (Labomid CXR2) at 400× magnification. Micrometric data were obtained using Coral DRAW 11.

2.6. Micronucleus assay procedure

The fresh blood samples collected in EDTA tubes were used to make thick and thin blood smears on clear slides and allowed to dry. 2-3 drops of Conroy's (methanol and glacial acetic acid) fixative were used for fixation. After 10 minutes, the slides were stained with Giemsa stain for 30 minutes and viewed with the help of a microscope. Nuclear anomalies per 1,000 white blood cells were counted and analyzed at 1000× according to Çelik et al. (2013)[30] for the classification of nuclear anomalies.

2.7. Data analysis

To collect observations from the photographs opened in Corel DRAW 2019, we counted number of these cells inside a square of 10 cm^2 at 3 random positions on each photomicrograph. 10x10 cm square gives enough size for counting the number of these cells and cover maximum area of slide. 10 photomicrographs from each liver were used to maximize number of observations[31-33]. The data obtained from liver and blood smear micrometry were then analyzed statistically by using one-way analysis of variance (ANOVA) followed by Tukey's test using SPSS software.

3. Results

3.1. Mean body weight

There was no difference statistically among mean initial body weight of mice randomly divided in control and treated groups. While analysis using one way ANOVA revealed that DEHP treatment resulted in significant (P<0.01) reduction in mean body weight of mice after 1st, 2nd, 3rd and 4th (P<0.05) week of exposure as compared to that of control group. Similarly, DEHP treatment showed a significant decrease in mean body weight of mice after 1st (P<0.01) and 3rd week (P<0.05) week of the experiment, as compared to the garlic given group. Effect of toxicant was also noticed in DEHP+ Garlic co-treatment as significant (P<0.05) reduction in mean body weight after the 1st, 2nd, and 4th (P<0.01) week as compared to the control group was observed (Table 1).

3.2. Mean liver weight

Statistical analysis showed that DEHP exposure for four weeks caused a highly significant (P<0.001) increase in mean liver weight of female mice as compared to control and Garlic given animals. DEHP+Garlic treatment resulted in a significant (P>0.01) reduction in the mean liver weight of female mice as compared to only DEHP exposed group. Garlic administration showed rehabilitative role against DEHP induced effects (Figure 1).

3.3. Mean number of mononucleated, binucleated hepatocytes, oval and Kupffer cells per unit area (10 cm^2)

According to one way ANOVA, a significantly higher number of mononucleated hepatocytes (P<0.001) was observed in DEHP treated mice liver as compared to that of control, garlic and DEHP+Garlic (P<0.01) administered mice. Similarly, the mean number of binucleated hepatocytes was significantly increased (P<0.05) in the DEHP treated group as compared to control, garlic and DEHP+garlic (P<0.01) groups. In the same way, the mean number of oval cells per unit area was significantly (P<0.001) increased in the DEHP treated group as compared to the control, garlic and DEHP+Garlic groups. No significant difference in the mean number of kupfer cells per unit area was noted among control and all treated groups (Figure 2).

3.4. Mean cellular and nuclear diameter of mononucleated hepatocytes and cross-sectional area of the central vein of liver

Statistical analysis by ANOVA showed that DEHP+ Garlic treatment caused significant decrease (P<0.01) in the mean cellular diameter of hepatocytes as compared to the control, Garlic and DEHP (P<0.001) groups (Figure 3A). However, the difference in the nuclear diameter of mononucleated hepatocytes was non-significant among control and treated groups (Figure 3B).

Table 1. Effect of 28 days oral exposure of DEHP (500 mg/kg), Garlic (500 mg/kg), and DEHP+Garlic (500 mg/kg each) on mean body weights of female mice.
Week 1 weights include the weight taken at day 0 before injecting the dose. The data in the Table 1 are presented as a mean weight. The comparison was made with the control group to know the comparative weight changes over a period of 4 weeks.

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
Control	30.33 ± 0.14	32.58 ± 0.97	32.20 ± 0.54	32.09 ± 0.56	33.60 ± 0.44
Garlic	29.49 ± 0.63	31.63 ± 0.77	30.96 ± 0.73	31.09 ± 0.82	29.69 ± 0.66
DEHP	29.31 ± 1.24	26.47 ± 1.46^{a**b**}	28.17 ± 1.10^{a**}	27.68 ± 1.22^{a**b*}	29.15 ± 1.10^{a*}
DEHP+Garlic	29.61 ± 0.66	28.61 ± 0.31^{a*}	28.57 ± 0.89 ^{a*}	29.37 ± 0.50	28.29 ± 1.61^{a**}

Values are presented as Mean±SEM, a=Control vs treated groups, b=Garlic vs DEHP and DEHP+Garlic groups. **P<0.01, *P<0.05.
There is no significant difference in DEHP+garlic vs control. difference of P<0.05 is in case of DEHP vs DEHP+Garlic.

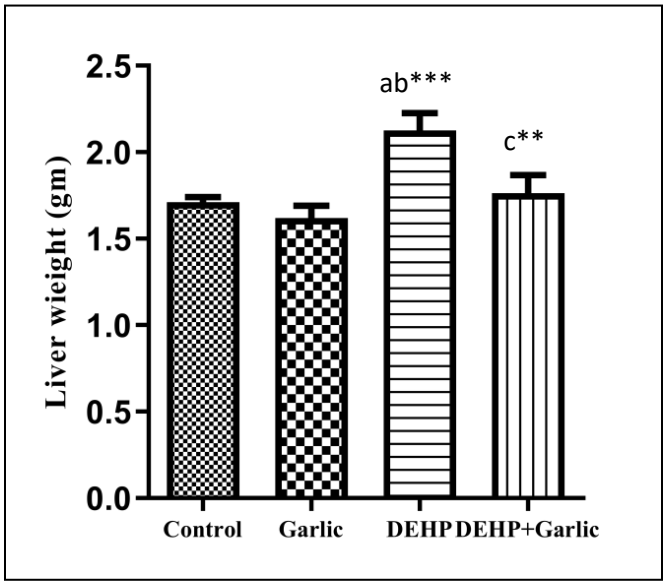

Figure 1. Mean liver weight of control and treated female mice after 28 days exposure to Garlic (500 mg/kg), DEHP (500 mg/kg) and DEHP+Garlic (500 mg/kg each).
Values are presented as Mean±SEM,
a=Control vs treated groups, b=Garlic vs DEHP and DEHP+Garlic groups and c=DEHP vs Combined treatment. ***P*<0.01, ****P*<0.001.

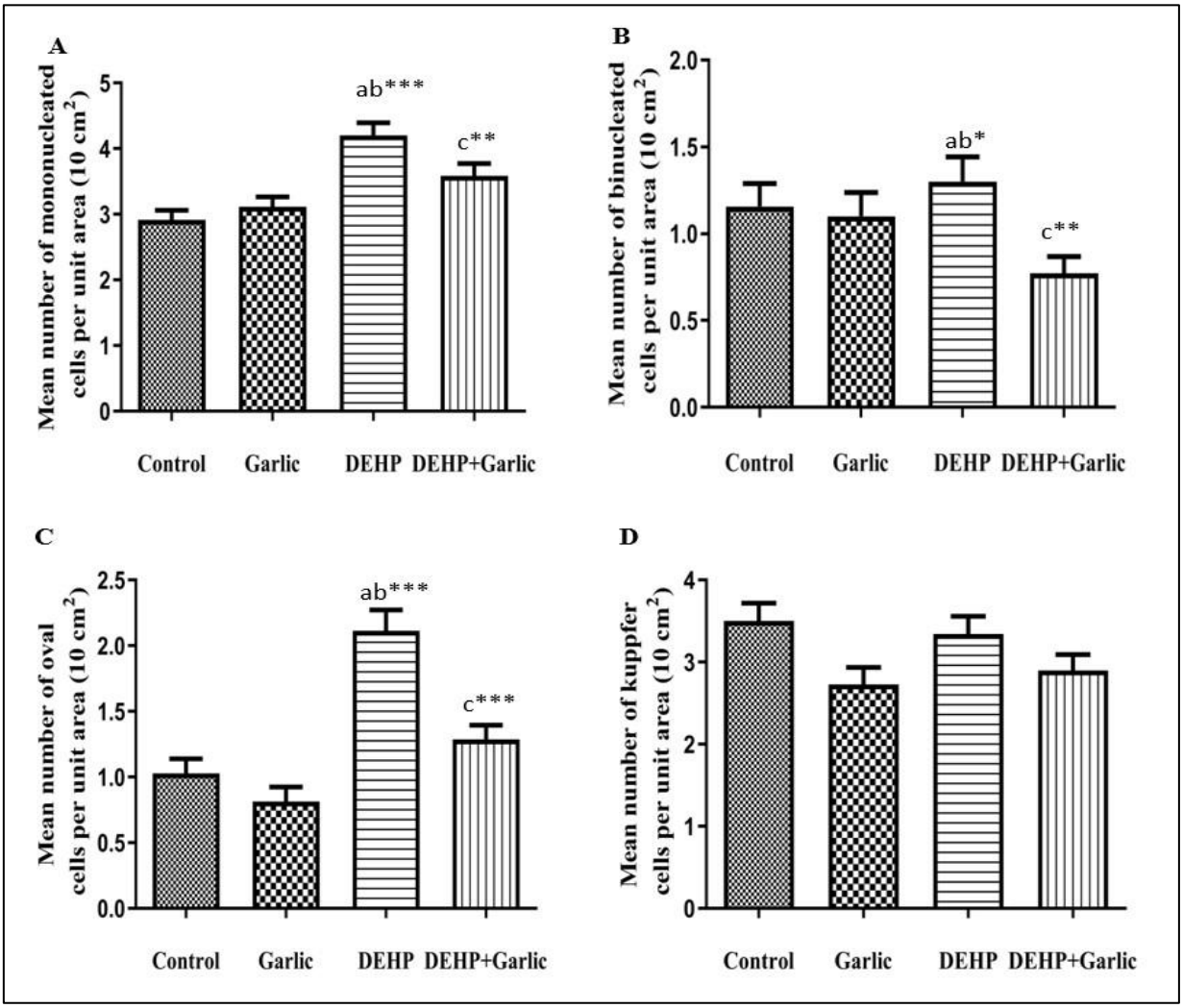

Figure 2. Mean number of mononucleated (A), binucleated (B), oval (C) and kupfer cells (D) per unit area (10 cm^2) in Control and mice exposed to Garlic (500 mg/kg), DEHP (500 mg/kg) and DEHP+Garlic (500 mg/kg each) through gavages for 28 days. One Way Analysis of Variance (ANOVA) followed by a Tukey's post-hoc analysis was performed to determine the significance. Data represented as Mean±SE.

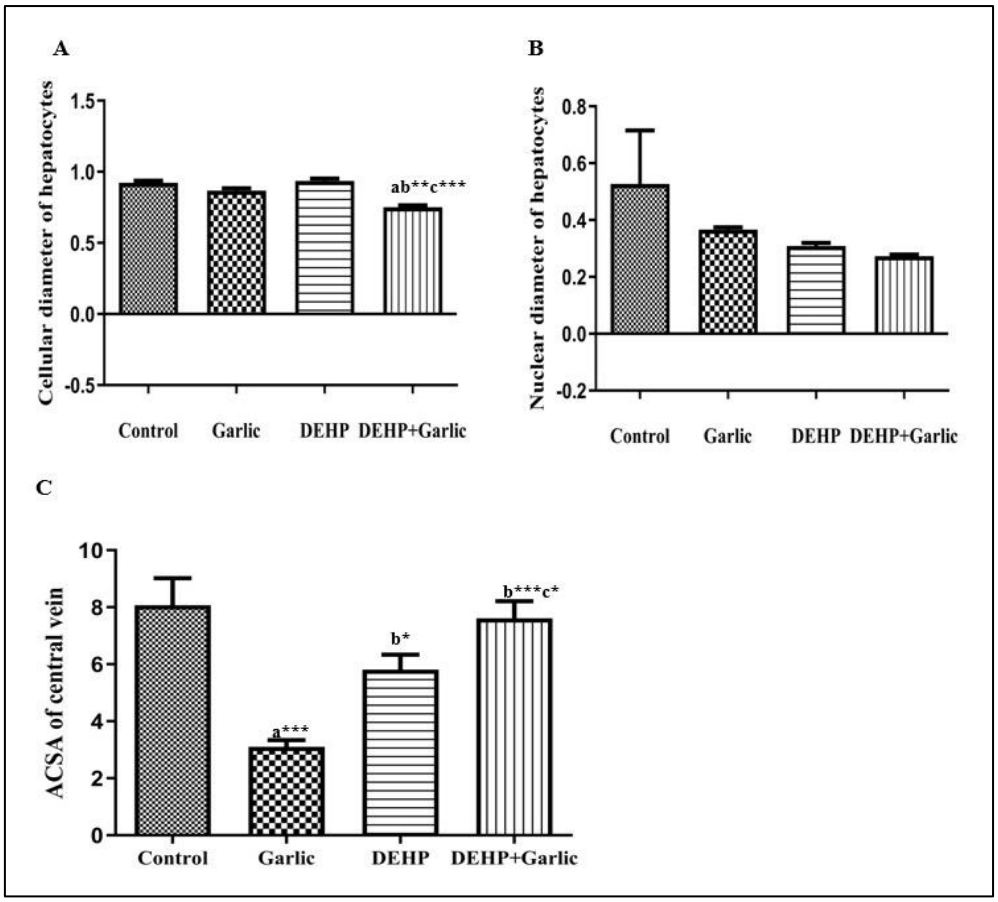

Figure 3. Mean cellular (A) and nuclear (B) diameter of mononucleated hepatocytes and average cross-sectional area of central vein (C) of lobule of liver of female mice exposed to Garlic (500 mg/kg), DEHP (500 mg/kg) and DEHP+Garlic (500 mg/kg each) through gavages for 28 days. One Way Analysis of Variance (ANOVA) followed by a Tukey's post-hoc analysis was performed to determine the significance. Data is represented as Mean±SE.
a=Control vs treated groups, b=Garlic vs DEHP and DEHP+Garlic groups and c=DEHP vs Combined treatment. *P<0.05, **P<0.01, ***P<0.001.

reduced (P<0.001) as compared to the control, DEHP (P<0.05), and DEHP+Garlic treated groups (P<0.001). A significant increase (P<0.05) in CSA of central vein was observed in the DEHP+Garlic given group as compared to DEHP administered group. (Figure 3C).

3.5. Biochemical analyses

There was no significant difference observed in the mean serum level of albumin, globulin, A/G ratio, and total protein contents among mice of all experimental groups (Table 2).

Statistically higher mean serum level of ALT (P< 0.05) was found in the DEHP+Garlic group as compared to that of control and DEHP groups. Mean AST serum level of DEHP treated mice showed a significant increase (P<0.001) as compared to that of control and garlic treated animals. Similar trend was found in DEHP+Garlic group. However, the value of serum AST was significantly decreased in DEHP+Garlic treated mice in comparison to only DEHP exposed group. Mean serum concentration of ALP was statistically higher in DEHP (P<0.001) and DEHP+Garlic (P<0.05) co-exposed group as compared to control. DEHP+Garlic treatment resulted in significant decrease in serum ALP (P<0.05) level as compared to only DEHP treated mice. Serum bilirubin level was not statistically different among control and all treated groups (Table 3).

3.6. Histological analyses

The microscopic examination of the liver tissues revealed several histological abnormalities in DEHP and DEHP+Garlic treated groups, while control group and Garlic exposed mice's liver tissues showed histologically normal structures such as regular portal morphology and spaces (Figure 4A and 4C), normal shaped hepatocytes in the hepatic parenchyma divided by regular sinusoidal spaces organized around the central veins. In DEHP treated mice liver's sections, there was mild sinusoid dilation in lobules-primarily around the central vein and deformed mononucleated and binucleated cells with swollen nuclei were also observed. Apparently, portal spaces were also minimized might be due to the increased number of inflammatory cells, (Figure 4B). The administration of DEHP+Garlic also led to mild sinusoid dilation, however, a compact central vein was clearly visible (Figure 4D).

Table 2. Mean serum albumin, globulin level, A/G ratio, and total protein contents in female mice orally exposed to DEHP (500 mg/kg), Garlic (500 mg/kg), and DEHP+Garlic (500 mg/kg each) for 28 days.

Groups	Albumin	Globulin	A/G Ratio	Total Protein
Control	3.57 ± 0.24	3.60 ± 0.17	1.02 ± 0.07	6.92 ± 0.16
Garlic	3.05 ± 0.06	3.35 ± 0.17	0.92 ± 0.02	6.40 ± 0.14
DEHP	3.60 ± 0.21	3.27 ± 0.17	1.07 ± 0.10	6.87 ± 0.17
DEHP+Garlic	3.50 ± 0.10	3.42 ± 0.07	0.97 ± 0.06	6.92 ± 0.16

Values are presented as Mean±SEM

Table 3. Mean serum ALT, AST, ALP, and bilirubin level in female mice after intake of DEHP (500 mg/kg), Garlic (500 mg/kg), and DEHP+Garlic (500 mg/kg each) for 28 days.

Groups	ALT(U/L)	AST(U/L)	ALP(U/L)	Bilirubin(mg/dl)
Control	33.50 ± 2.32	36.75 ± 4.87	117.00 ± 4.96	0.62 ± 0.09
Garlic	42.25 ± 5.18	32.50 ± 3.66	116.50 ± 20.20	0.57 ± 0.02
DEHP	38.25 ± 1.88	$178.25 \pm 14.65^{ab***}$	$188.50 \pm 6.00^{a***}$	0.50 ± 0.04
DEHP+Garlic	$63.50 \pm 10.53^{ac*}$	$99.00 \pm 6.16^{abc***}$	$138.00 \pm 11.15^{ac*}$	0.52 ± 0.04

Values are presented as Mean±SEM, a=Control vs treated groups, b=Garlic group vs DEHP and DEHP+Garlic groups and c=DEHP vs DEHP+Garlic. ****P*<0.001, ***P*<0.01, **P*<0.05

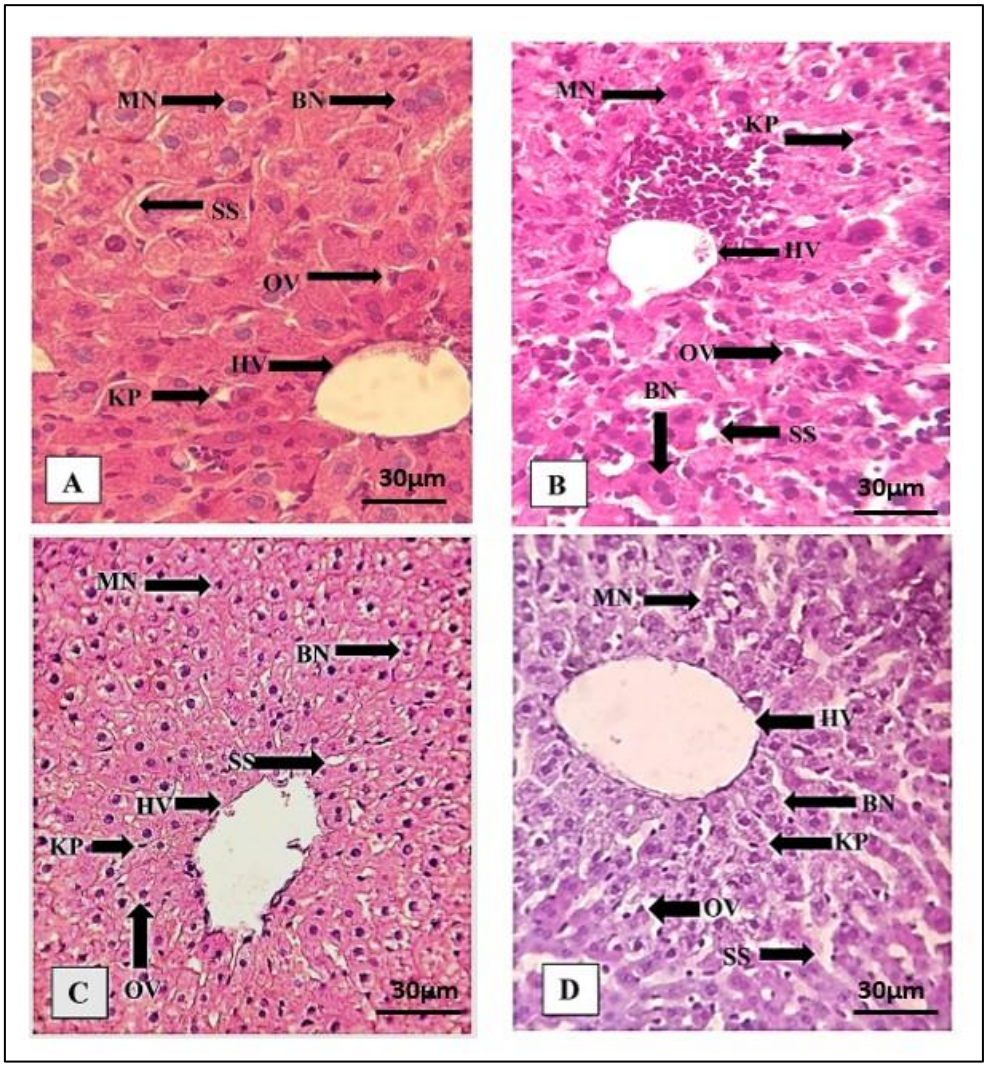

Figure 4. Hematoxylin and Eosin stained photomicrographs (400×) from representing liver sections of adult female mice exposed to Garlic (500 mg/kg), DEHP (500 mg/kg), and DEHP+Garlic (500 mg/kg each) through gavages for 28 days. Control (A): liver exhibited a well-organized structure of mononucleated (MN) and binucleated (BN) hepatocytes radiating from the central hepatic vein (HV) separated by regular sinusoidal spaces (SS). Kupfer cells (KP) lined in sinusoids and free oval cells (OV) were noticed in control sections. DEHP group (B): showed sinusoidal dilation around the central vein, deformed mononucleated cells (MN), and binucleated cells (BN), increased KP and few OV. Garlic group (C): presented compact arrangement of hepatocytes with widened central vein, normal cellular number and structure. DEHP+Garlic group (D) has loose cords of hepatocytes around compact central vein; cellular population was effected by phthalate treatment in co administered group.

3.7. DEHP-Induced genetic damage in the peripheral blood cells

By using micronucleus assay, some major nuclear abnormalities were observed in white blood cells of mice such as binucleated cells, nuclear bud, and micronucleus formation. Karyolytic and karyorrhectic cells were also observed which indicated cell death (Figure 5)[29]. Figure 5 is only pictorial representation of types of nuclear anomalies observed in white blood cells in this study while anomalies count and their difference in each group is given in Table 4.

Statistical analysis using ANOVA revealed that DEHP+Garlic treated group showed a significantly increased number of (P<0.001) binucleated white blood cells as compared to control and other treated groups. There were significantly (P<0.001) high number of micronuclear white blood cells in all treated groups as compared to the control group. Significantly more (P< 0.001) white blood cells with nuclear bud formation were

Table 4. Mean number of different white blood cells nuclear anomalies per 1,000 cells in peripheral blood of Control and treated female mice exposed to DEHP (500 mg/kg), Garlic (500 mg/kg), and DEHP+Garlic (500 mg/kg each) for 28 days.

Groups	Binucleated cells	Micronuclei	Nuclear bud	Karyolytic cells	Karyorrhectic cells
Control	1.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Garlic	1.20 ± 0.00	1.00 ± 0.00^{a***}	1.00 ± 0.00	6.66 ± 0.08^{a***}	0.00 ± 0.00^{a***}
DEHP	1.06 ± 0.03	1.00 ± 0.00^{a***}	1.00 ± 0.00	7.23 ± 1.10^{a***}	1.00 ± 0.00^{b***}
DEHP+Garlic	1.00 ± 0.00	1.13 ± 0.08^{a***}	3.76 ± 0.44^{abc***}	6.16 ± 2.41^{a***}	1.16 ± 0.03^{abc***}

Values are presented as Mean±SEM, a=Control group vs treated groups, b=garlic group vs DEHP and DEHP+Garlic groups and c=DEHP vs DEHP+Garlic given group. ***P<0.001.

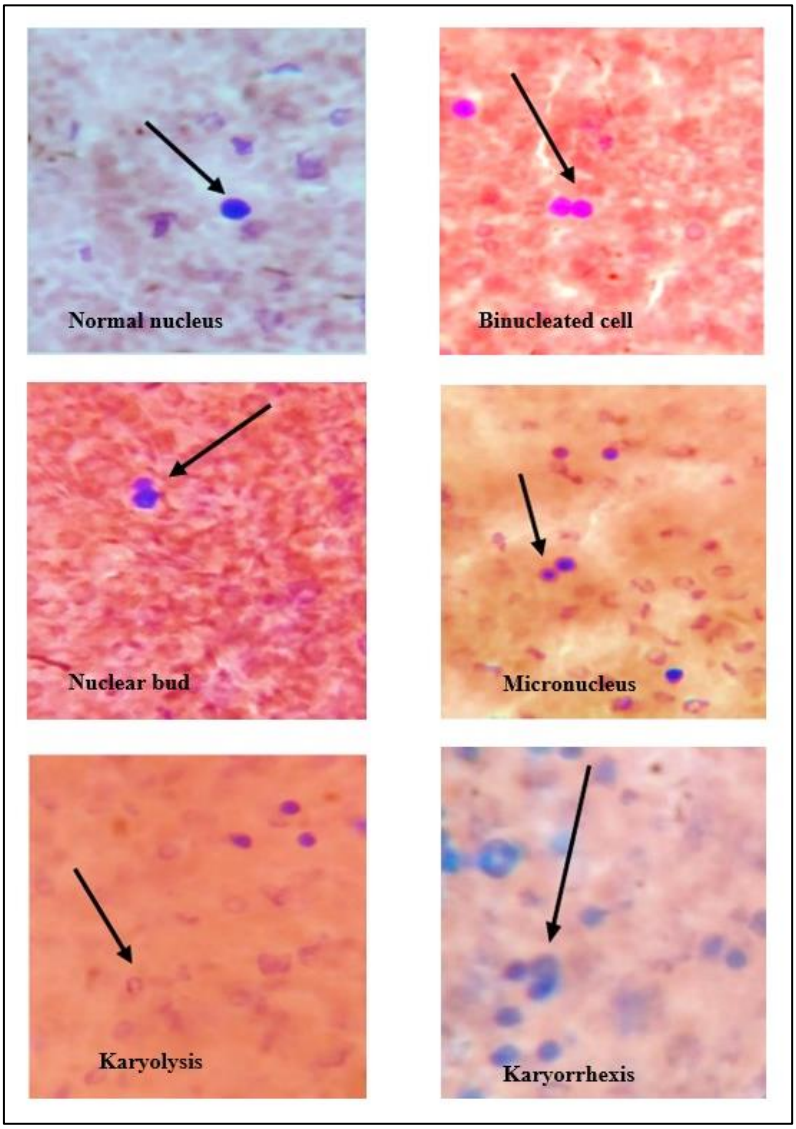

Figure 5. Different types of nuclear anomalies observed in the white blood cells of female mice exposed to Garlic (500 mg/kg), DEHP (500 mg/kg), and DEHP+Garlic (500 mg/kg each) through gavages for 28 days.

found in DEHP+Garlic given mice as compared to control, Garlic and DEHP groups. Karyolysis was significantly (P<0.001) increased in all treated groups including garlic group as compared to the control group.

It was observed that there was a significant decrease in karyorrhectic white blood cells (P<0.001) in the Garlic group and a significant increase (P<0.001) in DEHP+ Garlic group in comparison to the control group. Among treated groups, DEHP and DEHP+Garlic exposed groups showed a significant increase in (P<0.001) karyorrhectic cells in contrast to the Garlic treated group. DEHP+Garlic group showed a significant increase ~~of~~ in (P<0.001) karyorrhectic cells than DEHP treated mice. Hence, a significant difference in nuclear anomalies was noticed in all treated groups as compared to the control group (Table 4).

4. DISCUSSION

The objective of the current investigation was to assess the efficacy of garlic extract on hepatotoxicity and genotoxicity caused by DEHP in peripheral blood of female mice. In the present study, when compared with control a significant decrease (P<0.001) in the mean body weight was observed in female mice treated with 500 mg/kg of DEHP which might indicate the overall deteriorating health of the mice. There was also a significant difference in the mean liver weight of DEHP, and DEHP+Garlic groups as compared to the control group (Figure 1). A study demonstrated that dietary concentration of DEHP (500 mg/kg) did not affect the relative liver weight of mice following 2 or 4 weeks of treatment[34]. However, our results indicated a highly significant increase in the mean liver weight of the DEHP group as compared to the control group. DEHP+Garlic group showed a significant decline in the mean liver weight as compared to the DEHP group.

Histological observations of the present study indicated that there was a significant difference in the mononucleated, binucleated, and oval cells of the liver in treated groups as compared to the control group (Figure 2). A significant increase in the mean cross-sectional area of the liver's central vein was obvious among DEHP and DEHP+Garlic groups as compared to the control group. The cellular diameter of hepatocytes was found to be significantly (P<0.01) reduced in the DEHP+Garlic treated group as compared to the control group with no significant difference in the nuclear diameter of hepatocytes (Figure 3). Nuclear-cytoplasmic balance can be disturbed by toxicants. It has been reported that DEHP induce an increase in the hepatocyte's diameter in rat's liver at a dose of 500 mg/kg[35]. An increase in cellular diameter might be caused by the proliferation of peroxisomal proteins[10]. DEHP (2,500 ug/kg) is known to cause inflammatory cellular disruptions, hepatocellular necrosis, and dilation of sinusoids when compared to the control group[36].

Liver function test revealed a significant increase of serum ALT, AST and ALP of female mice exposed to DEHP and DEHP+Garlic group as compared to the control group. Serum AST (P<0.001), and ALP (P=0.05) levels were significantly reduced in the DEHP+Garlic group as compared to the DEHP group (Table 3). In previous studies, DEHP treatment in rats caused a significant increase in serum ALT, AST and ALP levels. An increase in ALT, ALP, and AST might be due to proteins from non-peroxisomal metabolism[37-38].

The present study indicated that in DEHP group, there was a significant increase (P<0.001) in the number of micronuclei as compared to control group and also a significant increase in karyorrhectic cells was observed as compared to the Garlic group. Micronucleus assay observation of DEHP+Garlic group indicated a significant increase (P<0.001) in the number of micronuclei as compared to the control group. The mean number of binucleated cells, karyorrhectic, karyolytic cells, and nuclear bud were also found to significantly increased (P<0.001) in DEHP+Garlic treated animals as compared to the control, DEHP and Garlic groups (Table 4). DEHP-induced genetic damage may be due to the production of ROS and peroxisome proliferation. DEHP has been documented to be highly cytotoxic on sea bass embryonic cell line data from the CBMN (cytokinesis block micronucleus assay) assay, with a corresponding effect on cell proliferation and a dose-dependent increase in the frequency of MN (micronucleus)[39]. DEHP exerts genotoxic effects presumably due to oxidative stress; lysosomes and mitochondria are the critical targets of di-(2-ethylhexyl) phthalate[15]. In the present study, garlic showed a significant protective effect against DEHP, although garlic also showed some genotoxic effects. It has been reported that high doses of garlic can be toxic to organisms[19]. Garlic has been reported to induce anti-hepatotoxic effects in the CCl^4 induced cytotoxicity in primary cultured rat hepatocytes *in vitro*. Garlic has also been reported to substantially inhibited the free radical generation in the target organisms[40]. Garlic exhibits antioxidant activity against lead-induced genotoxicity in human lung cells by scavenging ROS, and by activating cellular antioxidant enzymes within the cells (W1-38)[41]. Consequently, our current study supports the idea that DEHP is highly toxic to the liver of female mice and the aqueous extract of *Allium Sativum* ameliorates some of the DEHP-induced liver anomalies. It can be concluded that garlic extract can be beneficial for individuals exposed to large amounts of phthalates such as general population and industry workers to protect from the damage caused by these hazardous chemicals.

5. ACKNOWLEDGMENT

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval

This study was approved by the Bio-safety and Ethical Review Committee of the University of Sargodha, 40100 Pakistan (Ref: SU/Acad/1167/2, Date: November 06, 2019).

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