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Research article

Exposure to environmentally relevant phthalate mixture during pregnancy alters the physical and hemato-biochemical parameters in Black Bengal goats

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ABSTRACT

Several environmental pollutants, mostly chemicals and plasticizers, have an effect on the reproduction of small ruminants, causing abortion, delayed estrus, and decreased fertility. Phthalates are common in our environment and have been identified as endocrine disrupting chemicals (EDCs). The research work investigated the impact of dietary exposure to a phthalate mixture on physical and hemato-biochemical parameters in pregnant Black Bengal (BB) goats. A total of 20 clinically healthy, 1–2 months pregnant, aged 6–8 months with a body weight of 10–12 kg BB goats were collected and divided into two (n = 10) groups. The treatment group received a standard goat ration with a combination of different phthalates mixture while the control group was provided the same ration with the vehicle of aphthalatemixture until parturition. The physical parameters were measured with appropriate tools and blood samples were collected for hemato-biochemical tests. The results showed that the physiological parameters (body condition score, respiration rate and heart rate) were significantly ($P < 0.05$) reduced in phthalate-exposed goats without altering rectal temperature and rumen motility. The hematological parameters: RBC count, WBC count, hemoglobin concentration, hematocrit values and RBC indices were significantly ($P < 0.05$) lower in phthalate-exposed goats. Phthalate-exposed BB goats had significantly ($P < 0.05$) higher neutrophil and lower lymphocyte counts. Serum glucose, total protein, albumin and total cholesterol levels were significantly ($P < 0.05$) lower in phthalate-exposed BB goats but higher the values of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and blood urea nitrogen (BUN) levels in treated BB goats. It may be concluded that exposure to a phthalate mixture during pregnancy alters the physical, hematological and biochemical parameters in BB goats.

1. Introduction

Environmental pollution is a major global problem resulting from the rapid pace of urbanization, industrialization and the indiscriminate or improper use of chemicals in the environment. It has a significant impact on the health and physiology of human and

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animals. Different chemicals including pesticides, plasticizers, heavy metals, and fluorine are the major causes of environmental toxicity which affects humans, animals, plants and wildlife [1]. Most of the environmental pollutants are endocrine-disrupting chemicals (EDCs). Chronic exposure to EDCs such as phthalates and bisphenol A has resulted in a dose-dependent loss of fertility and embryo damage in animals [2]. Phthalates are widely used in common consumer products, including various plastics, cosmetics, personal care products, children's toys, polyvinyl chloride pipes, vinyl flooring plastic medical devices, processed foods, insecticide formulation and high fat dairy and meats [3]. Because of the ubiquitous characteristics of phthalates, animals are constantly exposed through ingestion, inhalation, and dermal contact [4]. The six most familiar phthalate metabolites such as di (2-ethylhexyl) phthalate, diethyl phthalate, dibutyl phthalate, dibenzyl phthalate, di-isopropanol phthalate were declared environmental pollutants by the United States Environmental Protection Agency [4,5]. Phthalates affect hormonal regulation and the normal endocrine system, consequently affecting the health and reproduction of animals and humans [6]. One of the most serious phthalate effects is on fetal development and conception abnormalities, which are referred to as "phthalate condition." [7]. Endocrine disruption effects of phthalates on rats and mice have already been published.

The administration of phthalate brings about significant changes in hematological and biochemical parameters. Hematological and biochemical variables of blood are generally used to monitor and evaluate the health, nutritional and physiological status of ruminants [8,9]. The evaluation of blood constituents has been widely used as a marker to determine the efficacy of feed nutrient content and supplements [10,11] but also as an index of transportation stress [12]. The hematological and biochemical profiles can also be utilized to evaluate goat immunity [13]. These profiles may change throughout pregnancy [14] and seasonal variations also have an effect on these profiles [15]. Finally, nutrition, stress, reproductive status, age, sex, genetics, management, housing and other environmental factors such as pollutants, temperature, relative humidity etc. are known to have a profound effect on the hematological and biochemical profiles of small ruminants [16,17]. The importance of determining the hematological and biochemical parameters of domestic animals has been widely documented [18] and the changes in these parameters have been studied in cattle [19] sheep [20] and goats [14]. There is a wide range of hematological and biochemical parameters seen between breeds of goats [14].

The emphasis on the risks of phthalates to reproduction is primarily on human medicine, whereas food-producing animals are often disregarded. Due to continuous exposure through food, water, air and skin absorption, phthalate contamination is regarded as a global and ubiquitous public health problem. Therefore, the focus on farm and companion animals should be addressed in order to reduce the human exposure and study their impacts on fertilityand economic productivity. The studies on cattle regarding phthalates mostly focus on the residual interest compromising transfer to cow's milk and pharmacokinetic characteristics [21,22]. There is a lack of documented research on the impact of these prominent environmental contaminants, specifically endocrine-disrupting chemicals, on the fertility parameters of small ruminants. Goats, particularly BB goats, possess significant potential in contributing to the provision of sustainable livelihoods and nutritional security for impoverished individuals, thereby contributing to the overall increase in the Gross Domestic Product (GDP) of Bangladesh [23,24] Hence, it is regarded as a poor man's cow [25,26]. The BB goat breed is well-known for its notable characteristics, including its high fertility, prolificacy, superior skin and meat quality, early sexual maturity, resistance against common diseases, and short kidding interval [27]. This research aims to determine the baseline and alterations of physiological, hematological, and biochemical data in BB goats exposed to phthalates, with the intention of contributing to the enhancement of sustainable goat productivity in Bangladesh.

2. Materials and methods

2.1. Experimental animals

A total of 20 clinically healthy, 1–2 months pregnant, aged 6–12 months with a body weight of 10–12 kg Black Bengal goats (*Capra hircus*) were used for the experiments. Before being used in the experiment, goats were closely monitored in a common isolation room for 7 days after collection in order to adjust to the new environment and conformation of pregnancy. During the experimental period, uniformity of management practices was maintained in the experimental shed as much as possible.

2.2. Experimental chemicals

Four different chemicals were used in the experiment such as Diethyl Phthalate (DEP), Dibutyl Phthalate (DBP), Diisobutyl Phthalate (DIBP) and Dipropyl Phthalate (DPP) which were purchased from Sigma-Aldrich Company, USA and these chemicals were dissolved in corn oil (vehicle) as stock before administration. Phthalates were handled very carefully to avoid the chance of being exposed.

2.3. Ethical approval

All experimental procedures were approved and performed according to the guidelines for the care and use of animals as established by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh.

2.4. Experimental design

The experimental pregnant Black Bengal goats were randomly divided into two (n = 10) experimental groups. The control group (n = 10) was provided only standard goat ration and the treatment group (n = 10) was provided a standard goat ration with phthalates mixture up to parturition 100 mg/kg body weight daily for 100 days respectively(Fig. S1).

2.5. Collection of blood samples

Approximately 5 ml of blood per goat was drawn aseptically from a jugular vein puncture, of which 3 ml of blood was transferred into a sterile vial containing disodium EDTA in order to estimate hematological parameters. The remaining 2 ml of blood was transferred into a vacutainer tube for serum separation in order to analyze biochemical parameters.

2.6. Physiological parameters analysis

The physiological parameters such as respiration and heart rate were estimated by the stethoscope. Body temperature was recorded by the clinical thermometer and rumen motility rate was counted with the help of hand placement in the flank region. The body condition score was measured according to the standard method.

2.7. Hematological parameters analysis

Collected blood samples were analyzed for different hematological parameters including – i) Total Erythrocyte Count (TEC), ii) Total Leukocyte Count (TLC), iii) Hemoglobin Concentration (Hb), iv) Packed Cell Volume (PCV), v) Mean Corpuscular Volume (MCV), vi) Mean Corpuscular Hemoglobin (MCH), vii) Mean corpuscular hemoglobin concentration (MCHC), viii) Erythrocyte sedimentation rate (ESR) and ix) Differential leukocyte count (DLC) such as Neutrophil (%), Eosinophil (%), Basophil (%), Lymphocyte (%) and Monocyte (%) by using the automated hematology analyzer Sysmex XN-2000.

2.8. Biochemical parameters analysis

The prepared serum sample was analyzed for different biochemical parameters include-i) Glucose (mg/dl), ii) Total protein (g/dl), iii) Albumin (g/dl), iv) Total cholesterol (mg/dl), v) Creatinine (mg/dl), vi) Uric acid (mg/dl), vii) Blood urea nitrogen (BUN) (mg/dl), viii) Aspartate aminotransferase (AST) (U/L), ix) Alanine transaminase (ALT) (U/L), x) Alkaline phosphatase (ALP) (U/L) calorimetrically by using the automatic biochemical analyzer Vitros −5600 (J&J)/Beckman Coulter AU480.

2.9. Statistical analysis

The obtained data was loaded and stored in the Excel spread sheet. Then the data was analyzed using IBM SPSS Statistical Package Version-22 followed by one-way ANOVA. All results are expressed as means ± standard error (SE) and a probability $P < 0.05$ or less was considered statistically significant.

3. Results and discussion

3.1. Impact of phthalate mixture on physiological parameters of Black Bengal goats

The physiological parameters of Black Bengal goats including body condition score (BCS), respiration rate, heart rate, rectal temperature and rumen motility values are presented in Table 1. It was found that the BCS values of phthalate-exposed goats were significantly ($P < 0.05$) lower than the control. It is possible that phthalate exposure caused severe dehydration and prolonged anorexia in animals as well as adverse developmental effects such as reduced growth, skeletal, visceral and external malformations in animals. The present findings are supported by the findings of [28]. Phthalate exposure is a considerable threat to health worldwide [29–31]. Respiration rate and heart rate were significantly ($P < 0.05$) higher in phthalate-exposed goats compared to normal goats. Phthalates may induce inflammation in the lungs associated with allergies and asthma resulting in an increased respiration rate [32]. reported that phthalates exposure is linked to allergies and asthma. Phthalate may be involved in the activation of ace and inhibition of the bradykinin-no pathway resulting in vasoconstriction and an increase in blood pressure and heart rate. The present findings are supported by Ref. [33]. The rectal temperature and rumen motility didn't differ significantly ($P > 0.05$). Chronic exposure to plasticizers may not affect the hypothalamic thermoregulatory center, but it may result in prolonged anorexia and decreased ruminal

Table 1
Physiological parameters (Mean ± SE) of Black Bengal goats exposed to environmentally relevant phthalates mixture.

Physiological parameters	Control goats	Phthalate exposure goats
Body condition score (BCS) (%)	3.68 ± 0.06	2.30[a] ±0.07
Respiration rate (breath/min)	28.40 ± 0.51	39.20[a] ±0.86
Heart rate (beat/min)	78.80 ± 1.07	94.60[a] ±1.63
Rectal temperature (^{0}F)	102.00 ± 0.45	103.70[ns] ± 0.20
Rumen motility (no/min)	1.40 ± 0.24	0.80[a] ±0.20

[a] Values in a row of the table differ significantly at the 5% level, ($P < 0.05$). NS: Not Significant.

function [34]. However, over time, this plasticizer causes rumen motility to decrease, resulting in ruminal atony and impaction [35].

3.2. Impact of phthalate mixture on hematological parameters of Black Bengal goats

The hematological values of pregnant Black Bengal goats are presented in Table 2. The results demonstrated that dietary exposure to phthalate mixtures during the pregnancy period reduced TEC, Hb and PCV values ($P < 0.05$). In terms of RBC indices, MCV and MCHC were significantly ($P < 0.05$) lower in phthalate-exposed goats although MCH values didn't differ significantly ($P > 0.05$) from control. Phthalates may limit hemoglobin production or RBC lysis, or they may modify hemoglobin structure, which may reflect hemoglobin functions [36]. The current findings are similar to those of [37,38] who found that phthalates may deplete circulating iron from hemoglobin and cause a decrease in GSH reductase activity and Heinz body formation in red blood cells. The fall in hemoglobin concentration, which is related to a fall in erythrocyte count and packed cell volume leads to anemia. The TLC values in phthalate-exposure goats were significantly ($P < 0.05$) lower than in control goats. Phthalates may cause a significant decrease in viability, osteogenic differentiation in the bone marrow mesenchymal stem cells and abnormal development in bone leading to the production of insufficient WBC [39]. The phthalate-exposed goats had higher neutrophil counts ($P < 0.05$) but lower lymphocyte counts. Similar observations were reported by Refs. [38,40]. No significant changes ($P > 0.05$) were found in basophil, eosinophil and monocyte counts between control goats and phthalate-exposed goats. The results agree with [38]. The decrease in lymphocyte count implies a reduction in the ability of the body to respond to infection, whereas a rise in neutrophils adducted the ability of the neutrophil to phagocyte. It may be concluded that phthalate suppresses the immunity of goats. The present findings come in accordance with [41,42] who found that phthalate like other EDCs; BPA also induced a significant decrease in red cell count, Hb concentration and PCV.

3.3. Impact of phthalate mixture on biochemical parameters of Black Bengal goats

The biochemical values such as serum glucose (Fig. 1A), total cholesterol (Fig. 1B) serum total protein (Fig. 1C) and albumin (Fig. 1D) levels were significantly ($P < 0.05$) lower in phthalate-exposed goats than those of the control goats. The present findings are consistent with the findings of [38]. The hypoglycemic action of phthalate may be attributed to the enlargement of the liver. Phthalate inhibited glycogenesis and glycogenolysis in animals and lowered the activity of glucose-6-phosphate dehydrogenase [43]. Phthalate diminishes protein expression required for cholesterol transport [44]. Reduction of cholesterol in the current study suggested that phthalate may affect the cholesterol metabolism of animals. The present findings are congruent with those of [38,45] who reported that phthalate induced hypercholesterolemia in animals.

Creatinine (Fig. 2A), BUN (Fig. 2B) and uric acid (Fig. 2C) levels reflect kidney functions and these parameters were higher in phthalate-exposed goats and differed significantly ($P < 0.05$) except for uric acid which didn't differ significantly. Similar findings were reported by Refs. [46,47]. Bisphenols and phthalates have a nephrotoxic effect due to the accumulation of toxic metabolites and the inability of the kidney to eliminate them [48]. The metabolic enzymes of the liver function test (AST, ALT and ALP) (Fig. 2D–F) were significantly ($P < 0.05$) higher in phthalate-exposed goats than the values found in control goats. Similar findings were reported by Ref. [49]. The current study demonstrated that phthalates have adverse effects on the liver, as seen by increased activities of ALT, AST and ALP enzymes. When the liver is injured or inflamed, these enzymes are released into the blood stream [50]. BPA or phthalate may induce the formation of reactive oxygen species in the liver [51].

4. Conclusion

It is concluded that exposure to phthalate mixtures during pregnancy alters the physiological, hematological and biochemical parameters of Black Bengal goats. The haemato-biochemical values are an efficient tool for the evaluation of physiological status, metabolic disorders and management problems of the farm which have a great relation to health status and the diagnosis of some

Table 2
Hematological values (Mean ± SE) of Black Bengal goats exposed to environmentally relevant phthalates mixture.

Hematological parameters	Control goats	Phthalate exposure goats
TEC (M/μl)	14.84 ± 0.23	10.72[a] ±0.22
TLC (K/μl)	9.48 ± 0.10	7.20[a] ± 0.24
Hb (g/dl)	13.20 ± 0.37	9.16[a] ±0.51
PCV (%)	35.80 ± 0.66	23.60[a] ±0.75
MCV (fL)	24.10 ± 0.59	21.94[a] ±0.47
MCH (pg)	8.86 ± 0.30	8.48 ± 0.34
MCHC (%)	36.88 ± 1.25	38.82 ± 1.92
ESR (mm in 1st hour)	0.60 ± 0.24	1.60 ± 0.24
Differential Leukocyte Count (DLC)		
Neutrophil (%)	34.00 ± 0.55	40.40[a] ±0.75
Eosinophil (%)	2.40 ± 0.24	1.80 ± 0.37
Basophil (%)	0.40 ± 0.24	0.60 ± 0.24
Lymphocyte (%)	58.20 ± 0.80	47.40[a] ±1.00
Monocyte (%)	2.60 ± 0.24	2.20 ± 0.37

[a] Values in a row of the table differ significantly at the 5% level, ($P < 0.05$). NS**:** Not Significant.

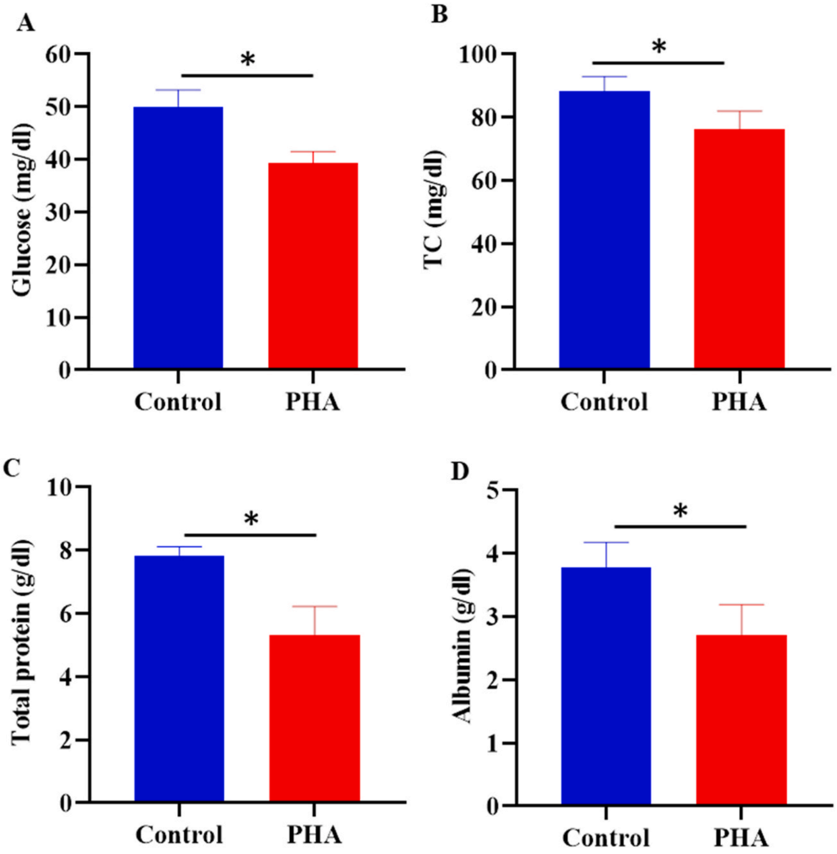

Fig. 1. Biochemical values (Mean ± **SE) of Black Bengal goats exposed to environmentally relevant phthalates mixture.** (**A) Glucose (mg/dl); (B) Total cholesterol (mg/dl); (C) Total protein (g/dl) and (D Albumin (g/dl).** *Denotes differ significantly at the 5% level, ($P < 0.05$). NS: Not significant.

pathos-physiological disorders in animals. Nonetheless, the data generated during the current study may be useful as reference values for the scientific community as this is the first study of its kind in case of Black Bengal goats exposure to phthalate mixture in Bangladesh. In this regard, the research may help to find out, reduce and concern the human and animal health hazard which are occurred by endocrine disrupting chemicals (EDCs) especially phthalates. Future research is required to determine how phalatates interfere with goat physiology at the molecular level through in vitro and in vivo experiments. It is also necessary to investigate the long-term effects of maternally exposed phthalates on the reproductive health of offspring goats.

Data availability statement

Data included in article/supplementary material/referenced in article.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Sajibul Hasan: Writing – original draft, Methodology, Investigation, Data curation. **Mohammad Alam Miah:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Afrina Mustari:** Writing – review & editing, Methodology, Formal analysis. **Khaled Mahmud Sujan:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Md Eliusur Rahman Bhuiyan:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Kazi Rafiq:** Writing – review & editing, Visualization, Methodology, Formal analysis.

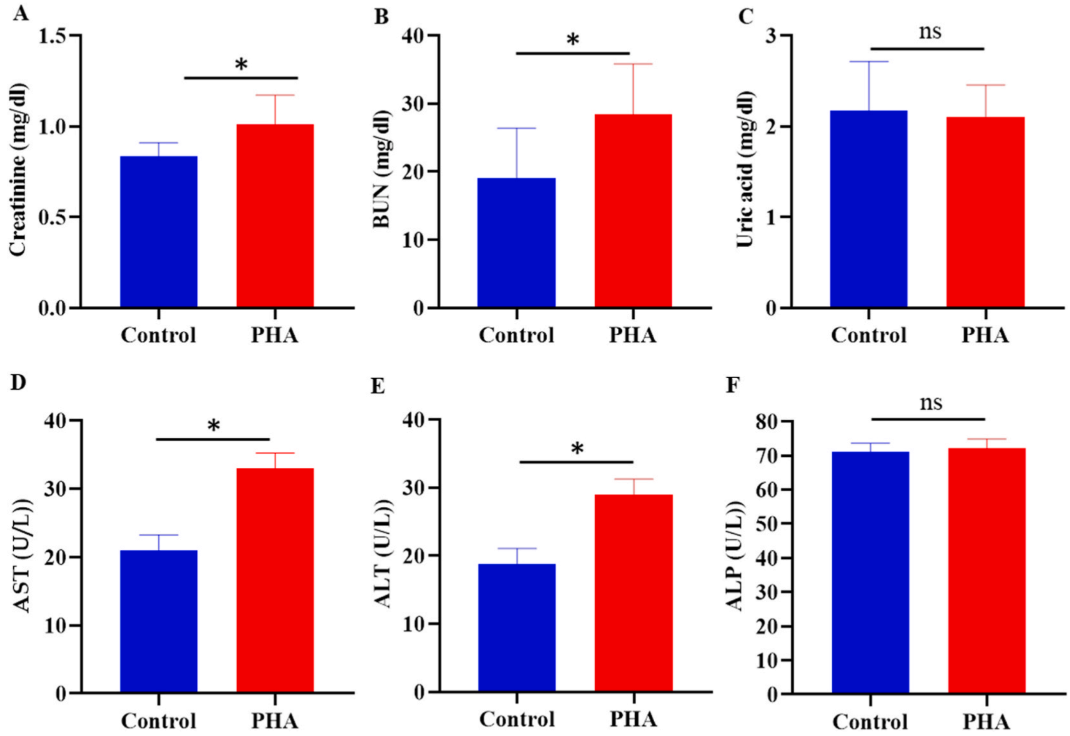

Fig. 2. Biochemical values (Mean ± **SE) of Black Bengal goats exposed to environmentally relevant phthalates mixture.** (**A) Creatinine (mg/dl); (B) BUN (mg/dl); (C) Uric acid (mg/dl); (D) AST (U/L); (E) ALT (U/L) and (F) ALP (U/L).** *Denotes differ significantly at the 5% level, ($P < 0.05$). NS**:** Not significant.

Declaration of competing 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25852.

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