



Antioxidant Role of Nano-Piperine and Ascorbic Acid in Some Blood Parameters and Liver in Dexamethasone Treated Male Rats

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Abstract | Glucocorticoids are extensively utilized in veterinary medicine because to their anti-inflammatory and immunosuppressive properties. Vitamin C and Nanopiperine are crucial in mitigating the effects of free radicals by acting as antioxidants. The study investigates the positive impacts of piperine nanoparticles and vitamin C on several blood parameters and liver tissue in male rats treated with dexamethasone. The forty-eight male rats were randomly divided into six groups. Nanopiperine (15 mg/Kg.B.W) was given orally to the T1 group. Vitamin C (25 mg/Kg.B.W) was given orally to the T2 group. The T3 group received orally dexamethasone (2 mg/Kg.B.W). Nanopiperine and dexamethasone were given orally to the T4 group at the same dose. The T5 group received a single oral dosage of vitamin C and dexamethasone. After the end of experiment the animals were dissected and blood samples were collected for measuring Hb levels, PCV, mean cell volumes, MCHC, and RBCs, and the livers samples were removed from all animals and reserved them in formalin 10% for histological examination. The results of the present study exhibited a notable rise ($p < 0.05$) in RBC count, Hb, PCV, MCV, MCH, and MCHC in T1 and T2 as compared with control group. While these parameters were significantly decreased ($p < 0.05$) in T3 group that drenched with dexamethasone. T4 and T5 exposed a substantial rise ($p < 0.05$) in all these blood parameters when compared to T3. Examination of liver tissue in control, T1 and T2 exposed normal tissue appearance. T3 exposed the normal radial arrangements of hepatocyte with a trifling infiltration of inflammatory cells and multiplying in kuffer cells. T4 and T5 revealed normal appear of hepatocyte with a clear central nucleus site. This research can be concluded that nano-piperine and Vit. C have a great role in reducing the side effect of dexamethasone on liver and red blood cells. The results related to the piperine nanoparticles was better than vitamin C.

Keywords | Nanopiperine, Ascorbic acid, Dexamethasone, Oxidative stress, antioxidant, Liver, Rats

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INTRODUCTION

One kind of corticosteroid drug is dexamethasone (DEX), which contains anti-inflammatory, anti-an-

giogenic, and estrogen-activity-controlling properties. In order to treat illnesses brought by an overactive immune system, doctors utilise glucocorticoids (Kadmiel and Cidlowski, 2013 and Jiang *et al.*, 2020). Numerous studies have examined how dexamethasone affects various bodily systems and activities. They discovered that DEX significantly

lowers RBC, PCV, and Hb (Razzaq *et al.*, 2020) and increases hepatotoxicity by weakening the defence system of antioxidants (Abou-Seif *et al.*, 2019). Every human being is dependent on red blood cells, they do, however, exhibit distinctive and noteworthy qualities, because of their capacity to carry oxygen, red blood cells have had to evolve special morphological and physiological characteristics, because erythrocytes are oxygen transporters, they have high quantities of the antioxidant glutathione (GSH), ranging from 0.4 to 3.0 mM, and many enzymes that guard against damage from reactive oxygen species (ROS) (van 't Erve *et al.*, 2013). Reduced GSH and its oxidised counterpart, GSSG, typically have a ratio of around 7:1.

According to Raftos *et al.* (2010), erythrocytes possess high-efficiency importers of amino acid components and enzymes for synthesising GSH. Vitamins are perfect antioxidants that can boost organ defence against oxidative stress and play a crucial part in nearly all biochemical events. Ascorbic acid, often known as vitamin C, or ascorbic acid is necessary component of several metabolic processes that the human body goes through, including the oxygen energy system's optimum operation. Furthermore, according to Amin and Al-Shehri (2018) and El-Borm *et al.* (2019), AA is a significant free radical scavenger in extracellular fluids, trapping radicals and shielding biomembranes from peroxide damage. Antioxidant supplements are being thoroughly studied in a number of illness situations and have been utilised to prevent or lessen damage to biological tissues. Compounds with antioxidant qualities may be able to prevent oxidative stress and shield against the damaging effects of free radicals.

According to a number of studies, piper nigrum Linn. is one of the most significant and ancient spices in the world and is used both in traditional Indian Ayurvedic and Siddha treatment and as a food ingredient. Piperine has a broad range of pharmacological characteristics, including antioxidant, a liver-protect, anti-inflammatory, as immune-modulating, and anticancer activity. Its nature makes it very lipophilic (Haq *et al.*, 2021). The goal of the study was to look at the beneficial effects of vitamin C and piperine nanoparticles on a number of blood parameters in male rats receiving dexamethasone treatment.

MATERIALS AND METHODS

ETHICAL APPROVAL

The students have gotten ethical approval from the research Ethical Approval Committee of the University of Al-Qadisiyah.

PREPARATIONS OF NANO_PIPERINE

Nanopiperine is using low-energy creation (thermal ap-

proach) and spontaneous emulsification (SE), this process creates nano-emulsions using components of food grade. This manufacturing process was modified in a novel way. 200 mg. of pure piperine, ground into a fine powder, was mixed with 100 ml of double-distilled water that had been deionized (W/V ratio: 2 mg-/ml). This oil-water phase, which included the hydrophilic surfactant Tween 80 in a concentration of 3%, was stirred for seven hours at 40°C at 3000 rpm using a magnetic stirrer. After 10 minutes of sonication, the mixture was filtered using a 200 nm nanofilter.

EXPERIMENTAL ANIMALS

In this experiment, forty eight adult male Wister rats were used, about four month old, with average weight about 162.5±13gm. Animals housed in well ventilated wire-plastic cages with dimensions 40×60 cm and reared under controlled conditions about 12 hour light and 12 hour dark at 22°C. The animals were allowed to acclimatize for 14 days before experimentation.

EXPERIMENTAL DESIGN

The forty eight animals weighed (162.5±13gm) were partitioned to 6 groups (8 male rats of each group) and remedied by intubated orally for (30) days as follow;

- Control (G1) group: rats of this group were received half ml of distilled water.
- The first treated group (T1): animals in this group were intubated nano-piperine (15 mg/kg B.W.) dissolved in half a ml of distilled water.
- The second treated group (T2): animals in this group were intubated vitamin C (25 mg/kg B.W.) in a solution of half a millilitre of distilled water.
- The third treated group (T3): animals in this group were intubated dexamethasone (2 mg/kg B.W.) in distilled water.
- The fourth treated group (T4): animals in this group were intubated nano piperine (15mg/kg B.W.) and dexamethasone (2 mg/kg B.W. in distilled water.
- The fifth treated group (T5): animals in this group were intubated Vit. C (25 mg/kg B.W.) and dexamethasone (2 mg/kg B.W.) in distilled water.

The experimental periods have been accompanied by continuous monitoring of male rats. Dissecting the animals after the trial ended allowed us to measure RBCs, Hb levels, PCV, MCH and MCHC. Sample from liver was taken for histological study.

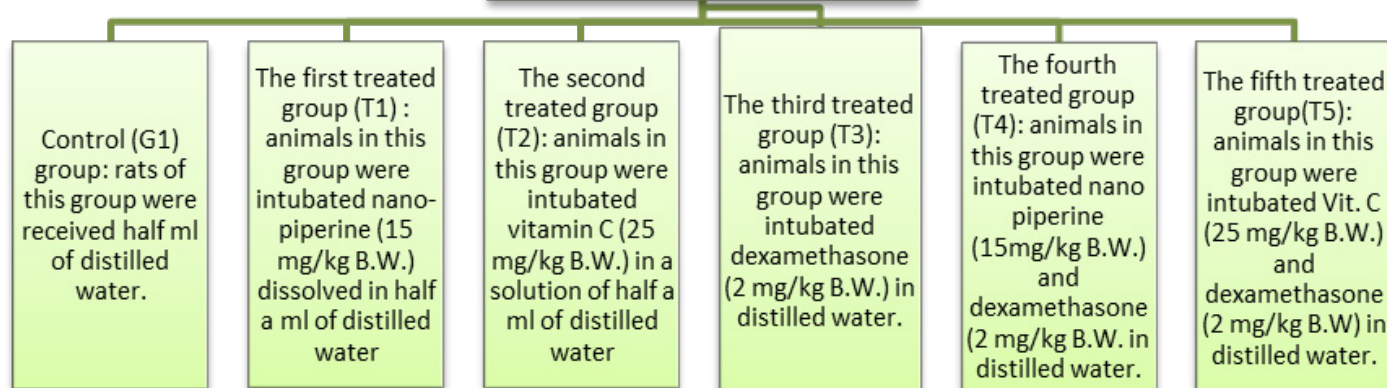
LABORATORY MEASUREMENTS

RBCs, Hb, PCV, MCV, MCH and MCHC were estimated by using Hematology Analyzer (United Kingdom).

HISTOPATHOLOGICAL PREPARATION

Slide preparation was undertaken according to method proposed by Luna, (1968).

The forty eight animals weighed ($162.5 \pm 13 \text{ gm}$) were partitioned to 6 groups(8 male rats of each group) and remedied by intubated orally for (30) days as follow:



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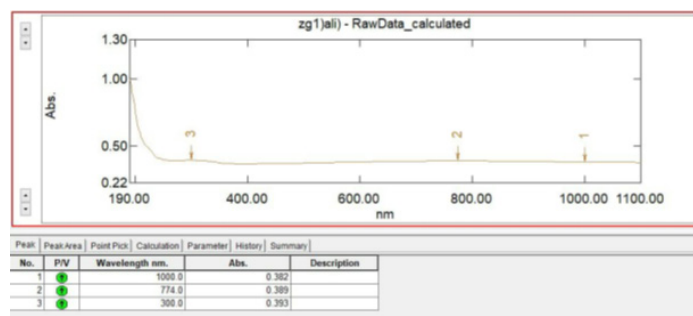


Figure 1: UV visible spectrum of Piperine nano particles.

STATISTICAL ANALYSIS

The results were detailed as mean $\pm$ standard error of the mean (SEM). Contrasts were achieved using one way analysis of variance (ANOVA), when $p < 0.05$. All statistical analysis by (SPSS, version 22) (Joda, 2008).

RESULTS

CHARACTERIZATION OF NANO PARTICLES

UV- Visible spectrophotometer analysis of nano-piperine: Nanoparticle stability and optical characteristics are being investigated through the crucial function that absorption of UV-visible spectroscopy plays in this regard (Figure 1). After diluting a small portion of the sample in distilled water, the UV-visible spectrum of the reduction medium was measured to track the bio-reduction of piperine nanoparticles. Using a UV-Visible Spectrophotometer, the surface plas-

mon resonance (SPR) in the 300 nm wavelength region was measured. electromagnetic waves using ultraviolet-visible spectroscopy with wavelengths between 300 and 1100 nm. The surface plasmon resonance of nanoscale particles exhibits peak features in the UV-visible absorption spectrum. At 300 nm of wave length, the absorption peak (lambda max) was measured, and the absorption was 0.393.

FOURIER TRANSFORM INFRARED SPECTROSCOPY ANALYSIS (FTIR):

The dried particle of produced black pepper nanoparticles was scanned using an FTIR spectroscopy to determine the functional groups present in the specimens (Figure 2), the peak location throughout a wavelength range of 400–4000 cm^{-1} is utilized. BPNPs solution was centrifuged at 10,000 rpm for 30 minutes in order to measure FTIR. Using 20 millilitres of deionized water, the pellet rinsed three times. After being dried, the black pepper NPs was examined. displayed the distinctive band at 2981.9 and 2827.6 cm^{-1} , which signify the existence of the alkane (C-H) group; the carbonyl (C=O) the group operates maximum value at 1639.49 cm^{-1} . Strong N-O stretching nitro compound was seen at 1562.3 and 1512,19 cm^{-1} , on the other hand,, at 1415.75 and 136.6 cm^{-1} , the carboxylic acid O-H was verified.strong C-O for sulfoxide and primary alcohol was measured at 1157.2. The esters (S-OR) group at 964.4 and 7813.96 cm^{-1} .

Table 1: Effect of nano-piperine, dexamethasone and vit. C on some blood parameters.

MCHC (g/dl)	MCH (pg)	MCV (fl)	PCV (%)	Hb (g/dl)	RBC (10 ⁶ /ml)	GROUPS
31.49±0.02 ^A	15.05±0.05 ^A	47.03±0.03 ^A	41.63±0.04 ^A	13.12±0.02 ^A	8.77±0.01 ^A	C
36.84±0.03 ^B	17.94±0.28 ^B	50.96±0.07 ^B	43.04±0.27 ^B	14.98±0.06 ^B	9.16±0.01 ^B	T1
35.05±0.01 ^C	16.11±0.41 ^C	49.03±0.18 ^C	42.27±0.41 ^C	14.15±0.17 ^C	10.24±0.13 ^C	T2
26.60±0.45 ^D	12.92±0.36 ^D	30.10±0.06 ^D	31.87±0.35 ^D	8.48±0.05 ^D	5.68±0.05 ^D	T3
30.86±0.67 ^{AE}	14.75±0.59 ^E	44.87±0.16 ^E	37.76±0.58 ^E	10.45±0.15 ^E	7.87±0.07 ^E	T4
29.18±0.31 ^F	13.50±0.43 ^F	40.69±0.04 ^F	36.89±0.39 ^F	9.39±0.03 ^F	6.58±0.07 ^F	T5
1.49	1.02	1.51	0.46	0.57	0.33	LSD

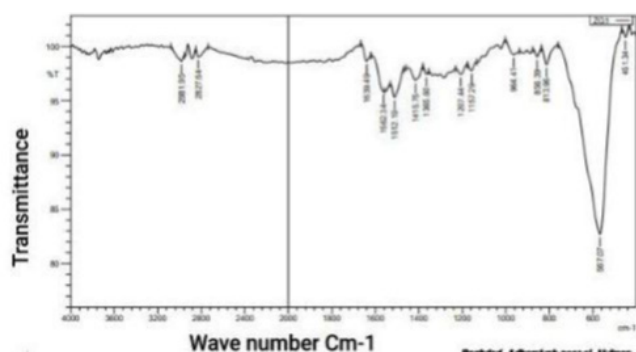


Figure 2: FTIR spectrum synthesised Piperine nano particles.

SCANNING ELECTRON MICROSCOPY ANALYSIS (SEM): SEM was used to assess the piperine nanoparticles' size and shape. thin films of the dried black pepper nanoparticle pellet, which were created on a cover slip grid by applying a little amount of the solution there and letting it air dry before seeing it under a scanning electron microscope. Using SEM, the distribution and format of BPNPs were investigated. According to the FE-SEM pictures in **Figure 3**, piperine particles have irregular forms that range from cubic to triangle and have a smooth outer surface. The resulting NPs' average diameter, as determined by SEM analysis, ranged from 33.8 to 200 nm. With a minor shift in the PDI value, the particle size was significantly enhanced at around 100 nm, indicating that Piperine NPs were satisfactorily stable throughout storage.

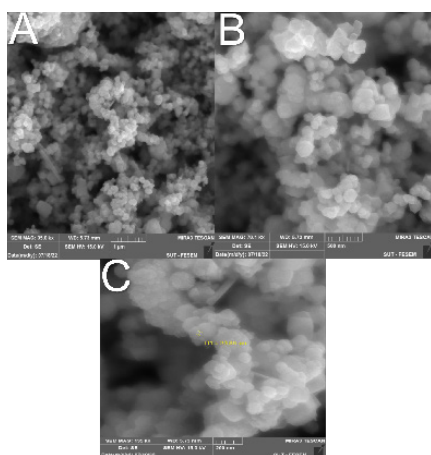


Figure 3: SEM images synthesized nano particles show cubic to triangle shape of about 33.8 nm.

X-RAY DIFFRACTION(XRD): The Paul Scherrer equation is used to calculate the size of powdered crystals. The size, crystalline nature, and fashioning of BPNPs were all described using the XRD pattern (**Figure 4**). After placing the sample on a glass slide and measuring the Bragg's angle θ at 2° , the XRD measurements were completed. The black pepper NPs solution was centrifuged for 30 minutes at 10,000 rpm. Twenty millilitres of distilled water were used to wash the granules three times. After the dried mixture of BPNPs was gathered, XRD patterns were computed. The Scherrer equation may be expressed as follows: $D_p = (0.94 \times \lambda) / (\beta \times \cos \theta)$. In this case, β stands for line broadening in radians, for Bragg angle, for X-ray wavelength, and D_p for average crystallite size.

With their FWHM 0.59, 0.2952, 0.295, 0.29, 0.295, 0.2, 0.344, 0.39, 0.590400, and 0.393600, the three peak 2θ in the current XRD data were recorded at 18.3, 30.25, 35.64, 37.26, 43.2, 53.68 and 57.133470, 71.357240, 74.280820 to recorded size at 14.77, 29.59, 29.54, 29.6, 30.2, 27.3, 70.06, 44.22, and 26.46 nm, respectively (4). The isolated piperine nanomaterial loaded with ferric chloride, as shown by the preceding equation, has very tiny crystal sizes, ranging from 14 to 70 and yet less than 100 nm.

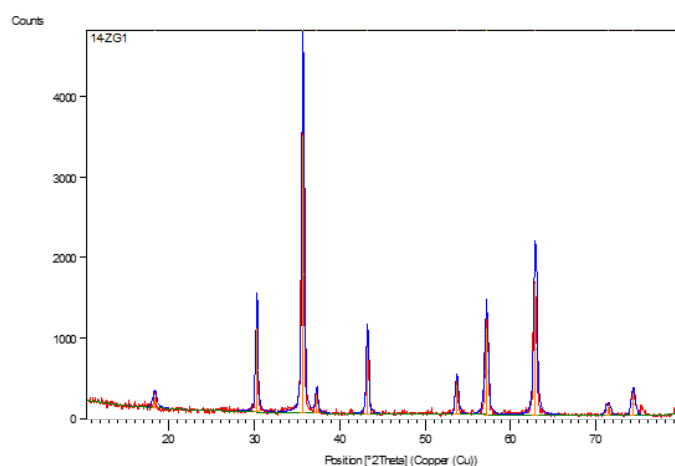


Figure 4: XRD analysis of Nano-Piperine.

The outcomes in Table 1 demonstrated that there were substantial differences ($p < 0.05$) between the experimental groups in terms of RBCs count, Hb, PCV, MCV, MCH, and MCHC. The current study's findings indicate that T1 treated with nano-piperine and T2 that received vitamin C had a substantial rise ($p < 0.05$) in RBC count, Hb, PCV, MCV, MCH and MCHC when related to the control group. While these parameters were significantly decreased ($p < 0.05$) in T3 group that drenched with dexamethasone. Following infusion of a combination of dexamethasone and piperine NPs. in T4 exposed a substantial rise ($p < 0.05$) in RBCs, PCV, Hb, MCV, MCH and MCHC as compared with T3. T5 which was given a combination of dexamethasone and piperine nanoparticles, had significantly higher ($p < 0.05$) in all these blood parameters when compared to T3.

HISTOLOGICAL CHANGES OF LIVER

In control group the hepatocytes seem grouped in a hexagonal radial form round the central vein (red arrow), as seen in Figure 5, and the bile duct appears normal. In T1 group results as shown in Figure 6. suggested hepatocytes are hexagon-shaped and seem normal (red arrow). Featuring a distinct central nucleus site, the bile duct seems normal, and the hepatocytes' usual geometric structure (black arrow) is present. The hepatocytes are oriented radially around the normal central vein. The liver's histological section in T2 group as shown in Figure 7. suggested the hepatocytes have a typical hexagonal appearance. Having a distinct central nucleus site, the bile duct seems normal (red arrow), and the hepatocytes exhibit their typical geometric form, appearing radially distributed around the normal central vein with the exception of steatosis and bile duct hyperplasia. Histological Section of the Liver in T3 group as shown in Figure 8. shown the ordinary radial organization of hepatocytes (red arrow). a small infiltration of inflammatory cells (black arrow), and a multiplying in kuffer cells (green arrow). Hepatocytes seem ordinarily with clear central nuclei. Histological analysis of the rats' liver in T4 group as shown in Figure 9. illustrated present the hepatocytes' typical geometric structure (red-arrow), which is oriented radially around the usual central vein, together with the bile duct's normal appearance. The form has a clearly defined central nucleus location. Despite existence of simple steatosis and simple hyperplasia of the bile ducts, hepatocytes look properly with distinct central nuclei and a little infiltration of inflammatory cells (black arrow) and a proliferation in kuffer cells (green arrow). In T5 group the histological analysis as shown in Figure 10. demonstrates how typical and hexagonal the hepatocytes seem. The hepatocytes have a clear central nucleus site and are organized radially round the normal central vein (red arrow), the bile duct also looks normal, with a small infiltra-

tion of inflammatory cells (black arrow) and a multi-plying in kuffer cells. The hepatocytes have a clear central nucleus site and are arranged normally, with exception of simple steatosis and simple hyperplasia of the bile ducts (green arrow).

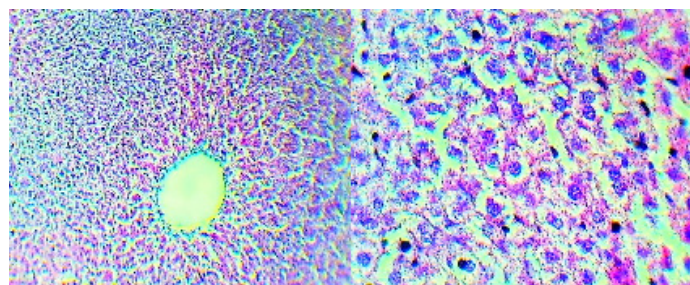


Figure 5: Section of liver tissues male rats drenched with distilled water (0.5 ml). (H&E)

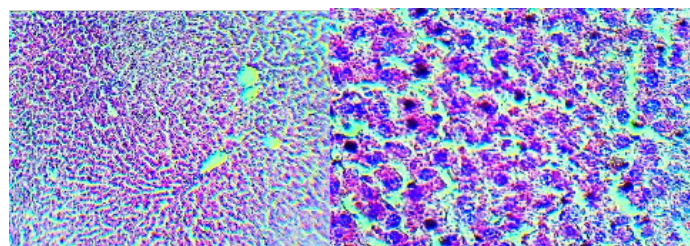


Figure 6: Section of liver tissues male rats drenched with Nanoparticle Piperine (15mg/kg suspended in 0.5 ml of distilled water). (H&E stain.40X).

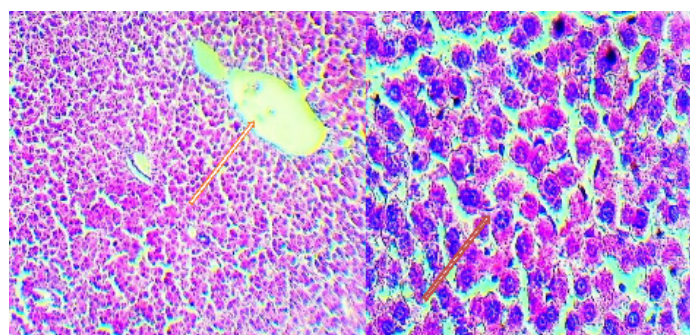


Figure 7: Section of liver tissues from male rats drenched with Vit. C (25mg/kg/B.W suspended in 0.5 ml of distilled water). (H&E stain.40X).

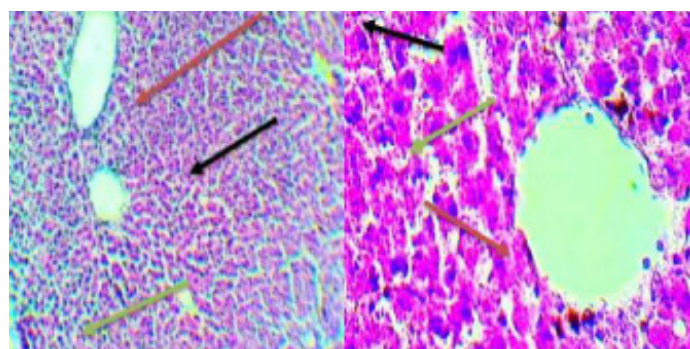


Figure 8: Section of liver tissues from male rats drenched with Dexamethasone (2 mg/kg/B.W suspended in 0.5 ml of distilled water). (H&E stain.40X).

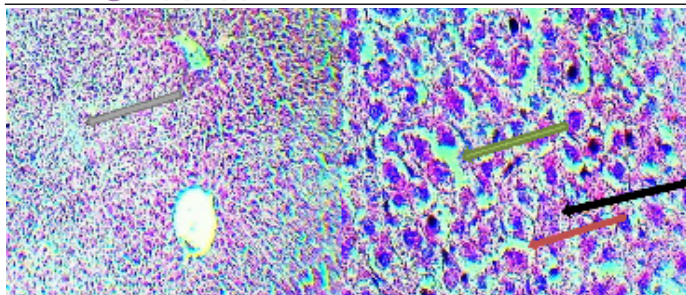


Figure 9: Section of liver tissues from male rats drenched with Nanoparticle Piperine+ Dexamethasone (suspended in 0.5 ml of distilled water). (H&E stain.40X).

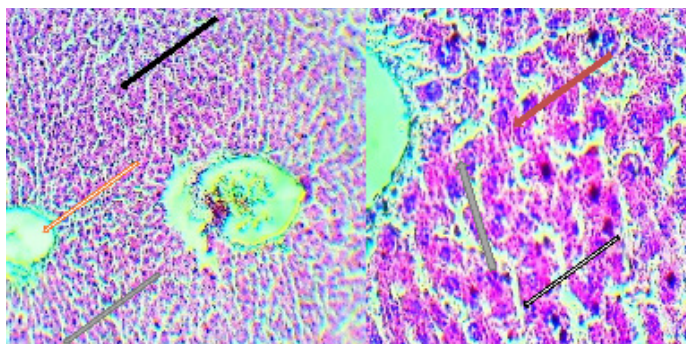


Figure 10: Section of liver tissues from male rats drenched with Vit. C + Dexamethasone (suspended in 0.5 ml of distilled water). (H&E stain.40X)

DISCUSSION

The results indicated that a noticeable decrease in the RBCs, Hb, PCV, MCV, MCH and MCHC in rats receiving dexamethasone. Antioxidants are reduced and MDA levels are raised by dexamethasone (El-Sawy *et al.*, 2018). RBCs' unique function as oxygen transporters exposes them to oxidative damage during normal aerobic functioning. Strong enzymatic and non-enzymatic antioxidant systems in normally healthy persons counteract this stress; a breakdown in these systems is likely to impair capillary perfusion (Geetha *et al.*, 2007). Reactive carbonyl species (ROS) are produced in high quantities as a result of macromolecule damage caused by ROS (Li *et al.*, 2010). Within the confines of the bone marrow, erythropoiesis takes place in a particular microenvironment. Human bone marrow stromal cells are known to be affected by dexamethasone (Kim *et al.*, 1999). An essential part of the bone marrow are stromal cells, which release extracellular matrix proteins that act as binding sites for progenitor and hematopoietic stem cells (Gordon *et al.*, 1987). The way dexamethasone affects haemoglobin, packed cell volume, and red blood cells in our investigation is consistent with the findings of a research by Stoicescu (2014), which suggested that using dexamethasone continuously might depress the bone marrow. These results agreed with those of Razzaq *et al.* (2020) and Jeje and Raji (2015). The findings indicated that rats

given vitamin C and piperine nanoparticles had higher blood parameters. Piperine has the potential to prevent free radical-induced lipid peroxidation. This suggests that it stops cell death or drastically lowers it. Piperine seems to support antioxidant-fighting enzymes (Selvendira *et al.*, 2003). According to Mittal and Gupta (2000), piperine possesses direct anti-oxidant actions against a variety of free radical types, which is consistent with the findings of our group. Previous research has shown that consuming vitamin C can raise blood haemoglobin levels (Shankar *et al.*, 2000; Makonen *et al.*, 2003 and Alarcon *et al.*, 2004). According to Rahfiludin and Ginandjar (2013), vit. C is necessary for improved red blood cell synthesis, especially for the creation of hemosiderin, an iron component. Because of its hydrophilic nature, vitamin C is an effective scavenger of free radicals. It guards against oxidative damage to biomembranes because it is highly effective at capturing free radicals in water. According to Mrityunjaya *et al.* (2020), it is capable of neutralising reactive oxygen species such as hydrogen peroxide. Rat liver sections treated with dexamethasone showed a minor infiltration of inflammatory cells and a doubling of kuffer cells. According to Diviari *et al.* (2020), dexamethasone may have produced adrenal insufficiency as a result of glucocorticoid withdrawal, which may have hindered glycogen synthase's enzymatic activity and or promoted glycogen autophagy in a cow's liver. Ni *et al.* (2020) claim that dexamethasone was produced and included reactive oxygen species (ROS) and other free radicals. In rats treated with piperine and vitamin C, histological sections of the liver showed that the hepatocytes had a distinct central nucleus location and seemed normal. This is in line with the typical geometric shape of hepatocytes, which is brought about by the antioxidant action of piperine, which averts free radicals and lipid peroxidation (Mittal, 2000). Both humans and animals have a network of antioxidants that serve as defences in the body against damaging free radicals. This process keeps the body in a balance (Powers *et al.*, 2010). According to stated study results, vitamin C possesses hepatoprotective properties. this used to do with its antioxidant property. It has been demonstrated that vitamin C can reduce the harm some chemical agents produce to the liver, especially in animals. This is supported by the study done by Bashandy and Alwasel (2011). As a hydrophilic free radicals as demonstrated scavenger, vitamin C eliminates superoxide, oxygen, hydroxyl, water-soluble peroxy radicals, and hypochlorous acid. Additionally, studies have demonstrated that vitamin C is antioxidant qualities may aid in liver protection (Adikwu and Deo, 2013). According to Oullibi *et al.* (2016), vitamin C has demonstrated a notable protective effect against hepatotoxicity caused by certain medicines and chemical agents. According to EL-Gendy *et al.* (2010), our findings indicate that vitamin C mitigates the hepatotoxicity caused by DEX. This is likely achieved by lowering oxidative damage and lipid peroxidation, as well

CONCLUSIONS AND RECOMMENDATIONS

The trial's results showed that Dexamethasone affects negatively on Liver and blood parameters. Nano-piprine and Vit. C have a great role in reducing the side effect of dexamethasone on Liver and blood parameters. The results related to the piperine nanoparticles was better than results related to the vitamin C. We also recommend studying the influences of various dose of nano piprine on different organs of body for long period.

ACKNOWLEDGMENTS

This work would not have been possible without the resources and assistance provided by the Branch of Physiology and Pharmacology at the College of Veterinary Medicine / Al-Qadisiyah University, to which the author is profoundly grateful.

NOVELTY STATEMENT

The originality of the research is in its emphasis on the physiological action of piperine nanoparticles and vitamin C to ameliorate the effects of Dexamethazone poisoning in male rats.

AUTHOR'S CONTRIBUTION

The contributions of all authors are equal.

DATA AVAILABILITY

Upon reasonable request, the authors will furnish all data.

CONFLICT OF INTEREST

No conflict of interest has been disclosed by the writer.

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