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The interaction effect of bee and date palm pollens against the toxicity of Ibuprofen on hematological parameters and oxidative stress in male albino rats

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Abstract: Ibuprofen (Ibu), a non-steroidal anti-inflammatory drug, is widely used for pain and inflammation relief. Bee pollens (BPs) are a natural honey bee product. They are rich in flavonoid and phenolic compounds, which possess antioxidant activities that prevent free radicals from being generated in the biological system. Date palm pollen (DPP) is an important source of natural antioxidants as it has a lot to do with the content of vitamins and flavonoids. This study's objective was to investigate the combined effects of DPP and BP against Ibu-induced toxicity on the hematological and oxidative stress parameters in male albino rats over one month. Seven groups of forty-two male albino rats were created. A control group is represented by the first group, the second group was administrated with Ibu (60 mg/kg body wt.) the third group was supplemented with BP (100 mg/kg body wt.), the fourth group was supplemented with DPP (100 mg/kg body wt.), the fifth group was treated with BP before Ibu, the sixth group was treated with DPP before Ibu, whereas the seventh group was treated with BP combined with DPP before Ibu. Results indicated that Ibu induced significantly altered hematological and oxidative parameters compared to control. DPP and BP normalized the hematological indices but affected the oxidative stress parameters. combined DPP and BP treatment mitigated Ibu toxicity, normalizing hematological indices, and significantly altering oxidative stress parameters. Therefore, BP and DPP alongside Ibu offer protective effects against Ibu-induced haematological and oxidative stress damage in rats. Also, these findings provide insights into BP and DPPs antioxidants, and protective influence-against Ibu toxicity.

Keywords: Ibuprofen, Bee pollen, Date palm pollen, Hematological parameters, Oxidative stress, Male albino rats, Antioxidant properties.

1. Introduction

Non-steroidal, anti-inflammatory drugs, or NSAIDs, are a class of medications that includes ibuprofen (Ibu) [1]. The body produces prostaglandins, which are what cause fever, pain, and inflammation. It is well known that Ibu inhibits cyclooxygenase, the enzyme responsible for producing prostaglandins. [2] resulting in a reduction in prostaglandin levels [3]. So, the pain, fever, and inflammation are reduced. Ibu was approved by the FDA in 1974 as an inflammatory drug. Ibu is a widely produced drug that may be found in many formulations across the globe and is included on the World Health Organization's Essential Drugs List [3] as one of the main medications. However, the widespread use of Ibu is likely to increase the prevalence of its adverse effects.

The toxicity of Ibu on hematological and oxidative stress parameters has been studied in aquatic animals. It has been stated that fishes (rainbow trout) which were exposed to two different doses of Ibu at concentrations of 50 and 500 µg/L for 96 hrs. induced changes in the hematological parameters indicated by RBCs and MCHC were seen to have significantly decreased, while HB, WBCs, MCV, and MCH were shown to have significantly increased [5]. Exposure of African sharp tooth catfish to Ibu at concentrations of 3.0, 3.5, 4.0, 4.5, and 5mg/L for 15 and 30 days induced a significant increase in

RBCs, HB, PCV, WBCs, and Mon. After 15 days of Ibu exposure, there was a significant drop in the levels of lymph, MCV, and MCH, but no discernible change in the levels of neutrophils, basophils, eosinophils, or MCHC. On day 30, there was no discernible variation between the control group and the values of RBCs, HB, PCVs, lymph, Neut., Mon., Bas., and Eos. However, there was a large drop in MCV and MCH levels and a considerable increase in MCHC values [5]. Exposing common carp to 0.25, 0.50 and 1.0 mg/l for 7, 14, and 21 days induced a significant decrease value in RBC counts after 21 days, compared to the control, while the fish-treated with Ibu caused an increase after 21 days. Also, after 21 days of Ibu exposure, Neut. decreased significantly and lymph. increased significantly at all treatments used. On the other hand, Ibu-treated fishes did not show any significant alterations in Mon., bas., and Eos. [6].

The toxicity of Ibu on the hematological and oxidative stress parameters has been seen in several aquatic species. In Indian major carp, Ibu at 14.2 mg/L for 35 days caused alteration in the hematological parameters [7]. Exposure of African catfish for Ibu with concentrations (0.28. 0.33, 0.38 0.43, and 0.48 mg/L for 24, 48,72, and 96 hrs produced increasing significantly in the number of red blood cells, hemoglobin concentration, platelet count, and white blood cell count as compared to the control, in a way that depends on concentration and duration. However, a non-significant decrease in MCV, MCH, and MCHC was documented [8]. In zebrafish, after 28 days of Ibu administration at doses ranging from 0.0001 to 25 mg/L, the oxidative stress markers changed, [9] also in common carp, which was subjected to 17.6 mg/L for 12 to 96 hours [10]. It has been determined that Ibu is harmful to hematological and oxidative stress markers in terrestrial animals. In male albino rats, administration of Ibu daily for 28 days at doses of 20 and 40 mg/kg body weight caused significant changes in the hematological parameters [11]. Also, in male rats exposed to 40 mg/kg body wt. of Ibuprofen for 28 days, hematological changes were observed [12]. Oral administration of Ibu at 200 mg/kg body wt. for 28 days to Wistar rats of both sexes showed no alterations in many of the hematological parameters except the differential leukocyte counts, which demonstrated a significant increase, in comparison to the control [13].

Since ancient times, rural and tribal groups have used herbal plants to heal a variety of illnesses. Herbal products have been utilized in developmental countries due to their affordability and ease of use in treating a variety of illnesses when compared to advanced Western synthetic medicine [14, 15]. It has been reported that about 80% of population in the developing countries such as Egypt depends on herbal medicines for the treatment of many diseases [16, 17]. These days, natural plant products account for over 50% of all chemical medications in use, which is crucial for pharmaceutical companies' drug development initiatives. This has prompted scientists to investigate additional medicinal plants in pursuit of cutting-edge substitute medication sources [18, 19]. Among these herbal medicinal plants are bee and date palm pollens which grow in a wide range of climates. All parts of the date palm are useful. Its fruits have been used as main food for more than 6000 years; it has been consumed by millions of people all over the world, especially in the Middle East and northern Africa [20].

Bee pollens are considered the major source of feeding for their growth [21, 22], which is regarded as particularly important for the reproduction and survival of these organisms and is identified as a flower kind that honeybees acquire. Due to their presence in the composition of honey, the chemicals found in these pollens have a substantial impact on both consumer health and the activities of honeybees. The nutritionally beneficial components that make up bee pollen include significant levels of phenolic and flavonoid chemicals, which are powerful antioxidants [23], and at the same time have free radical scavenging activity [24]. Bee pollen has the ability to improve health and immunity [25].

Date palm pollen is among the date palm's components. Pollens are the male gametocytes of the palm tree which was cultivated in Arabic countries, the Middle East, North Africa, and southern Asia as the source of dates, documented in Holly Quran and modern scientific literature [26, 27]. Because it contains flavonoids, carotenoids, vitamins, fatty acids, enzymes, and hormones, date palm pollen is a significant natural antioxidant source. Date palm pollens are significant ancient

herbal medicines that are frequently used to treat various diseases because they have numerous pharmacological properties, such as being an anti-oxidant stressor [28, 29], both antibacterial and anti-inflammatory [30, 31].

It has been stated that prothrombin time and platelet count significantly increased and decreased after Ibu was administered at a dose of 1.2 ml/t three times a day for 21 days [32]. Administering orally with Ibu 200 mg/ kg b.wt. Wistar albino rats of both sexes for 28 days caused the RBC count, HB, HCT%, MCV, and MCH to decrease non-significantly, while there was a significant decrease in PLT and lymph. At the same time, in comparison to the control, WBCs, Mon, and Neut increased significantly [33]. It has been suggested that giving male rabbits orally Ibu for 30 days at a dose of 40 mg/kg b.wt. per day induced a significant decrease in red blood cell count, hemoglobin concentration, HCT%, MCV level, MCH level, MCHC level, PLT count, and Neut.% compared with control, while a significant increase in WBCs and lymph% was documented [34]. It has been reported that oral treatments of albino male rats with 40mg/ kg b.wt. of Ibu for 28 days produced, in comparison to the control group, a significant decrease in the number of red blood cells, Hb concentration, and HCT% [12]. In a Gomaa's study [35], injection intraperitoneally of Ibu at a dose of 60 and 120 mg/kg b.wt. to Swiss albino rats for 1 month caused a significant reduction in relative Neut.%, while a significant increase in relative % of Mon. and Bas. at the higher dose (120mg/kg b.wt., regarding the control group. However, no significant change in total WBC count at both doses was observed. In aquatic animals (Freshwater fish), the total number of WBC, Neut., Lymph. Mono. and the count of thrombocytes was significantly reduced in fishes exposed to 0.1 and 1µg/L of Ibu for 14 days [36].

The use of each DPP and bee pollen and their combination for improving or protecting against the toxicity of Ibu on the hematological and oxidative stress parameters have not previously been studied in rats. However, bee pollen incorporated into the basal diet of *Tilapia nilotica* at 1%, 2.5%, and 4% (W/V) for 10, 20, and 30 days significantly increased hematocrit value, leucocrit and counts of neutrophils, monocytes, and lymphocytes [37]. Administration with doses of 50 and 100 mg/kg body weight of bee pollen to female Wistar albino rats for 3 weeks caused a significant increase in malondialdehyde levels and the activities of CAT, SOD, and GSH-Px in tissue and blood [29]. Also, it has been reported that the addition of 4 and 8 mg/kg body wt. in the diet of male rabbits for two weeks resulted in a significant increase in RBCs, WBCs count, Hb concentration, and PCV, compared with the control [38]. Also, it has been reported that the addition of 4 and 8 mg/kg body wt. of DPP in the diet of male rabbits for two weeks resulted in a significant increase in RBCs, WBCs count, Hb concentration, and PCV, compared with the control [39].

So, the current study aimed to evaluate the protective effect of bee and/or palm pollen and their combination against the toxicity of Ibu concerning hematological and oxidative stress parameters in rats. The current study bears originality regarding the existence of any previous study in which bee and/or palm pollens were used together for Ibu-induced toxicity in the hematological and oxidative stress parameters in the experimental animals. Our results could help to clarify the new target of BP and DPP in improving the toxic effect of Ibu on the biological system that was not fully recognized.

2. Materials and methods

Materials and methods:

Chemicals

Ibuprofen (Ibu) α-methyl -4- (isobutyl) phenylacetic acid, with a purity of 95% was purchased from Labo Chem (India). The drug was in powder form dissolved in distilled water and administrated as an aqueous suspension orally. Bee pollen (BP) in dried granules form was obtained from a professional beekeeper in Sohag Governorate, Egypt, dissolved in distilled water, and supplemented orally. Date palm pollen (DPP) grains were sourced from the Faculty of Agriculture farm, at Sohag University, Egypt, dried and dissolved in distilled water using a sonicator. The DPP solution was administrated as an aqueous suspension *via* oral gavage.

Animals:

Forty-two male white Albino rats (*Rattus rattus*) weighing (160-200 gm) were used in the study. The animals were obtained from the Zoology Department's Animal House at Sohag University, Faculty of Science, Egypt. They were housed in well-ventilated animal houses and cages. In accordance with the guidelines for laboratory animal care, Ethical Committee (no. CSRE-21-24) the animals were fed with standard rodent food and had free access to tap water *ad libitum*.

Experimental procedure:

Prior to experimentation, the animals were given two weeks to acclimatize. Seven groups of six rats each were created by random selection. Water and food were given to the first group, which was designated as the control. Ibu was administered at a dose of 60 mg/kg body weight to the second group [36]. 100 mg/ kg body weight of BP was administered to the third group [38], whereas the fourth group was treated with DPP at a dose of 100 mg/kg body weight [38] the fifth group was treated with BP along with Ibu, while the sixth group was treated with DPP along with Ibu. The seventh group was treated with combined BP and DPP along with Ibu. All treatments were administered orally once daily for 30 days using a stomach tube. The animals were monitored daily for clinical symptoms.

Blood collection:

Upon the end of the experimental period, the rats were sacrificed by cervical dislocation under light diethyl ether anesthesia after an overnight fast. Blood was collected and divided into two parts:

For hematological analysis: Blood was transferred into an anticoagulant tube and analyzed immediately.

For serum preparation: Blood was transferred into a tube without anticoagulant, and centrifuged at 3000 rpm for 15 minutes to collect serum, which was stored at -20°C for oxidative stress parameter analysis.

Determination of hematological indices:

A celltac hematological analyzer (Japan) was used to measure total erythrocytic count (RBCs), hemoglobin concentration (Hb; g/dl), hematocrit value (HCT %), mean corpuscular volume (MCV) (fL), mean cell hemoglobin (MCH) (Pg), mean corpuscular hemoglobin concentration values (MCHC) (g/dl), red cell distribution width (RDW %), total leucocytic count (WBCs) ($x10^3/\mu L$), platelet count (PLT; $x10^3/\mu L$), mean platelet volume (MPV; fL), platelet distribution width (PDW; fL), platelet crit value (PCT %), neutrophils count (Neut.; $x10^3$), lymphocytes count (lymph.; $x10^3$), monocytes count (mon.; $x10^3$), Eosinophil count (EOS; $x10^3$), and basophils count (BAS; $x10^3$).

Antioxidants analysis:

Serum was used to evaluate antioxidant enzymatic activities, including malondialdehyde (MDA) as an indicator of lipid peroxidation, superoxide dismutase (SOD), and catalase (CAT) using Bio Diagnostic kits from Diagnostic Research and Reagents Company, Giza, Egypt.

Statistical analysis:

The gathered data were presented in the form of mean ± standard deviation (SD). Using SPSS version 29.0.10 software, one-way analysis of variance (ANOVA) and Duncan's multiple range test were used to determine statistical significance. P values less than 0.05 were regarded as significant, < 0.01 was very significant, and < 0.001 was extremely significant.

3. Results and Discussion:

Results:

Hematological indices:

Highly significant ($p \leq 0.01$) and very highly significant ($p \leq 0.001$) decreases in RBCs count, Hb concentration, HCT value, and MCV were observed in the Ibu-treated group, in respect to the control, while a very highly significant ($p \leq 0.002$) and highly significant ($p \leq 0.01$) increase in MCH and MCHC, respectively were documented. However, a non-significant decrease was observed in RDW. A highly significant ($p \leq 0.03$) decrease was observed in the PLT count and PCT value, however, a non-significant decrease was noticed in MPV. Total WBCs, Neut., lymph., mon., Eos., and Bas. counts highly significantly ($p \leq 0.03$) increased in the Ibu-treated group, compared with the control (Table 1).

Table 1: The impact of ibuprofen (Ibu), bee pollen (BP), date palm pollen (DPP), bee pollen plus ibuprofen, date palm pollen plus ibuprofen, and bee pollen combined with date palm pollen plus ibuprofen on **Hematological Parameters** in Male Albino rats (*Rattus rattus*); The sign (•) is used to show non-significant $p > 0.05$, The sign (*) is used to show significant difference for control group with $p < 0.05$ and The sign (°) is used to show significant difference for ibuprofen group with $p < 0.05$.

Parameter	Control	Ibuprofen	BP	DPP	BP +Ibu	DPP +Ibu	BP+DPP +Ibu
RBCs (x10^6/UL)	4.53 ±0.49	3.95 ±0.046*	4.46 ±0.261●○	4.36 ±0.464●●	4.39 ±0.286●○	4.23 ±0.111●○	4.04 ±0.166●●
Hemoglobin (g/dl)	13 ±0.729	11.19 ±0.222*	12.05 ±0.507●○	12.56 ±0.535●○	12.01 ±0.412*○	12.61 ±0.437●○	11.88 ±0.303*○
Hematocrit (%)	36.11 ±2.623	33.02 ±0.906*	32.13 ±2.132●●	33.29 ±2.061●●	34.28 ±1.235●●	33.54 ±1.983●●	31.5 ±1.439*○
MCV (fL)	81.27 ±3.062	75.63 ±0.985*	83.2 ±1.084●○	81.73 ±3.779●○	84.11 ±2.914*○	85.16 ±2.654●○	81.64 ±1.926●○
MCH (Pg)	28.3 ±0.634	20.88 ±0.585*	29.23 ±1.316●○	29.15 ±2.156●○	29.83 ±1.088*○	34.15 ±5.36*○	30.8 ±1.191*○
MCHC (g/dl)	33.11 ±0.715	26.82 ±0.571*	33.02 ±1.669●○	34 ±1.153●○	33.37 ±0.543●○	34.1 ±3.891●○	35.77 ±1.155*○
RDW (%)	9.38 ±0.762	8.72 ±0.307*	9.62 ±0.61●○	8.73 ±0.188●●	8.98 ±0.256●○	10.11 ±2.162●●	8.78 ±0.144●●
Platelets (x10^3/ UL)	276.4 ±17.73	254.33 ±13.25*	281.27 ±24.54●○	294.33 ±39.75●●	295.93 ±11.26*○	292.87 ±30.02●○	287 ±15.845●○
MPV (fL)	9.66 ±0.77	7.23 ±0.432*	9.97 ±0.817●○	9.51 ±0.728●○	10.34 ±0.492●○	10.92 ±1.287*○	9.23 ±0.386●○
PDW (fL)	16.58 ±0.375	12.57 ±0.207*	17.12 ±0.549●○	16.55 ±0.602●○	16.9 ±0.285●○	17.59 ±1.286●○	16.45 ±0.518●○
PCT (%)	0.23 ±0.014	0.20 ±0.019*	0.29 ±0.006*○	0.25 ±0.035●○	0.26 ±0.012●○	0.22 ±0.045●●	0.23 ±0.017●○
WBCs (x10^3/ UL)	10.95 ±0.641	12.75 ±0.718*	10.33 ±0.802●○	10.58 ±0.523●○	10.15 ±0.451*○	11.27 ±0.969●●	10.02 ±0.685*○
Neutrophils (%)	6.79 ±0.397	7.97 ±0.403*	6.41 ±0.497●○	6.56 ±0.324●○	6.4 ±0.27●○	6.99 ±0.601●○	6.21 ±0.425*○
Lymphocytes (%)	3.36 ±0.261	3.83 ±0.215*	3.10 ±0.24●○	3.18 ±0.157●○	3.07 ±0.173●○	3.38 ±0.291●●	3.01 ±0.206*○
Monocytes (%)	0.58 ±0.034	0.67 ±0.033*	0.55 ±0.043●○	0.56 ±0.028●○	0.54 ±0.024*○	0.6 ±0.051●●	0.53 ±0.036*○
Eosinophils (%)	0.25 ±0.015	0.29 ±0.017*	0.24 ±0.027●○	0.24 ±0.012●○	0.23 ±0.01*○	0.26 ±0.022●●	0.23 ±0.017*○
Basophils (%)	0.44 ±0.027	0.510 ±0.03*	0.41 ±0.032●○	0.42 ±0.02●○	0.41 ±0.018*○	0.45 ±0.039●○	0.40 ±0.027●○

Table 2: The impact of ibuprofen (Ibu), bee pollen (BP), date palm pollen (DPP), bee pollen plus ibuprofen, date palm pollen plus ibuprofen, and bee pollen combined with date palm pollen plus ibuprofen on oxidative stress parameters in male Albino rats (*Rattus rattus*); The sign (•) is used to show non-significant $p > 0.05$, The sign (*) is used to show significant difference for control group with $p < 0.05$ and The sign (°) is used to show significant difference for ibuprofen group with $p < 0.05$.

Parameter	Control	Ibuprofen	BP	DPP	BP +Ibu	DPP +Ibu	BP+DPP +Ibu
LPO (nmol/ml)	59.71 ±2.041	72.02 ±0.236*	30.94 ±3.681*○	57.16 ±4.712●○	38.15 ±5.883*○	40.33 ±4.288*○	47 ±0.74*○
SOD (U /ml)	106.26 ±4.427	22.68 ±1.273*	152.25 ±4.441*○	136.21 ±17.293*○	148.36 ±4.561*○	135.08 ±16.502*○	195.65 ±7.339*○
CAT (U /L)	111.6 ±9.721	55.98 ±5.002*	137.12 ±11.104*○	139.31 ±3.474*○	130.28 ±8.682*○	124.38 ±8.528*○	123.41 ±12.158*○

No significant difference was observed in the hematological parameters in the groups treated with each BP and DPP, compared with the control except Hb concentration and PCT value in the group treated with BP, they showed a very highly significant ($p \leq 0.009$; $p \leq 0.00003$) decrease and increase, respectively (Table 1).

No significant difference was observed in the hematological indices in the group treated with each BP and DPP along with Ibu. However, administration of BP along with Ibu caused a remarkable significant ($p \leq 0.006$ and $p \leq 0.04$) decrease in Hb and MCV, while a significant ($p \leq 0.03$) increase in MCH, with respect to control was documented. Moreover, a significant ($p \leq 0.005$) increase in PLT; and a

remarkable significant ($p \leq 0.02$) decrease in WBCs, Mon., Eos., and Bas. counts were observed. The group that was treated with DPP along with Ibu showed a significant ($p \leq 0.03$; $p \leq 0.04$) increase in MCH and MPV, compared with the control (Table 1).

A significant ($p \leq 0.04$) decrease in Hb, WBCs; and polymorph nuclear leucocytes (Neut. and lymph.) and mononuclear leukocytes (Mon. and Eos.) was observed in the groups treated with Ibu along with both BP and DPP, whereas a highly significant ($p \leq 0.01$) increase in MCH and MCHC was observed, in respect to the control (Table 1).

No significant decrease or increase was observed in Hb, HCT, MCV, MCHC, and RDW in the group treated with BP, compared with the Ibu-treated group. But, a remarkably significant ($p \leq 0.006$) increase in RBC count was documented. As to PLT, WBCs, Neut., Lymph., mon., Eos. And Bas., a very highly significant ($p \leq 0.007$) decrease was observed. However, a very highly significant ($p \leq 0.0003$) increase in PCT; a non-significant increase in PDW and MPV was observed. A similar result about the hematological parameters was obtained on the administration of DPP when compared with the Ibu-treated group (Table 1). The dosage of each DPP and BP in addition to Ibu resulted in either a non-significant increase and decrease in Hb, HCT, MCV, HCH, and RDW, while they caused a remarkable significant ($p \leq 0.002$) decrease in red blood cell count (RBCs). However, DPP along with Ibu caused a highly significant ($p \leq 0.005$) increase in Hb concentration, compared with the Ibu-treated group. The administration of BP along with Ibu resulted in a very highly significant ($p \leq 0.0004$) decrease in WBCs, Neut., Lymph., Mon., Eos., and Bas. While a significant increase ($p \leq 0.03$) in PLT, MPV and PCT were documented. However, a remarkably significant ($p \leq 0.02$) increase in PDW was observed, with respect to the Ibu-treated group. As to the administration of DPP along with Ibu, a significant ($p \leq 0.03$) increase in PLT, MPV, and Neut., was noticed (Table 1) The administration of BP and DPP along with Ibu led to a highly significant ($p \leq 0.04$) decrease in HCT and MCV, whereas it caused a very highly significant ($p \leq 0.0005$) decrease in WBCs, Neut. Mon. Eos. and Bas. counts, compared with the Ibu-treated group. On the other hand, it caused a significant ($p \leq 0.04$) increase in PLT and PCT (Table 1)

Serum antioxidant enzymes:

The administration of Ibu significantly increased MDA by about 20% and reduced SOD and CAT by about 21% and 50% of the control. The administration of BP, DPP, BP along with Ibu, DPP along with Ibu, and combined BP with DPP along with Ibu removed the toxic effect of Ibu on the antioxidant enzymes. Moreover, they caused a very highly remarkable significant ($p \leq 0.0004$) increase, when compared with the control and Ibu-treated group (Table 2).

4. Discussion:

Non-steroidal anti-inflammatory drugs like ibuprofen are commonly used because of their global availability, and analgesic, anti-inflammatory, and antipyretic properties [10]. The study's conclusions demonstrated that ibuprofen has the potential to be harmful if used incorrectly and to harm both people and animals. Using this drug at overdose for a long period may result in alterations in the hematological parameters in the experimental animals [5].

Hematological indices are commonly used to assess the toxicity of the drugs on the physiological and functional state of the animal health. Also, it has been stated that the hematological indices may be sensitive to pharmaceutical care due to their relationships with defense, energy, and respiration mechanisms [40].

The current study's findings demonstrated that Ibu caused a significant decrease in RBC count, Hb, HCT, and MCV in comparison with the control. These results were in agreement with [34-36] who in a similar study observed a decrease in these parameters. However, this result did not agree with [5] who reported that the exposure of catfish to Ibu caused a significant increase in RBC count, HB concentration, and HCT%. The present study revealed that oral administration of Ibu induced a significant increase in MCH, MCHC, and MCV, while a significant reduction in RDW value was documented. These

findings were consistent with studies [5]. However, these results were in disagreement with the study [5] which indicated that exposure of African catfish to Ibu resulted in a non-significant reduction in the above Parameters. The observed increase in MCH, MCHC, and MCV may be due to the presence of a higher quantity of older or larger RBCs, since the increase in MCV indicates swelling of RBCs due to hypoxia, leading to reduced RBCs lysis in the circulation. The swelling of RBCs due to the toxicity of Ibu in organisms exposed to toxicants can lead to a noteworthy increase in MCV, MCH, MCHC, and a decrease in RDW value [5]. So, it can be concluded that the increased MCH, MCHC, and MCV and decreased RBCs, HB, HCT%, and RDW as a result of administration of Ibu to rats may lead to bone marrow failure causing erythropoietin deficiency, which is essential for the production of RBCs, consequently, render the formation of RBCs and finally causing microcytic anemia.

So, the reduction in these blood indicators suggested that Ibu can cause anemia, lower blood's ability to carry oxygen, and decrease the quantity of oxygen that reaches animal organs. The significant increase in MCH and MCHC in the Ibu-treated rats supports our assumption that Ibu is capable of causing anemia since the increase in MCH and MCHC values can often lead to anemia due to a deficiency of B vitamins which are required to make red blood cells. Also, it is known that MCV, MCH, and MCHC are referred to as red blood indices and are related to individual red blood cells. Hence, the alterations in these indices are often used to determine the animals' physiological state. The significant increase in WBCs, Neut., lymph., Mon, Eos. and Bas; and a significant decrease in PLT may act as an indicator of selective immune stimulation property and protective response to the drug because these are effector cells in the immune system. This result was in agreement with the study of [13] who in a similar study observed a significant increase in monocytes and neutrophils. Analyzing hematological indices is a very helpful way to understand how the compounds of herbal plants relate to blood [41-44].

Based on the hematological indices, the levels of Hb, HCT, MCV, and MCHC at each BP and DPP; and BP and DPP along with Ibu were either significantly lower or higher than that of the Ibu-treated group. It could be stated that the dose of BP and DPP may be low to allow enough response to the effect of Ibu on these parameters. So, the absence of significant changes in these parameters may suggest that the balance between the rate of the production and destruction of RBCs (erythropoiesis) was altered in the direction of destruction and this may be due to bone marrow failure. It is known that WBCs and differential leukocytic indices (Neut., Lymph., Mono., Eos. and Bas.) are normally used as indicators of body health, as these cells fall within the natural immunity that defends the body against toxins and diseases. The findings of the present study revealed that Ibu induced a significant increase in WBCs and the differential leukocytic indices count. These results were in accordance with the studies which indicated that administration of Ibu to the experimental animals resulted in an elevation of WBC count, Neut., Lymph. and Mono [5, 17, 34]. Additionally, research has shown that non-steroidal anti-inflammatory medications, such as sodium diclofenac and paracetamol, significantly increased white blood cell counts and differential leukocytic counts, which is consistent with the findings of the current investigation [37]. Increasing the WBC count can arise from an immunological defense system that attempts to shield the organism against stress-induced inflammatory alterations in the tissue that lead to the phagocytosis of harmful medications. Also, this increase in WBC count and decrease in PLT and PLT indices would suggest that the bone marrow is not destroyed.

The significant decrease in WBCs, Neut., lymph., Mon., Eos. and Bas.; and a significant increase in PLT in each BP and DPP, and BP and DPP along with Ibu may suggest that BP and DPP were able to attenuate the toxic effect of Ibu on these parameters and at the same time increase the immune response against the Ibu-toxicity. So, these herbal plants are very useful for human toxicity.

Oral administration of Ibu induced an imbalance between oxidative stress resulting in the generation of more reactive oxygen species, clarified by a remarkable alteration in the serum levels of MDA (as an indicator of LPO activity), SOD, and CAT. Ibu treatment in the current research increased the serum level of MDA by a highly significant amount. This finding was

consistent with the study which revealed that Ibu induced a significant increase in the serum levels of LPO [38]. Also, it has been stated that NSAIDs (Sodium diclofenac and Paracetamol) caused the blood level of LPO to increase significantly [37]. It is known that elevation of LPO serum level can decrease the membrane integrity, increase the membrane permeability, inactivate enzymatic activity, and loss of the essential fatty acid in the erythrocytic cell membrane, leading to deformation of erythrocytes, thereby reducing the survival of theerythrocytes This seems to be a case, since Ibu induced a significant decrease in RBCs count, HB concentration and HCT%. These results were in accordance with a study [45] that stated that Ibu caused a significant increase in LPO activity and a significant decrease in SOD and CAT in rats. On the other hand, these results were not in accordance with the study of Awad [46] who claimed that Ibu did not affect SOD activity, while it resulted in a significant increase in CAT activity in rats. It is known that SOD directs the antioxidant defense system by converting the reactive superoxide anion into H_2O_2 and did not regulate it, causing many types of cell damage. Decreased serum catalase levels inhibited the conversion of H_2O_2 into water and oxygen and this led to increasing ROS and cell damage. Thus, it can be concluded that Ibu may accumulate in the hemopoietic organs and thereby its conjugation with SOD and CAT inactivates these enzymes, leading to their reduction.

Bee and date palm pollens have been used since ancient times as an anti-toxin that could be exposed to the body during the day [47]. Also, these pollens have been used as herbal medicine for curing many diseases, especially in the north African [20] and Middle Eastern countries [13]. Due to their component of phenolic and flavonoid compounds [38] act as antioxidant and free radical scavengers [29, 41]. The current investigation found that oral treatment of both BP and DPP, either alone or in combination with Ibu, eliminated the toxicity of Ibu by elevating serum levels of SOD and CAT and lowering LPO levels. It has been reported that the increase in the antioxidant enzymes activities contributes to the elimination of the reactive oxygen species. So, it can be concluded that BP and DPP have a protective effect against the oxidative stress induced by ibuprofen on hematological indices and antioxidant enzymes.

5. Conclusion:

It can be concluded from the present study that ibuprofen was not safe for continuous use for four weeks on the hematological and oxidative stress parameters in rats. It induced a remarkably significant decrease in the hematological and oxidative parameters. It has an adverse effect on the haemopoietic and immune systems. These adverse effects were removed with the treatment of bee and date palm pollens. So, treatment with ibuprofen could be safe if bee and date palm pollens are taken during the treatment Additionally, this work will open the way for additional research on the use of date palm and bee pollens as a potential intervention in ibuprofen toxicity, including preclinical and clinical studies. Finally, it could be said that how date palm and bee pollens reduce oxidative and hematological damage due to their anti-oxidative activities. In this respect, we recommended the transformation of this study from the experimental animals to humans in treatment with bee and date palm pollens when using ibuprofen for a long duration of time.

6. CRediT authorship contribution statement:

Conceptualixaton, M. M. Abdelreheim[1], M. F. El-Sayed[1*], S. Kh. Abd El-Ghaffar[2], W. M. Abdelsamie[1].; methodology, M. M. Abdelreheim[1], M. F. El-Sayed[1*], S. Kh. Abd El-Ghaffar[2], W. M. Abdelsamie[1].; software, M. M. Abdelreheim[1], M. F. El-Sayed[1*]; validation, , M. F. El-Sayed[1*], S. Kh. Abd El-Ghaffar[2], W. M. Abdelsamie[1] .; formal analysis, M. M. Abdelreheim[1] .; investigation,; M. M. Abdelreheim[1], M. F. El-Sayed[1*], S. Kh. Abd El-Ghaffar[2] .; resources, , M. F. El-Sayed[1*], S. Kh. Abd El-Ghaffar[2], W. M. Abdelsamie[1] .; data curation, , M. M. Abdelreheim[1] .; writing—original draft preparation, M. M. Abdelreheim[1] .; writing—review and editing, M. F. El-Sayed[1*], S. Kh. Abd El-Ghaffar[2], W. M. Abdelsamie[1] .; visualization, M. M. Abdelreheim[1] supervision, , M. F. El-Sayed[1*], S. Kh. Abd El-Ghaffar[2] All authors have read

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