APAP–induced organ toxicity in rats: The prophylactic role of *Acrocarpus fraxinifolius*

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Abstract

APAP (N-acetyl-p-aminophenol) is used over-the-counter analgesic and anti–pyretic drug. APAP overdose can seriously damage several organs. *Acrocarpus fraxinifolius* Wight and Arn leaves (AFL) family Fabaceae is a medicinal tree species native to Africa and Asia. Traditionally, AFL is used in the prevention/treatment of liver and kidney damages. This study investigates the possible protective effects of AFL *n*-hexane extract (nHEAFL) against the APAP–induced organ toxicity in adult male Wistar albino rats. Rats were randomly divided into four groups. Group, I was the healthy control group that received placebo, group II (nHEAFL 500): rats received nHEAFL (500 mg/kg, p.o), group III (APAP+vehicle): rats received APAP (750 mg/kg, p.o) for 7 days. Group IV (nHEAFL 500+ APAP): rats pre-treated with nHEAFL (500 mg/kg, p.o) before inducing the organs damage in the last 7 days by APAP. Twenty-four hrs after last administration of APAP, the rats were sacrificed. APAP–induced a significant body weight loss, with the rise in serum liver markers (ASAT & ALAT), urea, uric acid, creatinine and renal/splenic/cardiac MDA, as well as with a reduction of cellular GSH, GR, GPx, SOD, and CAT activities. nHEAF showed a remarkable organs protective effect against APAP as evidenced by reduction of serum cellular toxicity and cellular lipid peroxidation, as well as enhanced cellular anti–oxidant defense system in renal/splenic/cardiac tissues. These findings suggest a potential protective role of AFL against APAP–induced organ toxicity in rats.

Keywords: Leguminosae, anti–oxidants, heart, mundane, oxidative stress, paracetamol, pink cedar, shingle tree, spleen.

Abbreviations:

AFL, *Acrocarpus fraxinifolius leaves*; ALAT, Alanine aminotransferase; APAP, N-acetyl-p-aminophenol; ASAT, Aspartate aminotransferase; CAT, Catalase; COX, Cyclooxygenases; GR, Glutathione reductase; GSH, Reduced glutathione; GPx, Glutathione peroxidase; MDA, Malondialdehyde; NAPQI, N-acetyl-p-benzoquinoneimine; nHEAFL. AFL *n*-hexane extract; NSAIDs, Nonsteroidal anti-inflammatory drugs.

Introduction

Nonsteroidal anti–inflammatory drugs (NSAIDs) are a heterogeneous class that non-selective/selective inhibitors for cyclooxygenase (at least two isoforms: COX1 and COX1). Commonly prescribed for their NSAIDs (such as: aspirin, paracetamol, ibuprofen, naproxen, and diclofenac) are extensively used for the relief of pain, fever and treatment of inflammatory conditions [1,2]. The common mechanism of these drugs is the ability to decrease prostaglandin synthesis by inhibiting COX (COX also known as prostaglandin-endoperoxide synthase). NSAIDs vary in their relative inhibitory effects on COX-1 and COX-2. There are worried that the COX-2 inhibitors may increase cardiovascular diseases [3]. The overdose of NSAIDs is associated with an increased toxicity. Also, this toxicity can lead to multiple complications, and may be result in death.

N-acetyl-p-aminophenol (APAP), also known as paracetamol or acetaminophen, is a non-prescription drug used as an analgesic, and anti–pyretic drug globally, considered safe and effective at therapeutic doses. APAP toxicity is one of the main causes of poisoning world-wide. Several studies reported that excessive use or overdose of APAP can damage several organs (especially the liver: represents the site of formation of the toxic metabolites, and the kidney: represents the site of its clearance) and even death [4,5]. Its toxicity is mediated by the activity of its reactive metabolite (N-acetyl-p-benzoquinoneimine, NAPQI), that generated via cytochrome P450 in liver. NAPQI is detoxified by the antioxidant effects of intracellular glutathione (GSH). Thus, an overdose of APAP cause depletion of cellular GSH. Therefore, it led to a reduced GSH capacity to detoxify NAPQI. Elevation of NAPQI mediates oxidative damage. This subsequently enhances cellular injuries and organ dysfunction [6]. Other studies reported that acute renal damage/failure can occur by overdose APAP even in the absence of liver damage/failure [6,7].

Several studies reported that APAP exerts acute and/or chronic hepato–toxic, nephro–toxic effects, gastrointestinal complications and hyperplasia of splenic tissue as well as cardio–toxicity

[1,2,6,8-12]. 75% of blood advent to the liver arrives directly from gastro-intestinal organs, and then goes to the spleen by portal veins that bring drugs as foreign substances and xenobiotics in near-undiluted form. Following tissue injury, splenic monocytes enter the circulation migrating to inflammatory sites. These splenic monocytes differentiate into macrophages, that participate in pro/anti–inflammatory responses [11]. In dogs, the toxic effects of APAP include hepatic damage, kidney failure and serious hematologic disorders as Heinz bodies formation and hemoglobin damage (non-functioning hemoglobin) [9,13].

Medicinal plants such as Caesalpinieae may be play a significant role in both cases of disease conditions and as a possible material for maintaining health. So, Caesalpinieae (9 subtribes that have more than 47 genera) have a spectrum of biological activities including anti–oxidant, anti–inflammatory, anti–tumor, anti–diabetic, anti–fungal, anti–bacterial, hepato–protective, gastro–protective, analgesic, anti–arthritic, anti–filarial, anti–malarial, anthelmintic, amoebicidal, diuretic, anti–psoriatic, anti–estrogenic, anti–fertility, wound–healing, anxiolytic, cardio–protective, immune-modulatory, anti–HIV [2,14-18].

Acrocarpus fraxinifolius leaves (AFL), Fabaceae family and Caesalpiniaceae subfamily, is a native wide spread tree worldwide especially in Africa and Asia. Also, it is distributed in the tropical countries including Egypt. It common name pink cedar, mundani or shingle tree [2,15]. The extracts of the plant were reported to have anti–diabetic, anti–proliferative, anti–inflammatory, anti–oxidant, and hepato–protective activities *in vivo* [2,14,17,19,20] as well as antitumor activity *in vitro* [16]. Until now, there is not enough scanty information on the protective effect of nHEAFL on oxidative stress induced by APAP in some tissues like kidney, spleen and heart. So, this study was designed to investigate the possible protective activity of nHEAFL, may be for the first time, against APAP–induced organs toxicity (especially kidney, spleen and heart) in male albino rats. At the same time, it investigated any side effects caused by nHEAFL in healthy normal albino rats.

Materials and Methods

Chemicals

APAP was purchased from Sanofi-Aventisegypts.A.E. (El Sawah, El Amiriya, Cairo, Egypt). The kits used for biochemical measurements were all purchased from Bio-diagnostic Company (Dokki, Giza, Egypt).

Preparation of nHEAFL

AFL were prepared by following the previous method [2,20]. Briefly, two kg powder of AFL was soaked in methanol (80%) for four days then filtered. The filtrate was completely evaporated (*in vacuo* at ≈ 55 °C) till complete dryness. The dried extract was further successively fractionated with *n*-hexane (nH). The nHEAFL was evaporated *in vacuo* until dryness to give fifty grams of a sticky dark greenish material. nHEAFL obtained was preserved in a sterile glass container (4°C) until further use.

Animals

Adult male Wistar albino rats weighing between 130-140gm were purchased from the Animal Breeding House of the National Research Centre (Dokki, Cairo, Egypt). The animals were housed in polypropylene cages in well-ventilated animal room (Zoology Department, Faculty of Science, Ain Shams University) and fed with pellet diet (Agricultural-Industrial Integration Company, Giza, Egypt) and tap water *ad libitum*. The animals (male rats) were acclimatized for one week before to the start of experiments. All experimental rats were humanely-treated (accordance with WHO guideline for animal care) and the design of this study was approved by the Ain Shams University Research Ethics Committee.

Experimental design & treatment schedule

Rats were divided into four groups (n = 6), i.e., Group I (healthy control group): rats received orally distilled water only for 21 days; Group II (nHEAFL 500): rats received nHEAFL only (500 mg/kg b.wt, p.o) for 21 days. The selected dose of nHEAFL was based on Abd El-Ghffar et al. [2] and Alaa [20] who showed the hepato–protective effects of nHEAFL on the APAP–induced hepato–toxicity. Group III (APAP + vehicle): rats received orally distilled water then the animals received APAP (750 mg/kg b.wt; p.o) in the last 7 days to induced oxidative stress in organs [6]; and Group IV (nHEAFL 500 + APAP): rats received orally nHEAFL for 21 days then treated with APAP in the last 7 days.

Blood & tissues sampling

Animals were sacrificed after the last administration dose after an overnight fast (on day 22). The blood was collected into tubes with EDTA (for complete blood picture analysis) or without EDTA (for serum markers of cellular toxicity). The kidney, spleen and heart were separated out of the body, cleaned, and weighed then homogenized in 5mL cold buffer (0.5 g of Na_2HPO_4 and 0.7 g of NaH_2PO_4 per 500mL deionized water, pH 7.4) per gram tissue. Then, the homogenates were centrifuged (15 min/4000 rpm/4°C); and the obtained supernatants were divided into aliquots and preserved at –80°C until used for evaluating the oxidant/anti–oxidant parameters.

Measurements

Body weight (gain or loss) and relative organs weight were calculated. Serum amino transaminases enzymes (ASAT and ALAT), urea nitrogen, creatinine and uric acid were estimated according to Reitman & Frankel [21], Vassault et al. [22], Young et al. [23] and Fossati et al. [24], respectively. The malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and reduced glutathione (GSH) in tissue homogenates were estimated by the spectrophotometric methods described by Ohkawa et al. [25], Nishikimi et al. [26], Paglia & Valentine

[27], Goldberg & Spooner [28], and Beutler et al. [29], respectively. Red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), blood indices, white blood cell (WBC) and differential counts were determined by automated hematology analyzer (Hemat 8 analyzer; SEAC, Freiburg, Germany).

Statistical analysis

The results were expressed as mean values with their standard errors (SE). Data was analyzed using ANOVA, and the differences between groups were determined by Tukey's multiple comparison test using Graph Pad Prism. Differences at $P<0.05$ were considered statistically significant.

Results

In Fig 1, the body weight gain and relative kidney weight were significantly decreased and increased ($p < 0.05$-0.001), respectively, in APAP- intoxicated group in contrast to the control group. This was not accompanied by significant change in spleen and heart relative weights ($p>0.05$). Oral treatment of nHEAFL completely modulated the decrease in in the body weight loss ($p>0.05$; compared to the healthy control animals), and partially alleviated the increase in relative kidney weight ($p<0.05$/$p<0.001$; compared to the healthy/APAP intoxicated control animals, respectively).

Fig 1. Body weight (a) and organs weight (b) and relative organs weight (c) of control, nHEAFL 500 alone, APAP-only and nHEAFL500 plus APAP groups. Values are means (n = 6/group), with their SEM represented by vertical bars. nHEAFL: *n*-hexane extract of *Acrocarpus fraxinifolius* leaves; APAP: N-acetyl-p-aminophenol. ** p<0.05, ***p<0.001: compared with the healthy control group; ††$P<0.01$, †††$P<0.001$: compared with the APAP intoxicated group that received vehicle.

There was a slight but not significant decrease (p>0.05) RBCs, Hb, and HCT in APAP-intoxicated group compared with control rats (Fig 2). In addition, granulocytes and agranulocytes were a slight but not significant increase (p>0.05) in APAP- intoxicated group compared with control rats. All these hematological parameters did not significantly change (p>0.05) in APAP treated with nHEAFL compared with the healthy/ APAP- intoxicated control rats (Fig 2).

Fig 2. Hematological parameters (a) and blood indices (b), differential granulocytes (c), differential agranulocytes, (d) and total agranulo- agranulocytes counts (e) of control, nHEAFL 500 alone, APAP-only and nHEAFL500 plus APAP groups. Values are means (n = 6/group), with their SEM represented by vertical bars. nHEAFL: *n*-hexane extract of *Acrocarpus fraxinifolius* leaves; APAP: N-acetyl-p-aminophenol, Hb: hemoglobin, HCT: hematocrit, MCH: mean corpuscular Hb, MCHC: mean corpuscular, Hb concentration, MCV: mean corpuscular volume, RBCs: red blood corpuscles.

Fig 3 revealed that the serum markers for cellular toxicity (serum ALAT, ASAT activities, urea, uric acid and creatinine levels) significantly increased (p<0.05 to p<0.001) in APAP- intoxicated group compared with the healthy control animals.

Fig 3. Serum markers for cellular toxicity (a&b) of control, nHEAFL 500 alone, APAP-only and nHEAFL500 plus APAP groups. Values are means (n = 6/group), with their SEM represented by vertical bars. nHEAFL: n- hexane extract of *Acrocarpus fraxinifolius* leaves; APAP: N-acetyl-p-aminophenol, LAT: alanine aminotransferase, ASAT: aspartate aminotransferase. ** p<0.05, **p<0.001, ***p<0.001: compared with the healthy control group; †P<0.05, †††P<0.001: compared with the APAP intoxicated group that received vehicle.

As shown in Fig 4, kidney/spleen/heart MDA and non-enzymatic/enzymatic anti–oxidant (GSH, GR, GPx, SOD and CAT) were significantly increased and decreased (p<0.001), respectively, in APAP- intoxicated group compared with the healthy control group. Oral treatment of nHEAFL completely modulated the increase in heart MDA (p>0.05) compared with the healthy/APAP-intoxicated control group; But, partially alleviated the increase/decrease in MDA and non-

198 enzymatic/enzymatic anti–oxidant (p<0.05-0.001 to p<0.01-0.001) compared with the healthy/APAP-
199 intoxicated control group, respectively.

200 Fig 4. Cellular oxidant and anti–oxidant markers (a-f) in kidney, spleen, and heart of control, nHEAFL
201 500 alone, APAP-only and nHEAFL500 plus APAP groups. Values are means (n = 6/group), with their
202 standard errors (± SEM) represented by vertical bars. nHEAFL: n- hexane extract of *Acrocarpus*
203 *fraxinifolius* leaves; APAP: N-acetyl-p-aminophenol, CAT: Catalase; GPx: Glutathione peroxidase;
204 GR: Glutathione reductase; GSH: Reduced glutathione; MDA: malondialdhyde; SOD: Superoxide
205 dismutase. ** p<0.05, **p<0.001, ***p<0.001: compared with the healthy control group; ††P<0.01,
206 †††P<0.001: compared with the APAP intoxicated group that received vehicle.
207

208 All the above parameters measured in the present study were not significantly altered (p>0.05)
209 in healthy-treated rats that received nHEAFL compared with the healthy control rats (Figs 1-4).
210 Furthermore, the mortality rates in all groups that received nHEAFL were zero during the period of the
211 study. Subsequently, no deleterious effects were detected for the dose of nHEAFL used in this study.
212

213 # Discussion

214 APAP is now the most common drug in self-poisoning, with a high rate of morbidity and mortality
215 [30]. The present study showed that over dose of APAP–induced deleterious effects on some organs
216 (kidney, spleen and heart) as indicated by decreasing cellular anti–oxidant defense system (GSH, GR,
217 GPX, SOD and CAT and increasing serum liver/kidney biochemical markers (ALAT, ASAT, urea,
218 uric acid and creatinine) and cellular lipid peroxidation (MDA). The elevation in serum cellular toxicity
219 markers are indicative of cellular leakage and loss the functional integrity of cell membranes
220 [6,13,19,31,32]. The liver, kidney, spleen and heart are thought to form a toxic metabolite only when
221 their GSH content depleted by APAP [4,11-13]. Where, APAP is metabolized to NAPQI (highly
222 reactive free radicals) decreases the body's natural anti-oxidant GSH and can bind covalently to
223 proteins (selenium-binding protein and glutamine synthetase) and unsaturated fatty acids of cell
224 membranes, resulting in lipid peroxidation, cellular membrane disruption, depressed mitochondrial

function and elevated cellular injury markers and initiates cell death. The loss in body weight caused by APAP was also reported by Abdul Hamid et al. [6] and Manimaran et al. [33] in rats treated with 105 and 750 mg APAP/kg b.w, respectively. Relative kidney weight was significantly increased in APAP- intoxicated group. On the other hand, spleen/heart relative weights in the APAP experimental model did not significantly change, most probably due to the short treatment duration of APAP toxicity. But, we observed disturbance in anti–oxidant defense system in renal, splenic and cardiac tissues of male rats.

Several studies suggested that myocardial injury may be occurs due to a similar mechanism which causes hepatic injury by APAP through its a toxic metabolite, (NAPQI). Where NAPQI may acts as a direct toxin on the myocardium [8,10,12]. These results are in line with the research work of Hinson et al. [4] who reported that hepatic injury resulted from APAP may be suggested to toxic reactive metabolites (NAPQI) and/or their acidic moiety that bind to possibly critical cellular proteins in the liver, kidney, spleen and cardiac tissue And this damage results in the alteration of lipid/protein structure/function, loss of functional integrity of cell membrane and causes tissue injury [34]. APAP can deplete sulfydryl-groups, which interferes with nitric oxide (NO) production, and may results in coronary ischemia [8,12]. In addition, APAP may also cause organs toxicity (like liver, kidney, heart and spleen) by mechanisms leading to the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical, nitric oxide and peroxynitrite [6, 32,35,36]. Such assumption was confirmed in the present study by the observed significant decrease in cellular non-enzymatic/enzymatic anti–oxidant and increase cellular lipid peroxidation in kidney, spleen and heart. In addition, this may explain the observed changes in the serum urea, uric acid and creatinine [6,36].

Hematological parameters are frequently used as indicators of health status. Current data revealed that administration of APAP had no significant changes in hematological parameters (RBCs,

Hb, HCT, blood indices, total WBC, neutrophil, eosinophil, monocyte and lymphocyte counts), which suggest that the immune system have not been compromised or due to immune modulatory effects [34,37].

Pretreatment with nHEAFL produced partial protection against APAP induced organs toxicity (such as liver, kidney, spleen and heart) represented by significant reduction of serum cellular markers (ALAT, ASAT, urea, uric acid, and creatinine), cellular MDA concentration and significant elevation renal/splenic/cardiac anti-oxidants (GSH, GR, GPX, SOD and CAT) compared with non-treated APAP group. This finding may suggest nHEAFL's ability to restore the balance between generation and clearance of ROS and lipid peroxidation, and the stability of the function during organs injury [19,20]. Also, these modulations led to alleviate the loss in body weight gain [2] and relative kidney weight. This may be attributed to the fact that phenolic acids in AFL have anti–oxidant and free radical chelators/scavengers activities as with special impact over hydroxyl/peroxyl radicals, superoxide anions, and peroxynitrites [16].

Recently, bioactive phenolics such as brevifolin carboxylic acid, ellagic acid, gallic acid and methyl gallate were identified from the extract of AFL [16,17], and may be responsible for its radical scavenging activity [38].

Our previous studies have indicated that nHEAFL (500 mg/kg,p.o, for 7 to 21 days) plays an important role in improving GSH status and total anti–oxidant capacity in intoxicated rats with APAP [2,20]. In this study, the high level of GSH in response to nHEAFL may result from increased activity of cellular anti–oxidant enzymes and decreased cellular lipid peroxidation.

Higher activity in nHEAFL may be due to presence of α–tocopherol (an isoform of vitamin E and as an anti–oxidant agent), which has a powerful anti–oxidant activity in detoxifying free radicals, stabilization of the cell membrane and structure restoration [2]. α–Tocopherol may have inhibited the chain reactions of APAP–generated free radicals or scavenged the ROS before reaching its renal,

splenic and cardiac targets. Furthermore, α–tocopherol stimulated the upregulation of endogenous cytochrome P3 (A4 and A5) which metabolize APAP into reactive metabolite NAPQI [2,39].

The Polyphenols, flavonoids, and anthocyanins are known to possess antioxidant activities (scavenging of free radicals), due to their several phenolic hydroxyl groups [40]. Recently, El-Kashak et al. [17] reported that AFL have very strong anti–oxidant effect due to presence of flavonoids (such as: quercetin–3–O–β–D–glucopyranoside, quercetin–3–O–α–L–rhamnopyranoside, myricetin-3–O–β–D–galactopyranoside and myricetin–3–O–α–L–rhamnopyranoside). Abd El-Ghffar et al. [2] reported that the phytochemical screening of nHEAFL afforded varieties of polyphenolic components; α-tocopherol (18.23%), labda-8 (20)-13-dien-15-oic acid (13.15%), lupeol (11.93%), phytol (10.95%) and squalene (7.19%). Also, it has been reported that nHEAFL contains flavonoids, tri–terpenoids, α–tocopherol and steroids [2] which exhibited strong anti–oxidant activity. Another scientific report indicated certain flavonoids, tri-terpenoids and steroids have the protective effect on hepatic tissue due to its anti–oxidant properties [41]. The presence of these compounds in nHEAFL may be responsible for the protective effect against APAP only induced organs toxicity in rats.

Abd El-Ghffar et al. [2] and Alaa [20] proved that the anti–oxidant and hepato–protective activities exerted by nHEAFL against APAP-induced oxidative damage was attributed to its active constituents such as lupeol, squalene, and phytol. Also, another study proved that lupeol from *Ficus pseudopalma* Blanco (Moraceae) extract has the anti–oxidant and hepato–protective activities against APAP-induced oxidative damage [42]. Regarding squalene Sivakrishnan & Muthu [43] and Zuhan et al. [44] reported about the promising hepato–protective effects of squalene isolated from *Albizia procera* (Roxb.) Benth (Mimosaceae) against APAP– and CCl_4–induced toxicity. It has been reported that phytol, which is an acyclic diterpene alcohol, acts as a precursor for vitamin E and K1 and it has anti–oxidant as well as anticancer activities [2,45]. Taking this fact with gained results we could suggest that the amount of exogenous polyphenolic diets increases, endogenous anti–oxidant defense system

297 increase as well. Therefore, the medicinal anti–oxidant therapeutic will offers a promising way to
298 prevent and/or treat the diseases induced by the excessive exposure to oxidative stress (ROS/NOS).
299 Finally, our study demonstrated that nHEAFL has important anti–oxidant effects as ability to
300 scavenge ROS, inhibit lipid peroxidation as well as increase of anti–oxidant defense system in kidney,
301 spleen, and heart tissue organs; which was attributed to presence of mainly α–tocopherol, terpenoidal
302 and steroids compounds.
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305 Conclusion

306
307 In our observations, we have concluded that nHEAFL has great value as a source of compounds for
308 pharmaceutical applications. nHEAFL was found to be effective in protecting the liver, kidney, spleen
309 and heart tissue from oxidative damage in animal models of APAP -induced organs toxicity. The
310 possible mechanism by which nHEAFL exhibited significant protection against APAP-induced organs
311 damage may be due to its anti–oxidant effect and its constituents such as α–tocopherol, terpenoidal,
312 and steroids compounds. Additional experiments are necessary to confirm the mechanisms of action of
313 nHEAFL and the it's protective role that plays important role in APAP–induced oxidative damage to
314 these organs.
315
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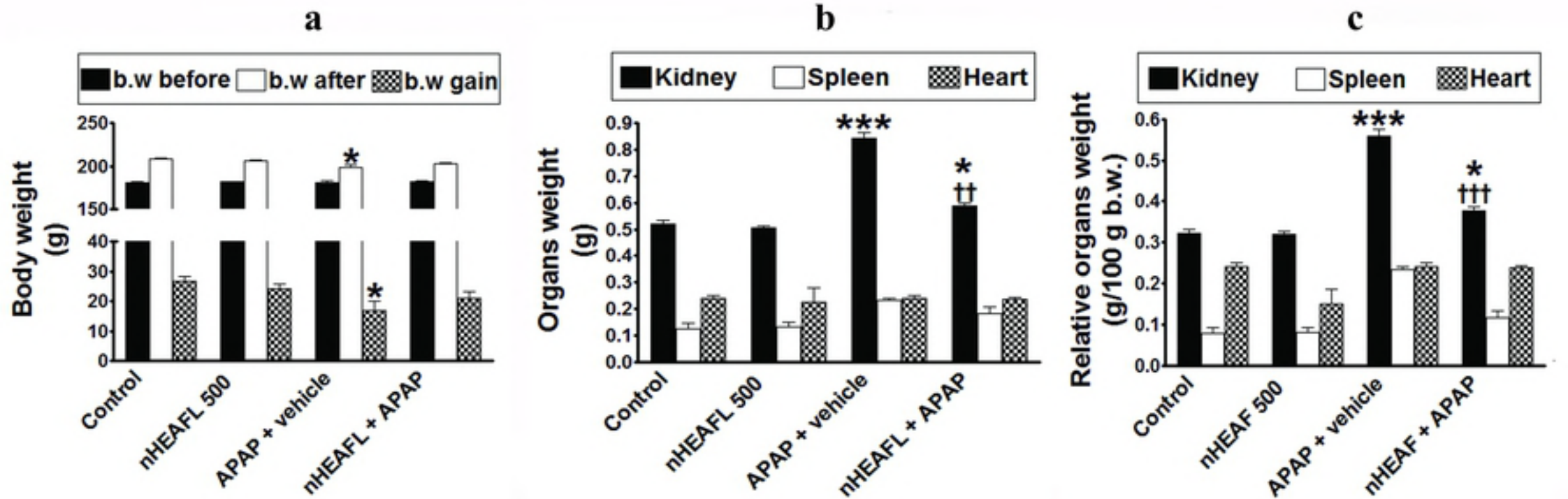
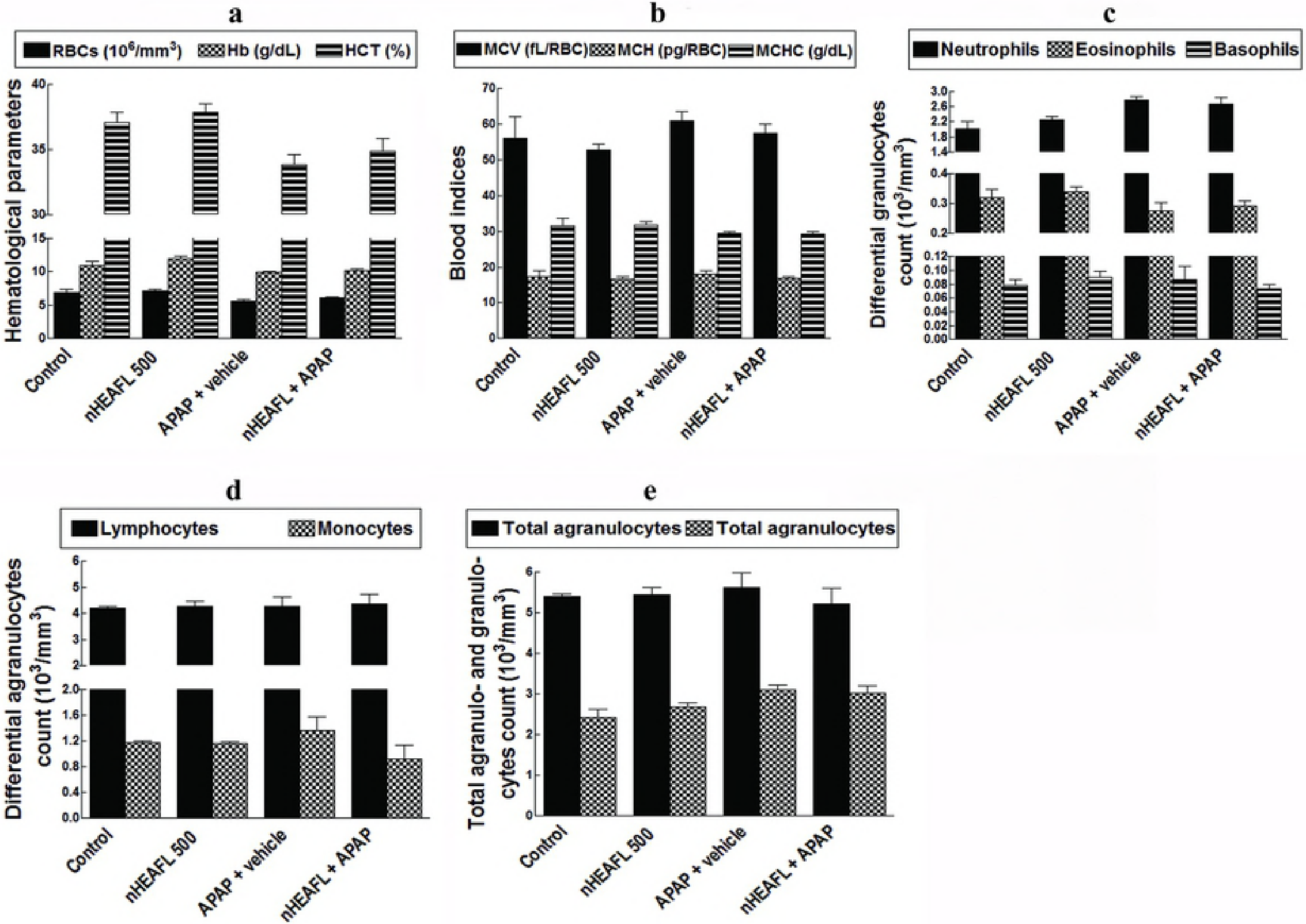
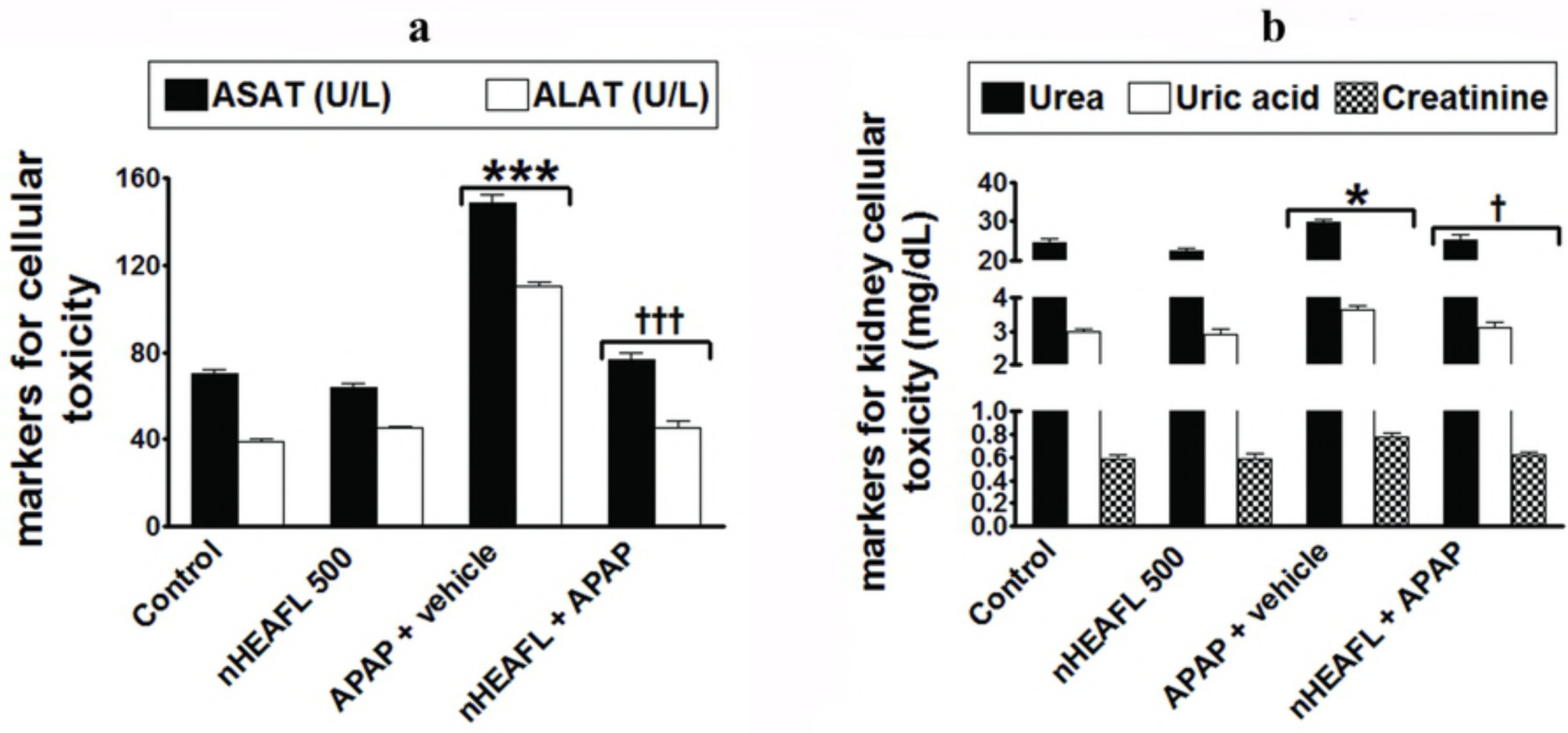
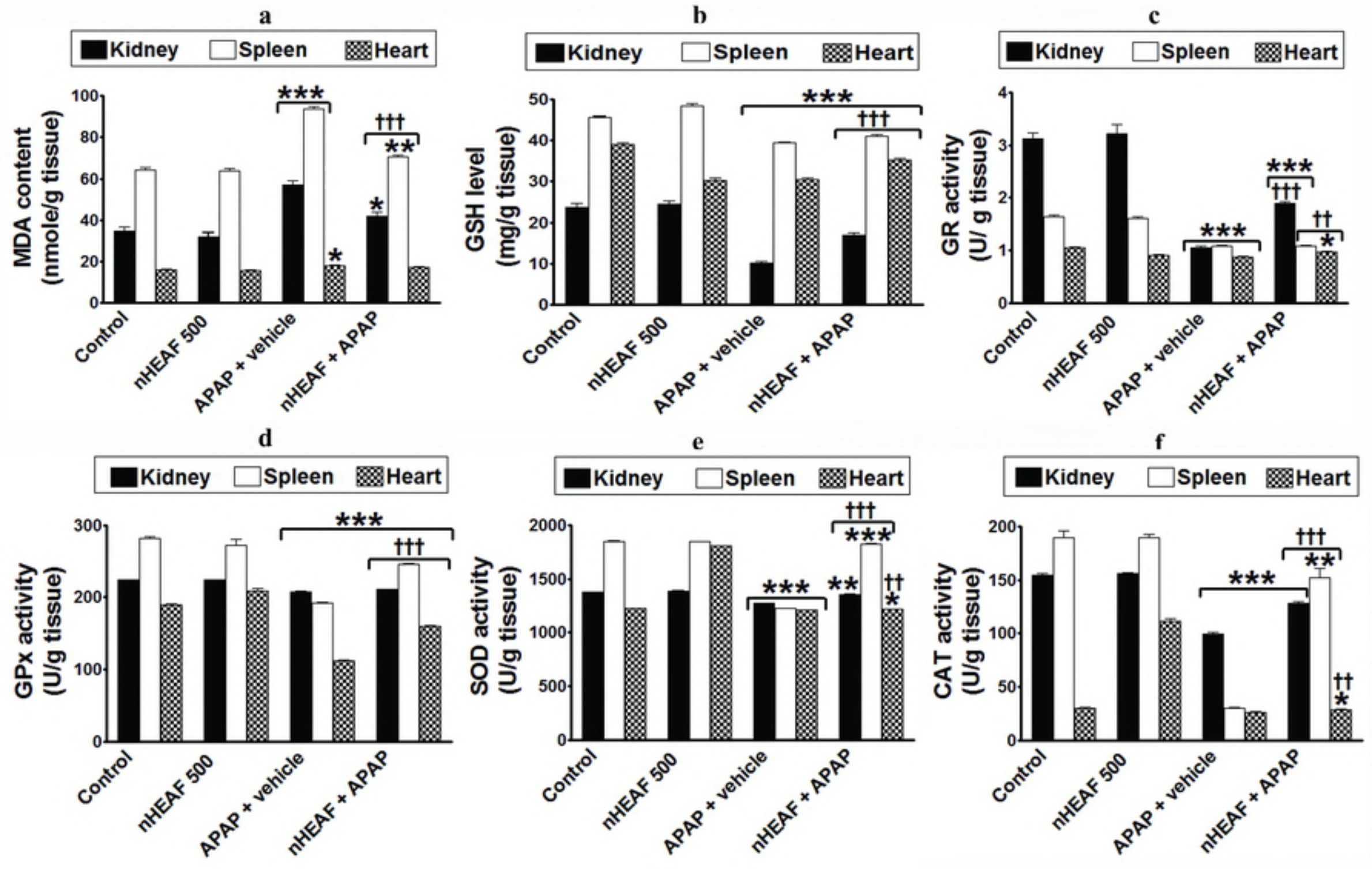

a
b.w before
b.w after
b.w gain
Body weight (g)
250
200
150
40
30
20
10
0
*
*
Control
nHEAFL 500
APAP + vehicle
nHEAFL + APAP
b
Kidney
Spleen
Heart
Organs weight (g)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
***
*
††
Control
nHEAFL 500
APAP + vehicle
nHEAFL + APAP
c
Kidney
Spleen
Heart
Relative organs weight (g/100 g b.w.)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
***
*
†††
Control
nHEAF 500
APAP + vehicle
nHEAF + APAP


a
RBCs (10^6/mm^3)
Hb (g/dL)
HCT (%)
Hematological parameters
Control
nHEAFL 500
APAP + vehicle
nHEAFL + APAP
b
MCV (fL/RBC)
MCH (pg/RBC)
MCHC (g/dL)
Blood indices
Control
nHEAFL 500
APAP + vehicle
nHEAFL + APAP
c
Neutrophils
Eosinophils
Basophils
Differential granulocytes count (10^3/mm^3)
Control
nHEAFL 500
APAP + vehicle
nHEAFL + APAP
d
Lymphocytes
Monocytes
Differential agranulocytes count (10^3/mm^3)
Control
nHEAFL 500
APAP + vehicle
nHEAFL + APAP
e
Total agranulocytes
Total agranulocytes
Total agranulo- and granulo-cytes count (10^3/mm^3)
Control
nHEAFL 500
APAP + vehicle
nHEAFL + APAP


a
ASAT (U/L)
ALAT (U/L)
markers for cellular toxicity
160
120
80
40
0
***
†††
Control
nHEAFL 500
APAP + vehicle
nHEAFL + APAP
b
Urea
Uric acid
Creatinine
markers for kidney cellular toxicity (mg/dL)
40
30
20
4
3
2
1.0
0.8
0.6
0.4
0.2
0.0
*
†
Control
nHEAFL 500
APAP + vehicle
nHEAFL + APAP


a
Kidney
Spleen
Heart
MDA content (nmole/g tissue)
100
80
60
40
20
0
***
†††
**
*
*
Control
nHEAF 500
APAP + vehicle
nHEAF + APAP
b
Kidney
Spleen
Heart
GSH level (mg/g tissue)
50
40
30
20
10
0
***
†††
Control
nHEAF 500
APAP + vehicle
nHEAF + APAP
c
Kidney
Spleen
Heart
GR activity (U/ g tissue)
4
3
2
1
0
***
***
†††
††
*
Control
nHEAF 500
APAP + vehicle
nHEAF + APAP
d
Kidney
Spleen
Heart
GPx activity (U/g tissue)
300
200
100
0
***
†††
Control
nHEAF 500
APAP + vehicle
nHEAF + APAP
e
Kidney
Spleen
Heart
SOD activity (U/g tissue)
2000
1500
1000
500
0
***
***
**
†††
††
*
Control
nHEAF 500
APAP + vehicle
nHEAF + APAP
f
Kidney
Spleen
Heart
CAT activity (U/g tissue)
200
150
100
50
0
***
**
†††
††
*
Control
nHEAF 500
APAP + vehicle
nHEAF + APAP