Biochemical appraisal of the underutilized *Hura crepitans* seed oil: functional and inflammatory responses in albino rats

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SUMMARY: *Hura crepitans* seed oil (HCSO) remains under-utilized, largely due to the scarcity in data regarding its biochemical properties. To investigate the functional and pro-inflammatory responses to HCSO, twenty-four male rats were grouped into four and received compounded diets containing 5%-HCSO; 10%-HCSO; 15%-HCSO; and 15%-AHO (as control) for eight weeks. The functional responses and the expression of pro-inflammatory cytokines and their receptors were appraised. The organ function biomarkers in rats fed with HCSO-supplemented diets were statistically similar to those of control rats, except for uric acid and creatine levels, which were significantly lower in the HCSO-fed groups, and the urea level, which was elevated in all HCSO-fed groups. Also, HCSO significantly downregulated the expression of pro-inflammatory cytokines (TNF- α , IL-1 α , IL-1 β , and IL-6) and their receptors (IL-1R and IL-6R), when compared to the control group. Our results highlight the reno- and cardio-protective potentials of HCSO, as well as its anti-inflammatory potentials.

KEYWORDS: Arachis hypogea; Hura crepitans; Inflammation; Organ function; Seed oil.

RESUMEN: *Evaluación bioquímica del aceite de semilla de* **Hura crepitans** *infrautilizado: respuestas funcionales e inflamatorias en ratas albinas.* El aceite de semilla de Hura crepitans (ASHC) sigue estando infrautilizado en gran parte debido a la escasez de datos sobre sus resultados bioquímicos. Para investigar las respuestas funcionales y proinflamatorias al ASHC, veinticuatro ratas macho se agruparon en grupos de cuatro y recibieron dietas compuestas que contenían 5%-ASHC; 10%-ASHC; 15%-ASHC y 15%-AC aceite de cacahuete (aceite de cacahuete control), durante ocho semanas. Se evaluaron las respuestas funcionales y la expresión de citocinas proinflamatorias y sus receptores. Los biomarcadores de la función de los órganos en ratas alimentadas con dietas suplementadas con ASHC fueron estadísticamente similares a los de las ratas de control, excepto por los niveles de ácido úrico y creatina, que fueron significativamente más bajos en los grupos alimentados con ASHC, y el nivel de urea, que fue elevado en todos los grupos alimentados con ASHC. Además, ASHC disminuyó significativamente la expresión de citocinas proinflamatorias (TNF- α , IL-1 α , IL-1 β e IL- β) y sus receptores (IL-1R e IL-6R), en comparación con el grupo de control. Nuestros resultados destacan los potenciales renoprotectores y cardioprotectores del ASHC, así como su potencial antiinflamatorio.

PALABRAS CLAVE: Aceite de semilla; Arachis hypogea; Función de los órganos; Hura crepitans; Inflamación.

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1. INTRODUCTION

Seeds, which are valuable sources of fats and oils, comprise an essential component of the human diet (Hao et al., 2020). Seed oils are finding more and more uses in various industries, e.g., as flavors and textures in food industries and as oleo-chemicals for petrochemical industries (Anyasor et al., 2009). Africa, like most other (sub-)tropical continents, has these seeds and nuts in abundance but is yet to wholly utilize them, owing to the scarcity in data concerning their physical, chemical, biochemical, and industrial properties (Abdulkadir et al., 2013). Nevertheless, some seed oils, like those from Arachis hypogea (groundnut) and Glycine max (soya beans), have received considerable attention and already play important roles in countries such as Nigeria (Esonu et al., 2014). However, most seeds remain underutilized, one of which is Hura crepitans (Abdulkadir et al., 2013; Hao et al., 2020).

H. crepitans L. (common name: Sandbox tree) is an evergreen, perennial and dicotyledonous plant of the spurge family (Euphorbiaceae). It has short, dark, thickly parked, and pointed spines on the trunk and branches and is often planted as shade trees in towns and villages in Nigeria (Ezeh et al., 2012). H. crepitans seeds embody a very vital source of oil, with diverse potentials. The seeds contain amino acids at levels comparable to the other utilized seeds, with even higher lysine, cysteine methionine, threonine, and histidine (Ezeh et al., 2012; Esonu et al., 2014). Moreover, the bark has been used as a traditional medicine to treat constipation, skin irritations, microbial and fungal diseases in humans and in veterinary practices (Adindu et al., 2015). In Nigeria, however, the seeds are discarded as waste since there is no definite use for the H. crepitans seed oil (Adewuyi et al., 2014). Oil from Arachis hypogea (AHO), known as groundnut oil or peanut oil, is one of the major vegetable oils, with as much as 6.05 million metric tons of AHO produced globally in 2019/20 (Akhtar et al., 2014; Arya et al., 2016). AHO is rich in essential vitamins and unsaturated fats but contains a low proportion of saturated fats; it also has good antioxidant properties (Arva et al., 2016). It is therefore not surprising that it is one of the most utilized seed oils, whereas that from H. crepitans remain under-utilized, despite boasting comparable physical and chemical properties. The physicochemical properties and fatty acid composition of the seed and seed oil of *H. crepitans* have been reported (Oyeleke *et al.*, 2012; Oyekunle and Omode, 2008). Besides, previous studies demonstrated the antimicrobial potentials of essential oil from *H. crepitans* (Abdulkadir *et al.*, 2013; David *et al.*, 2014) but left issues regarding its safety and other biochemical outcomes unexplored.

This study reports, for the first time, the functional and inflammatory responses to *H. crepitans* seed oil, in comparison with that of *A. hypogea* oil (a well-utilized oil), in a bid to fill the dearth of information regarding the biochemical characteristics of this under-utilized seed oil.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Tris salt, n-hexane, diethyl ether, heparin, Tris, EDTA, boric acid, ethidium bromide, agarose, and hydrogen chloride were obtained from British Drug Houses Chemicals Limited, Poole, England. These and all other reagents used were of the purest grade available.

2.2. Plant materials and oil extraction

H. crepitans seeds, of good quality, were collected from Covenant University in Ota, Ogun state. The seeds were handpicked, air-dried, and then preserved. The seeds were identified and authenticated at the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, with the herbarium voucher number - FUNAABH-0082. The oil was extracted from *H. crepitans* seeds using the Soxhlet extraction technique, with analytical grade n-hexane as extraction solvent (Oniya, 2017), while unadulterated A. hypogea oil was purchased from the Kurmi market in the city of Kano, Kano State, Nigeria. Both oils were stored at 25 °C, in glass vials. Yield (%) was calculated as the percentage of the weight of oil divided by the weight of the seeds (Brühl, 1997). The color and smell of the oils were determined by visual observation and sense of smell.

2.3. In-vitro assays

Peroxide value. An oil sample (1 g) was weighed into a 200-ml conical flask, followed by 25 ml of glacial acetic acid:chloroform solvent (2:1 v/v); saturated potassium iodide (1ml) was then added, and the mixture was left in the dark for 1 minute. Next, 30 ml of water were added, and the mixture was titrated with a

Composition	5% HCSO diet (g/100g)	10% HCSO diet (g/100g)	15% HCSO diet (g/100g)	15% AHO diet (g/100g)
Maize	40	40	40	40
Flour binder	10	10	10	10
Soy beans	7	7	7	7
Groundnut cake	10	10	10	10
Fish meal	9	9	9	9
W/Offal	5	5	5	5
Bone	1.4	1.4	1.4	1.4
Premix (Broiler)	2	2	2	2
Lysine	0.1	0.1	0.1	0.1
Salt (NaCl)	0.2	0.2	0.2	0.2
Methionine	0.3	0.3	0.3	0.3
A. hypogea oil	10	5	-	15
H. crepitans seed oil	5	10	15	-

TABLE 1. Composition of H. crepitans seed oil (HCSO) and A. hypogea oil (AHO) -compounded diet.

0.02 N thiosulphate solution using 5 M starch as the indicator. A blank determination was similarly carried out. Peroxide value was calculated from the equation:

Peroxide value (mEq/kg) = [100(V1 - V2)]/W

W = weight of sample (g); V1 = volume (ml) of thiosulphate solution in test; V2 = volume (ml) of thiosulphate solution in blank (Brühl, 1997).

Acid value. The acid value of the oil sample was determined by dissolving 0.20 g of oil in 2.5 ml ethanol:diethyl ether solvent (1:1 v/v) and titrating with 0.1 N potassium hydroxide (KOH) while swirling using phenolphthalein as indicator. The calculation is as follows:

Acid Value (mg KOH/g) = $[56.1 \times N \times V]/W$

N = Normality of NaOH; V = Volume (ml) of NaOH; W = Weight of sample (Brühl, 1997).

Saponification value. The Sample oil (1 g) was weighed and transferred into an Erlenmeyer flask, after which 4 mls of ethanol and 2 mls of KOH were added. The flask (equipped with a reflux condenser) was heated in a water bath for 30 minutes with occasional shaking. The flask was cooled, a few drops of phenolphthalein were added, and the excess KOH was immediately titrated with 0.5 M hydrochloric acid (HCl). A blank test was also carried out.

Saponification value (mg KOH/g) = $[(a - b) \times 28.05]/W$

a = volume (ml) of 0.5 M HCl consumed in the blank; b = volume (ml) of 0.5 M HCl consumed in the test; W= weight (g) of sample (Brühl, 1997).

2.4. Experimental animals

Twenty-four (24) male albino rats (150-170g) used in this study were obtained from a reputable animal farm in Ota, Ogun State Nigeria, housed in separate cages under ambient conditions in the animal house of our department, and served food and water *ad libitum*. This study received ethical approval from the ethics committee of the Department of Biochemistry, Federal University of Agriculture, Abeokuta (Ref No: FUNAAB/CBS/BCH/PG/14-0054). All conditions of animal experimentation conformed to the guidelines outlined by Percie du Sert *et al.* (2010).

After a two-week acclimation period, the rats were divided randomly into four groups of six animals each. The first group received a compounded diet containing 5% H. crepitans seed oil (HCSO), and the second group received a 10% HCSO-compounded diet, the third group received a 15% HC-SO-compounded diet, while the last group (serving as the control) received a 15% AHO-compounded diet. The 15% AHO group was used as the control since this group received the standard seed oil (AHO) to which HCSO was being compared. The diets, compounded as shown in table 1, were given daily for eight (8) weeks with fresh water *ad libitum*.

2.5. Sample collection

After eight weeks, the animals were sacrificed after an overnight fast under diethyl ether anaesthesia. Blood samples, collected via cardiac puncture, were centrifuged immediately for 10 minutes at 4000 rpm to obtain the plasma. Small portions of organs (liver, kidney, and heart) were stored in RNase-free water (-80 °C) for gene expression profiling.

2.6. Biochemical analysis

Liver function tests [direct and total bilirubin levels, and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT)], kidney function tests (levels of uric acid, creatinine, and urea), and heart function tests (lactate dehydrogenase (LDH) and creatine kinase activities) were determined in the plasma, using diagnostic kits obtained from Randox Laboratories Limited (Crumlin, United Kingdom).

2.7. Gene expression profiling

The gene expression profiles for pro-inflammatory cytokines and their receptors [tumor necrosis factor-alpha (TNF- α), interleukin-1alpha (IL-1 α), interleukin-1beta (IL-1 β), IL-1 receptor (IL-1R), interleukin-6 (IL-6), and IL-6 receptor (IL-6R)] were assessed using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) techniques. RNA was extracted from the organs using an Easy-Spin plus RNA mini extraction Kit (Sigma-Aldrich, Germany). The RT-PCR was carried out using the Transgen *EasyScript*® one-step RT-PCR super mix kit with gene-specific primers (Table 2). The intensity of the amplicon bands on 1% agarose was analyzed using a UV Transilluminator and the image band was quantified with Image J software (Rotimi *et al.*, 2017).

2.8. Statistical analysis

Values are expressed as mean \pm standard error of mean (SEM). The levels of homogeneity among the groups were tested using one-way analysis of variance and Tukey's test, with (p < 0.05) consid-

Genes	Sequence (5`-3`)	Template	TM (°C)
TNF-α	Forward: ATCCGAGATGTGGAACTGGC Reverse: AAATGGCAAATCGGCTGACG	NM_012675.3	55.0
IL-6	Forward: CACTTCACAAGTCGGAGGCT Reverse: AGCACACTAGGTTTGCCGAG	NM_012589.2	55.0
IL-6R	Forward: GTCAACGACACTGGGCACTA Reverse: TTCAGCGGTCCCAAGGGATA	NM_017020.3	50
IL-1R	Forward: CTATACTTGCCGCACGTCCT Reverse: AAGCACAGAACACGGCTGTA	NM_013123.3	54.4
IL-1β	Forward: GGGCCTCAAGGGGAAGAATC Reverse: ATGTCCCGACCATTGCTGTT	NM_031512.2	56.2
IL-1 α	Forward: CCATCCAACCCAGATCAGCA Reverse: TCTCCTCCCGATGAGTAGGC	NM_017019.1	55.7
β-Actin	Forward: GTCAGGTCATCACTATCGGCAAT Reverse: AGAGGTCTTTACGGATGTCAACGT	NM_031144.3	54.4

TABLE 2. Gene-specific primers

TABLE 3. Some physicochemical properties of A. hypogea oil (AHO) and H. crepitans seed oil (HCSO)

Sample	Saponification value (mg KOH/g)	Peroxide value (meqO ₂ /kg)	Acid value (mg KOH/g)	Color	Odor
AHO	185.95 ± 1.67	15.70 ± 1.44	1.29 ± 0.19	Light brown	Agreeable
HCSO	167.93 ± 3.75	10.50 ± 1.16	0.64 ± 0.02	Golden yellow	Agreeable
P value	0.011	< 0.0001	0.028	-	-

Values are mean ± standard error means of three replicates, with the level of significance determined by student's T-test.

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ered significant. For the *in-vitro* assays (two sample groups), the post-hoc test used was student's T-test. All analyses were done using GraphPad Prism (version 8.0).

3. RESULTS

3.1. In-vitro and physiochemical results

The saponification value, peroxide, and acid value of AHO were significantly (p < 0.05) higher than that of HCO by 10, 33, and 50%, respectively. HCSO had a golden-yellow color compared to light

brown for AHO. At room temperature (25 $^{\circ}$ C), the two oils were in the liquid state, and both had agreeable odors; while the observed percentage yield for HCSO and AHO were 35.28 and 32.00%, respectively (Table 3).

3.2. Effect of HCSO on liver function biomarkers compared to AHO

There was no significant difference (p < 0.05) in the assessed markers for liver function (i.e., levels of direct and total bilirubin, as well as activities of AST, ALT, and GGT) across the groups (Figure 1A-E).



FIGURE 1: Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on liver function markers in the plasma of experimental rats. The oils were compounded into the animal diets, with varying percentage compositions (5 – 15%). AST = Aspartate transaminase; ALT = Alanine transaminase; GGT = Gamma-glutamyl transferase. Bars are mean ± standard error of mean (n=6). Bars bearing different letters are significantly different (p < 0.05; one-way ANOVA and Tukey test were used to analyze the results).



FIGURE 2: Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on kidney function markers in the plasma of experimental rats. The oils were compounded into the animal diets, with varying percentage compositions (5 - 15%). Bars are mean \pm standard error of mean (n=6). Bars bearing different letters are significantly different (p < 0.05; one-way ANOVA and Tukey test were used to analyze the results).

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FIGURE 4: Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on the relative gene expression of pro-inflammatory cytokines and their receptors in the liver of experimental rats. The oils were compounded into the animal diets, with varying percentage compositions (5 – 15%). TNF- α = Tumour necrosis factor alpha; IL-1 α = Interleukin 1 alpha; IL-1 β = Interleukin 1 beta; IL-1R = Interleukin 1 receptor; IL-6 = Interleukin 6; IL-6R = Interleukin 6 receptor. Bars are mean ± standard error of mean (n=3). Bars bearing different letters are significantly different (p < 0.05; one-way ANOVA and Tukey test were used to analyze the results).

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FIGURE 5: Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on relative gene expression of pro-inflammatory cytokines and their receptors in the kidney of experimental rats. The oils were compounded into the animal diets, with varying percentage compositions (5 - 15%). TNF- α = Tumour necrosis factor alpha; IL-1 α = Interleukin 1 alpha; IL-1 β = Interleukin 1 beta; IL-1R = Interleukin 1 receptor; IL-6 = Interleukin 6; IL-6R = Interleukin 6 receptor. Bars are mean ± standard error of mean (n=3). Bars bearing different letters are significantly different (p < 0.05; one-way ANOVA and Tukey test were used to analyze the results).

3.3. Effect of HCSO on kidney function biomarkers compared to AHO

Plasma urea levels were significantly higher (p < 0.05) in all HCSO groups compared to the 15%-AHO; while both uric acid and creatinine levels were significantly (p < 0.05) decreased in the plasma of the HCSO groups compared to the 15%-AHO group (Figure 2).

3.4. Effect of HCSO on cardiac function biomarkers compared to AHO

The activities of CK and LDH were significantly (p < 0.05) lower in 10% and 15%-HCSO groups compared to the 15%-AHO; while in the 5%-HCSO group, CK and LDH enzyme activities were not significantly (p > 0.05) different from that of the 15%-AHO (Figure 3).

3.5. Effect of HCSO on relative gene expression of pro-inflammatory cytokines and their receptors in the liver of albino rats, compared to AHO

The gene expressions of TNF- α , IL-1 α , IL-1R, and IL-6R, compared to β -actin, followed a similar pattern in the liver (Figure 4A, 4B, 4C, and 4F). Compared to the 15%-AHO group, the relative expressions of these genes were significantly (p < 0.05) decreased, with the least expression of these



FIGURE 6: Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on the relative gene expression of pro-inflammatory cytokines and their receptors in the heart of experimental rats. The oils were compounded into the animal diets, with varying percentage compositions (5 – 15%). TNF- α = Tumour necrosis factor alpha; IL-1 α = Interleukin 1 alpha; IL-1 β = Interleukin 1 beta; IL-1R = Interleukin 1 receptor; IL-6 = Interleukin 6; IL-6R = Interleukin 6 receptor. Bars are mean ± standard error of mean (n=3). Bars bearing different letters are significantly different (p < 0.05; one-way ANOVA and Tukey test were used to analyze the results).

pro-inflammatory mediators observed in the 15%-HCSO group, followed by the 10%-HCSO group, and then the 5%-HCSO group (i.e., in a dose-dependent manner). The HCSO groups had downregulated expression of IL-6 compared to the 15%-AHO group, with the 15%-HCSO group having the lowest expression; while the 5% and 10%-HCSO groups had statistically similar 1L-6 expression levels (Figure 4E). No significant difference (p < 0.05) was observed in the expression of IL-1 β (relative to β -actin) across all experimental groups of animals (Figure 4C).

3.6. Effect of HCSO on relative gene expression of pro-inflammatory cytokines and their receptors in the kidney of albino rats, compared to AHO

In a similar manner, the relative expression of gene coding for the pro-inflammatory cytokines and their receptors were significantly (p < 0.05) lower in the kidneys of the HCSO groups compared to the 15%-AHO group, except for the IL-1 receptor, where the 5%-HCSO showed no significant (p > 0.05) difference from the 15%-AHO group (Figure 5). Notably, the decreased relative expressions of TNF- α , IL-6, and IL-6R followed a dose-dependent trend.

3.7. Effect of HCSO on the relative gene expression of pro-inflammatory cytokines and their receptors in the heart of albino rats, compared to AHO

Similarly, in the heart, the relative expression of the pro-inflammatory cytokines and their receptors were significantly (p < 0.05) downregulated, particularly by the 10 and 15%-HCSO diet, when compared to the 15% AHO group (Figure 6).

4. DISCUSSION

The unprecedented surge in the world's population, along with the resultant increased consumption of food and fuel, has made the search for alternative food and fuel sources a priority in the area of study. Seed oils now occupy influential positions in human diets and as alternative biofuels, owing mostly to their tolerability, availability, inexpensiveness, and a plethora of applications (Anyasor et al., 2009; Lei et al., 2018; Pachuau et al., 2019). Despite the intensifying enthusiasm to elucidate the diverse health relevance and bioactivities of these seed oils, many are still under-studied, and one of such is H. crepitans (Abdulkadir et al., 2013), partly due to lack of information regarding its biochemical properties. This study thus investigates the functional and pro-inflammatory responses to H. crepitans seed oil (HCSO), in comparison with A. hypogea oil (AHO), in a bid to fill that dearth of information.

Saponification value correlates inversely with average molecular weight (or, by extension, chainlength) of fatty acids in oil, implying that the lower the saponification value the higher the average molecular weight (or longer the fatty-acid chain) and vice-versa (Gunstone, 2009). Peroxide value is used to monitor the oxidative deterioration (rancidity) of oils. Thus, a high peroxide value may indicate increased oxidation and formation of hydroperoxides (Gordon, 2004). The acid number is a measure of the number of carboxylic acid groups, i.e., the acidity of the oil sample, and a high acid value indicates oil with a reduced quality (Kardash and Tur'yan, 2005). In-vitro evaluations revealed similar physical properties (color, odor, and yield) between HCSO and AHO. However, the saponification, peroxide, and acid values were lower in the HCSO, indicating that HCSO may be of even better quality than AHO. Moreover, the values of these chemical properties obtained for HCSO compared favorably with previous values obtained for more utilized seed oils like those of soy bean, sunflower, olive, linseed, etc. (Gunstone, 2009), which is suggestive of the quality of HCSO.

We also examined the effects of HCSO on the function of some vital organs. The liver, the largest

internal organ, performs various metabolic functions that are essential for the continuity of life. Due to its strategic position and diverse roles, it is particularly susceptible to diseases (Owojuvigbe et al., 2020). Liver disease, currently among global health issues (Byass, 2014), can be detected by carrying out liver function tests in the blood, such as levels of bilirubin and albumin, as well as the activities of some liver enzymes (Cheesbrough, 2006; Owojuyigbe et al., 2020). Bilirubin is formed from the breakdown of heme. This water-insoluble bilirubin (unconjugated or indirect bilirubin) is transported to the liver, via the blood, where it is conjugated with glucuronic acid (by glucuronosyltransferase) to form water-soluble bilirubin glucuronides (conjugated or direct bilirubin), which are then excreted via biliary excretion. Total bilirubin refers to both direct and indirect bilirubin (Cheesbrough, 2006). Liver enzymes (such as AST, ALT and GGT) are generally useful and rather sensitive markers of liver disease. Localized within the hepatocytes, these enzymes are released into circulation, following the compromise of the cell membrane (resulting from liver injury or disease) (Niemelä and Alatalo, 2010). Increased levels or activities in these biomarkers typically characterize liver damage or disease (Owojuyigbe et al., 2020). Indeed, the hepatoprotective properties of many treatments are assessed based on the reductions in the blood levels of these liver biomarkers. In this study, the activities and levels of these biomarkers in rats fed with HCSO-supplemented diets were not significantly different from that in rats fed with 15% AHO, indicating that HCSO did not damage the liver or impair its function. These findings are corroborated by Igwenyi et al. (2017), who reported decreased liver biomarkers (AST, ALT, total and direct bilirubin) following treatment of diabetic rats with H. crepitans seed extract (HCSE). They attributed these effects to its rich phytochemical constituents, such as alkaloids, carotenoids, flavonoids, etc., previously characterized by Adindu et al. (2015).

The kidney, another major organ, primarily excretes wastes from the blood and are involved in other regulatory processes. However, because they are metabolically active and receive a quarter of cardiac output (despite weighing below 1% of total body weight); while also filtering out water from the filtrate (and may thus concentrate and accumulate toxic substances), the kidneys are particularly vulnerable to

injury (Gheshlaghi, 2012; Shaterzadeh-Yazdi et al., 2018). Clinically relevant biomarkers of renal damage include urea, uric acid, and creatinine. Creatinine is produced by the muscle during unaltered catabolism excreted by the kidney, and may thus serve as an important criterion for kidney function. Urea, a waste product from dietary protein, is also filtered into the urine by the kidneys (Burns and Wortmann, 2011). Uric acid, a product of purine metabolism, is a normal component of urine produced in conditions where there is cellular destruction and thus, degradation of the nuclear material and release of purine bases. These purines are, via a series of reactions, converted into either hypoxanthine or xanthine, which are then broken down by xanthine oxidase into uric acid. The excessive build-up of uric acid results in gout (Kanwal et al., 2018). However, these biomarkers (creatinine, urea, and uric acid) are liberally filtered by the glomerulus and efficiently excreted via the urine with negligible metabolism by the kidney. Thus, increased levels in the blood, in most cases, may evince the onset of kidney failure (Burns and Wortmann, 2011). While this current study did not carry out urinalysis, in light of the observed significantly (p < 0.05) reduced plasma levels of uric acid and creatinine in all HCSO groups compared to the AHO group, we hypothesize that HCSO promotes renal clearance of these excretory products, suggestive of its reno-beneficial potentials. Interestingly, the levels of urea were significantly (p < 0.05)higher in rats fed with the HCSO-supplemented diet compared to those fed the AHO supplemented diet. We attribute this elevation in urea level to the high amino acid content of the H. crepitans seed oil (Esonu et al., 2014), especially glutamate (about 14.41 g/100 g protein), as per the reports of Fowomola and Akindahunsi (2007). Thus, the increased urea level may be a consequence of increased amino acid catabolism, which obligates increased urea production (Higgins, 2016).

The heart, a muscular organ, pumps blood that carries nutrients and oxygen to other parts of the body and metabolic waste away from these parts through the blood vessels of the circulatory system (Gaze, 2012). Clinically relevant cardiac tests include CK and LDH activities in the plasma, which are quantifiable markers of the health/disease condition of the heart. CK couples the phosphorylation of creatine to phosphocreatine (PCr) with the dephosphorylation

of ATP to ADP. PCr serves as an *in-situ* energy store for the swift regeneration of ATP. Creatine kinase (CK) is primarily cytosolic and is examined as a damage biomarker of CK-rich tissues, like the heart (Moghadam-Kia et al., 2016). LDH, another cytoplasmic enzyme, converts pyruvate to lactate during anaerobic respiration and is extensively expressed in metabolically active tissues like the heart (Hu et al., 2015). Following cardiac injury/damage, these cytosolic enzymes are released into the blood. Thus, elevated activities correlate positively with various heart diseases (Hu et al., 2015; Moghadam-Kia et al., 2016). Our results showed that the CK and LDH activities in rats fed the HCSO-supplemented diet were not significantly different from those obtained from rats fed with AHO. From these results, the HCSO did not provoke any significant damage to the organs (kidney, liver, or heart), at least in comparison with AHO.

Gene analyses revealed a dose-dependent inhibition of the relative expression of genes coding for pro-inflammatory cytokines (TNF- α , IL-1 α , IL-1 β , and IL-6) and receptors (IL-1R and IL-6R) in the HCSO-fed groups compared to the 15%-AHO group. To the best of our knowledge, this current study is the first to provide data regarding the in-vivo anti-inflammatory effects of H. crepitans seed oil following oral supplementation. The previously available study on the anti-inflammatory effect of H. crepitans focused on its hexane and ethyl acetate extracts and used topical application on rat paws (Avoseh et al., 2018). Although inflammation is a defence mechanism in response to noxious stimuli, e.g., infectious agents, irritants, damaged tissues, etc., if left uncontrolled, it quickly becomes damaging, and this is a contributing factor to the pathogenesis of a plethora of chronic inflammatory diseases (Chen et al., 2018). During the inflammatory response, immune cells are typically activated and they, in turn, secrete cytokines that initiate inflammatory pathways. These pro-inflammatory cytokines (e.g., $TNF-\alpha$, IL-1 β , and IL-6) facilitate inflammation via interaction with the receptors of TNF (TNFR), IL-1 (IL-1R), IL-6 (IL-6R), as well as the Toll-like receptors (TLRs). Following activation, the receptor initiates intracellular signalling cascades, such as the nuclear factor kappa-B (NF-kB), mitogen-activated protein kinase (MAPK), activator of transcription (STAT), and Janus kinase (JAK)-signal transducer cascades (Zhang *et al.*, 2019). These cascades play major roles in the development of many leading causes of death, like cancer, cardiovascular diseases, chronic obstructive pulmonary disease, diabetes, etc. (WHO, 2020).

The suppression of inflammatory mediators by HCSO, in a dose-dependent manner, clearly evinces its anti-inflammatory properties. It remains to be seen if these properties will prove beneficial in different models of disease conditions. Previous studies have characterized different parts of H. crepitans and enumerated various inherent phytochemical constituents, such as the nitrogen-containing alkaloids, aromatic ring-containing phenolics, and isoprene-containing terpenoids (Oyekunle and Omode, 2008; Oyeleke et al., 2012; Adindu et al., 2015). These phytochemicals have been reported to possess antioxidant and anti-inflammatory properties (Chen et al., 2018; Zhu et al., 2018) and may account for the observed effects of HCSO on organ function and inflammatory markers, and even contribute to the lower saponification, peroxide, and acid values obtained for HCSO.

CONCLUSIONS

Our results demonstrate that HCSO did not significantly affect the function of major organs, nor did it provoke adverse inflammatory responses. Instead, it suppressed the expression of pro-inflammatory mediators. Having characterized some of the biochemical effects of HCSO, further research may investigate its potential beneficial effects on various disease states, particularly those involving inflammation.

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