

# Unravelling the Potential of Trans-3-Hydroxycinnamic Acid on Metabolic Indices, Biochemical, and Haematological Parameters in Rats with Metabolic Disturbances

Aziz ASA<sup>a</sup>, Idris BA<sup>a</sup>, Suliman NA<sup>a</sup>, Bakar NAA<sup>a</sup>, Mohamed M<sup>b</sup>, Zakaria Z<sup>b</sup>, Othman ZA<sup>a</sup>

<sup>a</sup>Department of Anatomy and Physiology, Faculty of Medicine, Universiti Sultan Zainal Abidin, Jalan Sultan Mahmud, Terengganu, Malaysia.

<sup>b</sup>Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia.

## ABSTRACT

**INTRODUCTION:** Metabolic disturbances caused by high-calorie diets contribute significantly to global mortality. *Trans-3-hydroxycinnamic acid* (HCA), a bioactive antioxidant, may offer protective effects against such imbalances. This study evaluated the metabolic, biochemical, and haematological effects of HCA in rats fed a high-fat, high-fructose (HFHF) diet. **MATERIALS AND METHODS:** Male Sprague Dawley rats aged 7-8 weeks were fed with HFHF diet for 6 consecutive weeks. Rats with metabolic disturbances received HCA at 15 mg/kg, 30 mg/kg, or 60 mg/kg, and were continuously fed with HFHF diet for another 6 weeks. Normal control rats received normal pellets. The effects of HCA on metabolic indices, biochemical, and haematological parameters were evaluated. **RESULTS:** HCA treatment for 6 weeks ameliorates metabolic indices, including weight gain, Lee obesity index, abdominal and thoracic circumferences, TC/HDL ratio, glucose, and fat pad deposition. Improved AST and ALT levels indicated the hepatoprotective effect of HCA, supported by decreased fat vacuolation. The most significant effects were observed at 30 mg/kg. No significant haematological or renal toxicity was detected. **CONCLUSION:** Our results indicated HCA at 30 mg/kg is the optimum dose that exerts its ameliorative effects in reducing metabolic indices, along with no severe biochemical and haematological changes.

## Keywords

trans-3-hydroxycinnamic acid, metabolic indices, biochemical, haematological, metabolic disturbance

## Corresponding Author

Dr. Zaidatul Akmal Othman  
Department of Anatomy and Physiology,  
Faculty of Medicine, Universiti Sultan Zainal  
Abidin, Jalan Sultan Mahmud,  
Terengganu, Malaysia.  
E-mail: zaidaakmal@unisza.edu.my

Received: 2<sup>nd</sup> October 2025; Accepted: 23<sup>rd</sup>  
February 2026

Doi: <https://doi.org/10.31436/imjm.v25i03/3089>

## INTRODUCTION

Metabolic disease is a constellation of metabolic risk factors, including obesity, hyperlipidaemia, elevated glucose associated with insulin resistance, and elevated blood pressure.<sup>1</sup> The incidence is escalating rapidly, hence become the main priority among healthcare professionals due to its substantial health implications and fatality risk. The pathological state may develop into hepatic steatosis, atherosclerotic cardiovascular disease, kidney failure, gallbladder stones, and cancers.<sup>2</sup> A high-calorie diet coupled with a sedentary lifestyle has significantly contributed to increasing metabolic indices pertaining to body weight, lipid profiles, glucose, and insulin levels.<sup>3,4</sup>

Given the substantial morbidity and mortality burden related to metabolic disease complications, which show no abating signs, there is significant interest in identifying novel compounds with high pharmacological targets that could be effective in reducing the complications of metabolic disease. At present, treatment of metabolic disease relies on multiple drugs, which have multiple side effects.<sup>5</sup> Hence, a safer, cost-effective agent needs to be explored. Phenolic compounds have been exploited to promote health and serve as a potential source for discovering new therapeutic or preventive drugs.<sup>6,7</sup>

*Trans-3*-hydroxycinnamic acid (HCA), also known as *m*-coumaric acid, is one of the naturally occurring polyphenols found in a variety of plant-based foods. We previously found a high composition of *trans-3*-HCA in fermented bee bread harvested from the *Heterotrigona itama* bee species.<sup>8</sup> HCA exhibits potent antioxidant capacity by scavenging free radicals via its phenolic hydroxyl group and has demonstrated a positive effect in modulating glucose and lipid metabolism.<sup>9,10,11</sup> However, the *in vivo* evidence regarding the metabolic benefits of HCA remains sparse and inconsistent, primarily due to variations in animal models, dosage regimens, and treatment durations. Therefore, the present study was designed to evaluate the optimal dose of *trans-3*-HCA and assess its ameliorative effects on the metabolic indices, biochemical, and haematological parameters in rats fed a HFHF diet. The findings aim to provide foundational data supporting the potential therapeutic application of HCA in managing metabolic disturbances.

## MATERIAL AND METHODS

### Preparation of hydroxycinnamic acid

HCA with >98% purity was purchased from Sigma-Aldrich (France) and freshly dissolved in saline solution prior to each administration. A certificate of analysis was obtained from the supplier.

### Preparation of high-fat high-fructose (HFHF) diet

The HFHF diet was formulated using a mixture of 62% normal pellet (Gold Coin, China), 32% animal ghee (Cripto, Malaysia), 6% powdered milk, 12% cholesterol powder (Nacalai Tesque, Japan), and 300 IU of vitamin C and D (Eurobio Biocal D Complex, Malaysia). This formulation provided 560.09 kcal/100g and consisted of 11.93% protein, 40.47% fat, 37.03% carbohydrate, 6.68% moisture, and 3.89% ash. A 10% fructose drink was prepared weekly in a dilution with distilled water. The HFHF diet used in this study was a modified high-fat diet with increased caloric density, adapted from our previously established obesity model.<sup>12</sup>

### Animals

Twenty-five male Sprague-Dawley rats (7-8 weeks old),

weighing 220-250 g, were obtained from the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia, Kelantan. Each rat was housed separately in a polypropylene cage with a 12-hour light-dark cycle, a regulated temperature of 22±2 °C, and a controlled humidity level of 55±10%. All experimental procedures were approved by the UniSZA Animal and Plant Research Ethics Committee (UAPREC/008/010), and followed the guidelines provided by the Faculty of Medicine, Universiti Sultan Zainal Abidin, and the National Institute of Health (NIH), Malaysia.

### Experimental design

Following a one-week acclimatisation period, rats were fed either a normal pellet (*n*=5) or a high-fat high-fructose (HFHF) diet plus 10% fructose in drinking water (*n*=20) for 6 weeks. Metabolic disturbance was confirmed by increases in the Lee obesity index, total cholesterol (TC), and glucose, and by an abnormal total area under the curve following an oral glucose tolerance test. Rats with metabolic disturbances were randomly allocated into four groups, with the first group as a positive control group. Another three groups of rats were administered HCA at 15 mg/kg, 30 mg/kg, or 60 mg/kg via oral gavage for 6 weeks. All groups were allocated five animals each as follows.

- The normal Control group was fed with normal pellets and administered 1 mL of distilled water via oral gavage.
- The metabolic disturbances (MD) group was fed with a high-fat diet and 10 % fructose solution (HFHF diet), and 1 mL of distilled water.
- The MD+HCA1 group was fed with the HFHF diet and received HCA at 15 mg/kg body weight.
- The MD+HCA2 group was fed with the HFHF diet and received HCA at 30 mg/kg body weight.
- The MD+HCA3 group was fed with the HFHF diet and received HCA at 60 mg/kg body weight.

HCA doses were selected based on the previous study.<sup>13</sup> Body weight of the rats was monitored weekly throughout the experimental period. Body weight changes were recorded after the differences between the

initial and final body weights of the rats.

### **Blood collection and tissue sampling**

The animals were anaesthetised with ketamine 90 mg/kg and xylazine 5 mg/kg via intraperitoneal injection after 16 hr of fasting period. Blood samples were collected from the posterior vena cava and centrifuged at 4000 rpm for 15 min. The obtained serum was aliquoted and stored at  $-80^{\circ}\text{C}$  for subsequent biochemical analysis. The adipose tissue from the abdominal, epididymal, retroperitoneal, pericardial, and perirenal regions was carefully excised and weighed in grams (g). The liver, kidney, heart, and aorta samples were dissected and preserved in 10% formalin for histopathological examination.

### **Anthropometrical analysis**

The body weight of the rats was measured weekly using a digital weight balance. Naso-anal length was determined by measuring the longitudinal axis of the naso-anal. The abdominal circumference was determined by the circumference length of the midpoint between the anterior and posterior legs. The thoracic circumference was determined by measuring the circumference length immediately behind the foreleg region. The Lee obesity index was determined by dividing the cubic root of body weight (g) by the naso-anal length (cm).<sup>14</sup>

### **Biochemical analysis**

#### **Determination of oral glucose tolerance test**

Rats were fasted for 16 hr overnight and orally loaded with glucose at 2 g/kg/bw. Blood glucose was determined from the acquired blood sample of the tail vein using a glucometer (Accu-Chek, Malaysia) before (at 0 min) and after glucose loading (at 30, 60, 90, and 120 min). A total of glycaemic responses to OGTT were determined from the calculation of total area under the curve (AUC) following completion of 120 min observation period using the trapezoidal method.<sup>15</sup>

#### **Determination of serum lipid profile**

The protocols for serum total cholesterol (TC) and triglyceride (TG) levels were performed via an enzymatic

colorimetric method according to the provided commercialised kit protocol provided (ARCHITECT c kit, Abbott, IL, USA). Low-density lipoprotein (LDL) levels were determined using the formula provided by the previous protocol:  $\text{LDL (mmol/L)} = (\text{TC} - \text{HDL} - (\text{TG}/5))$ .<sup>16</sup> High-density lipoprotein (HDL) was determined after removal of LDL cholesterol, chylomicron, and VLDL cholesterol.

#### **Determination of liver function and renal function tests**

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined according to the method provided by the International Federation of Clinical Chemistry (IFCC) using standard spectrophotometric methods (Abbott Architect Ci8200, Abbott Park, IL, USA). The renal function test was evaluated using a standard calibrator machine, which comprised several methods involving an indirect ion-selective electrode, the urease method, phosphomolybdate, and uricase.

#### **Determination of serum full blood count**

The Multi-Angle Polarized Scatter Separation (MAPSS) system, a flow cytometric technology, was used to assess haematological parameters. This method characterises red blood cells (RBC), platelets (PLT), white blood cells (WBC), and nucleated other cells (NOC) in detail.

#### **Histological analysis**

The liver, heart, kidney, and aorta were stored in 10% formaldehyde solution. The grossed tissues were serially processed in an automated processor and embedded with paraffin wax into a block. The sample blocks were serially sectioned (5  $\mu\text{m}$ ) using a microtome and mounted on slides before being stained with Haematoxylin and Eosin. The images were analysed under an optical microscope (Olympus, Tokyo, Japan).

#### **Statistical analysis**

All the data obtained from the experimental findings are provided as mean (S.E.M). Data were statistically measured with GraphPad Prism 9.0. One-way ANOVA

with post-hoc Tukey test was applied to analyse the mean differences between multiple groups. A *P* value of less than 0.05 is considered to be statistically significant.

## RESULTS

### Effects of *trans*-3-hydroxycinnamic acid on anthropometrical parameters

As shown in Table I, rats in the MD group exhibited significantly higher weight gain compared to the Control group after 12 weeks of the HFHF diet. Following 6 weeks of HCA treatment, the MD+HCA1 and MD+HCA2 groups showed significant reductions in body weight gain compared with the MD group. Additionally, the Lee obesity index, abdominal and thoracic circumferences of the MD group were significantly higher than those of the Control group, indicating the successful induction of obesity. Administration of HCA, particularly at doses of 15 and 30 mg/kg, significantly decreased these parameters, suggesting attenuation of HFHF-induced adiposity.

**Table I:** Effects of *trans*-3-hydroxycinnamic acid on anthropometrical parameters.

Parameter	Control	MD	MD+HC A1	MD+HC A2	MD+HC A3
Body weight gain	185.72 (6.40)	245.93 (2.55)*	153.62 (2.82)**	179.28 (12.36)**	210.61 (9.36)***
Rate of body weight gain	0.38(0.02)	0.47(0.04)*	0.41(0.00)*	0.38(0.02)*	0.48(0.03)
Lee obesity index	321.76 (1.98)	369.91 (8.39)*	319.06 (9.26)**	329.77 (8.33)**	343.70 (4.85)
Abdominal circumference	17.75 (0.75)	20.17 (0.93)*	17.50 (0.50)**	18.00 (1.00)**	19.00 (0.58)
Thoracic circumference	17.00 (0.20)	18.38 (0.43)*	16.67 (0.33)**	17.00 (0.29)**	17.33 (0.73)

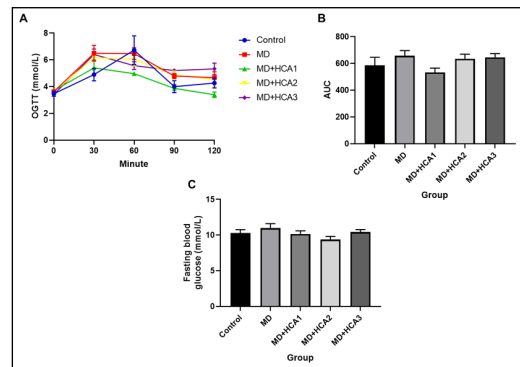
Data are presented as means (S.E.M.), *n*=5/group. Statistical analysis performed using ANOVA with Tukey's post hoc test for multiple comparisons. \* *p*<0.05 in comparison with Control group, \*\* *p*<0.05 in comparison with MD group, \*\*\* *p*<0.05 in comparison to MD+HCA1 group. Control: rats fed with normal pellet, MD: metabolic disturbance rats fed with high-fat high-fructose diet, MD+HCA1: metabolic disturbance rats with HCA at 15 mg/kg, MD+HCA2: metabolic disturbance rats with HCA at 30 mg/kg, MD+HCA3: metabolic disturbance rats with HCA at 60 mg/kg. MD, metabolic disturbances; HCA, *trans*-3-hydroxycinnamic acid.

### Effects of *trans*-3-hydroxycinnamic acid on oral glucose tolerance test (OGTT) and fasting serum glucose

The OGTT was performed in the final week of the experimental period. No significant changes were observed in the area under the curve and fasting blood glucose levels across all experimental groups.

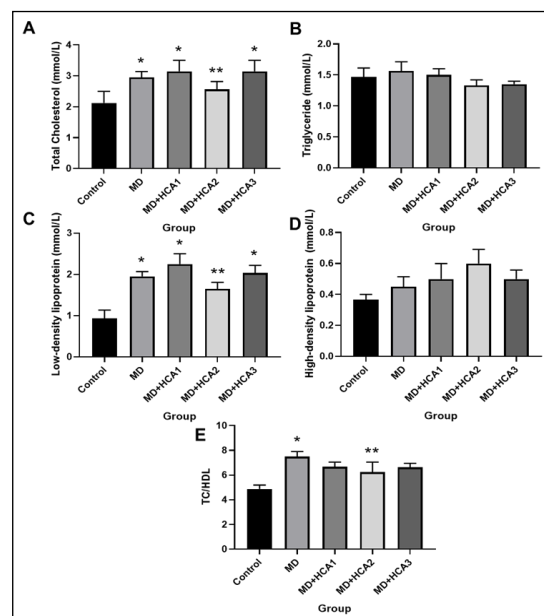
### Effects of *trans*-3-hydroxycinnamic acid on lipidomic profile

Figure 2 illustrates the lipidomic profiles of all experimental groups. Total cholesterol and low-density



**Figure 1.** Effects of *trans*-3-hydroxycinnamic acid on oral glucose tolerance test and fasting blood glucose in metabolic disturbance rats. Data are expressed as mean with S.E.M. (*n*=5). Statistical analysis performed using ANOVA with Tukey's post hoc test for multiple comparisons. AUC: area under the curve, Control: rats fed with normal pellet, HCA, *trans*-3-hydroxycinnamic acid, MD: metabolic disturbance rats fed with high-fat high-fructose diet, MD+HCA1: metabolic disturbance rats with HCA at 15 mg/kg, MD+HCA2: metabolic disturbance rats with HCA at 30 mg/kg, MD+HCA3: metabolic disturbance rats with HCA at 60 mg/kg, OGTT: oral glucose tolerance test. MD, metabolic disturbances.

lipoprotein in the MD, MD+HCA1, and MD+HCA2 groups were significantly higher than in the Control group. Conversely, the MD+HCA2 group showed a marked suppression of these lipid markers (Figure 2A, 2C). Additionally, the MD group showed a significant increase in TC/HDL ratio when compared to the normal Control group, with a significant reduction elicited in the MD+HCA2 group (Figure 2E). Meanwhile, no significant changes were observed in TG levels across all experimental groups (Figure 2B).



**Figure 2.** Effects of *trans*-3-hydroxycinnamic acid on lipidomic profile in metabolic disturbance rats. Data are expressed as mean with S.E.M. (*n*=5). Statistical analysis performed using ANOVA with Tukey's post hoc test for multiple comparisons. \* *p*<0.05 in comparison with Control group, \*\* *p*<0.05 in comparison with MD group. Control: rats fed with normal pellet, MD: metabolic disturbance rats fed with high-fat high-fructose diet, MD+HCA1: metabolic disturbance rats with HCA at 15 mg/kg, MD+HCA2: metabolic disturbance rats with HCA at 30 mg/kg, MD+HCA3: metabolic disturbance rats with HCA at 60 mg/kg. MD, metabolic disturbances; HCA, *trans*-3-hydroxycinnamic acid.

## Effects of *trans*-3-hydroxycinnamic acid on full blood count

Table 2 exhibited notable variations in full blood count parameters across all groups. Haemoglobin concentration was highest in the Control group, but consistently decreased across metabolic groups, with a significantly notable value observed in the MD+HCA3 group compared to the Control group. A similar trend was also seen for MCV value in the MD+HCA3 group. Other parameters were negligible across all groups.

**Table II:** Effects of *trans*-3-hydroxycinnamic acid on full blood count

Parameter	Control	MD	MD+HC A1	MD+HC A2	MD+HC A3
Total RBC ( $\times 10^{12}$ /L)	7.17(0.09)	7.30(0.30)	7.55(0.35)	7.67(0.13)	6.67(0.99)
Haemoglobin (gm/L)	141.25 (2.95)	131.33 (2.85)	130.00 (1.00)	130.33 (2.40)	129.67 (0.67)*
PCV (L)	0.36(0.01)	0.34(0.00)	0.34(0.01)	0.34(0.01)	0.30(0.04)
MCV (fl)	50.00(0.58)	46.00 (0.58)	46.00 (1.00)	45.75 (1.25)	45.33 (0.88)*
MCH (pg)	20.00(0.58)	18.00 (1.00)	17.50 (0.50)	17.00 (0.00)	20.33 (3.33)
MCHC (g/L)	393.33 (8.82)	385.00 (5.00)	380.00 (10.00)	376.67 (3.33)	446.67 (66.67)
RDW (%)	14.43(0.33)	14.70 (0.10)	15.30 (0.20)	15.33 (0.62)	13.97 (1.26)
WBC ( $\times 10^9$ / L)	12.07(0.61)	10.40 (1.80)	10.80 (1.60)	9.73(1.13)	8.55(1.39)
Polymorphs (%)	16.33(3.93)	18.00 (2.00)	21.00 (4.00)	17.67 (4.67)	20.67 (6.23)
Lymphocytes (%)	78.33(2.73)	77.00 (3.00)	72.00 (2.00)	76.00 (3.61)	73.33 (6.17)
Monocytes (%)	5.00(1.00)	5.00(1.00)	7.00(1.00)	6.00(1.00)	5.67(0.33)
Eosinophils (%)	0.33(0.33)	0.00(0.00)	0.50(0.50)	0.33(0.33)	0.33(0.33)
Basophils (%)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
Platelet Count $\times 10^9$ /L	1083.00 (101.49)	1064.50 (150.00)	969.00 (226.00)	1108.00 (30.66)	844.67 (263.44)

Data are presented as means (S.E.M.),  $n=5$ /group. Statistical analysis performed using ANOVA with Tukey's post hoc test for multiple comparisons. \*  $p<0.05$  in comparison with the control group. Control: rats fed with normal pellet, MD: metabolic disturbance rats fed with high-fat high-fructose diet, MD+HCA1: metabolic disturbance rats with HCA at 15 mg/kg, MD+HCA2: metabolic disturbance rats with HCA at 30 mg/kg, MD+HCA3: metabolic disturbance rats with HCA at 60 mg/kg. MD, metabolic disturbances; HCA, *trans*-3-hydroxycinnamic acid.

## Effects of *trans*-3-hydroxycinnamic acid on liver function test and renal profile

Table 3 summarises the effects of various doses of HCA on liver function tests and renal profile. Significant elevations in AST, ALT, and ALP levels were observed in the MD group compared with the Control group. Treatment with HCA2 and HCA3 substantially attenuated the AST levels. A notable reduction in ALP level was observed only in the HCA2 group. Whereas a substantial reduction of the elevated ALT was also observed in the HCA1 and HCA2 groups. The data on biochemical levels of renal profile indicated no significant alterations compared to the Control group.

**Table III:** Effects of *trans*-3-hydroxycinnamic acid on liver function test and renal profile in metabolic disturbance rats.

Parameter	Control	MD	MD+HC A1	MD+HCA 2	MD+HC A3
Total protein (g/L)	62.33(1.20)	63.00 (1.53)	67.33 (2.85)	65.00 (2.08)	63.33 (1.20)
Albumin (g/L)	25.33(0.88)	27.00 (0.58)	27.67 (1.33)	28.67 (0.33)	26.67 (0.33)
Globulin (g/L)	40.00(0.00)	36.67 (3.33)	40.00 (0.00)	36.67 (3.33)	40.00 (0.00)
AG Ratio	0.63(0.03)	0.77 (0.07)	0.67 (0.03)	0.77 (0.07)	0.67 (0.03)
AST (U/L)	128.00 (7.09)	168.00 (13.99)*	159.67 (25.50)	124.00 (3.79)**	108.67 (16.60)**
ALT (U/L)	54.80(4.89)	109.67 (3.53)*	115.00 (26.84)*	109.67 (6.06)*	94.67 (13.68)
ALP (U/L)	126.33 (16.70)	384.20 (60.01)*	356.67 (45.11)	295.00 (45.70)**	365.50 (29.50)
Sodium (mmol/L)	140.00 (0.58)	140.33 (0.88)	140.00 (0.88)	140.33 (1.20)	142.00 (1.00)
Potassium (mmol/L)	5.80 (0.38)	5.43 (0.38)	6.27 (0.88)	5.03 (0.20)	4.87 (0.33)
Chloride (mmol/L)	103.00 (1.00)	102.33 (0.88)	104.00 (0.58)	104.00 (0.58)	105.00 (0.58)
Urea (mmol/L)	7.13 (0.14)	3.60 (0.61)	3.57 (0.57)	3.80 (0.10)	3.73 (0.42)
Creatinine ( $\mu$ mol/L)	51.33 (1.45)	55.33 (1.76)	52.67 (5.78)	50.33 (3.18)	49.00 (1.73)
Uric acid ( $\mu$ mol/L)	108.00 (21.63)	92.00 (17.09)	104.00 (17.44)	84.00 (3.46)	82.00 (5.29)

Data are presented as means (S.E.M.),  $n=5$ /group. Statistical analysis performed using ANOVA with Tukey's post hoc test for multiple comparisons. \*  $p<0.05$  in comparison with Control group, \*\*  $p<0.05$  in comparison with MD group. Control: rats fed with normal pellet, MD: metabolic disturbance rats fed with high-fat high-fructose diet, MD+HCA1: metabolic disturbance rats with HCA at 15 mg/kg, MD+HCA2: metabolic disturbance rats with HCA at 30 mg/kg, MD+HCA3: metabolic disturbance rats with HCA at 60 mg/kg. MD, metabolic disturbances; HCA, *trans*-3-hydroxycinnamic acid.

## Effects of *trans*-3-hydroxycinnamic acid on adipose tissue deposition

Table 4 revealed notable differences in various adipose tissue deposition among experimental groups. Abdominal, epididymal, retroperitoneal, and perirenal fat pads were significantly higher in the MD group compared with the Control group. Co-treatment with HCA1 and HCA2 markedly mitigated these effects. The MD+HCA1 group showed a substantial decrease in abdominal, epididymal, and retroperitoneal fat pads when compared to the MD group. Similarly, the MD+HCA2 group also demonstrated a significant reduction in these fat depositions, with extensive reduction of perirenal fat when compared to the MD group. Whereas, the MD+HCA3 group exhibited a significant reduction in retroperitoneal fat deposition when compared to the MD group and did not achieve statistical significance in all areas. Differences in pericardial fat were minimal across all experimental groups.

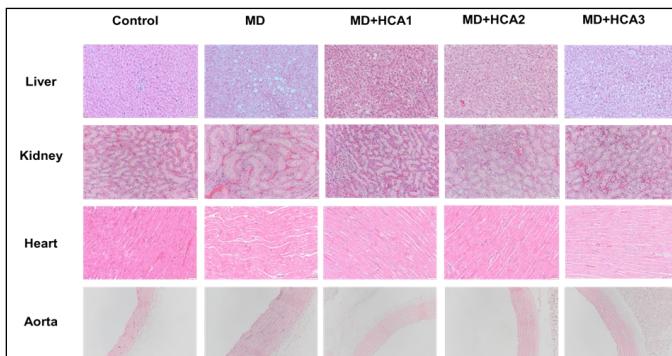
**Table IV:** Effects of *trans*-3-hydroxycinnamic acid on adipose tissue deposition

Parameter	Control	MD	MD+HCA1	MD+HCA2	MD+HCA3
Abdominal (g)	4.13 (1.07)	11.37 (1.48)*	4.478(0.63)**	6.46(0.28)**	7.57(1.09)
Epididymal (g)	4.47 (0.85)	7.91(0.52)*	4.08(0.05)**	4.44(0.50)**	5.50(0.64)
Retroperitoneal (g)	0.48 (0.15)	1.42(0.16)*	0.51(0.16)**	0.54(0.08)**	0.67(0.09)**
Pericardial (g)	0.15 (0.05)	0.34(0.09)	0.18(0.05)	0.19(0.03)	0.18(0.04)
Perirenal (g)	0.16 (0.04)	0.55(0.11)*	0.21(0.09)	0.19(0.07)**	0.20(0.05)

Data are presented as means (S.E.M.),  $n=5$ /group. Statistical analysis performed using ANOVA with Tukey's post hoc test for multiple comparisons. \*  $p<0.05$  in comparison with Control group, \*\*  $p<0.05$  in comparison with MD group. Control: rats fed with normal pellet, MD: metabolic disturbance rats fed with high-fat high-fructose diet, MD+HCA1: metabolic disturbance rats with HCA at 15 mg/kg, MD+HCA2: metabolic disturbance rats with HCA at 30 mg/kg, MD+HCA3: metabolic disturbance rats with HCA at 60 mg/kg. MD, metabolic disturbances; HCA, *trans*-3-hydroxycinnamic acid.

### Effects of *trans*-3-hydroxycinnamic acid on histopathological analysis

The liver, kidney, heart, and aorta in the Control group demonstrated a normal arrangement of histological architecture. The MD group showed a series of morphological alterations with multiple fat vacuoles in the liver, tubular dilatation in the renal cortex, disarray and hypertrophy of the myocardium, and rough endothelial surface of the aorta. Whereas these metabolic alterations seem to be ameliorated in HFHF diet rats that received HCA at various doses (Figure 3).



**Figure 3.** Illustrates the effects of *trans*-3 hydroxycinnamic acid on histological analysis of liver, kidney, heart, and aorta on Haematoxylin and Eosin staining, at x100 magnification (scale). The MD group exhibited morphological alterations, including fat vacuolation in the liver, tubular dilatation in the renal cortex, myocardial disarray and hypertrophy, and a roughened endothelial surface of the aorta. Control: rats fed with normal pellet, MD: metabolic disturbance rats fed with high-fat high-fructose diet, MD+HCA1: metabolic disturbance rats with HCA at 15 mg/kg, MD+HCA2: metabolic disturbance rats with HCA at 30 mg/kg, MD+HCA3: metabolic disturbance rats with HCA at 60 mg/kg. MD, metabolic disturbances; HCA, *trans*-3-hydroxycinnamic acid.

### DISCUSSION

Metabolic disease is a worldwide problem, affecting most developed and industrialised countries.<sup>17</sup> The alteration in metabolic parameters is associated with high-fat diet intake, thus implicated in cellular and biochemical changes that deviate from the normal body homeostasis.

These resulted in increased metabolic-related complications such as obesity, diabetes mellitus, hypertension, and hyperlipidaemia.<sup>18</sup> In the present study, the HFHF diet was administered for 12 weeks, with a concomitant HCA treatment delivered during the latter 6 weeks of the experimental period. We aimed to determine the ameliorative effects of HCA, a potential bioactive compound, on metabolic indices, biochemical, and haematological parameters in rats fed with the HFHF diet.

In the present study, rats fed with an HFHF diet for 12 weeks developed notable signs of metabolic disturbances, as evidenced by significant increases in body weight gain and Lee obesity index, as demonstrated in the MD group. This was associated with the significant increase in abdominal and thoracic circumferences, which serve as a key indicator for central obesity. The obesogenic effects are further supported by increased adiposity in abdominal, epididymal, retroperitoneal, and peri-renal regions. These findings are in agreement with a previous study showing that a prolonged high-calorie and high-fat diet can contribute to the accumulation of total and visceral fat mass, with ectopic deposition in the liver, heart, and muscle.<sup>19</sup> Furthermore, the high calories provided by the HFHF diet used in the present study could promote rapid lipid accumulation and systemic metabolic disturbances. These obesogenic effects could also be attributed to prolonged fructose intake that stimulates the lipogenic molecules through the activation of sterol regulatory element-binding protein 1 (SREBP-1) and carbohydrate-responsive element-binding protein (ChREBP), enhancing TG synthesis and fat accumulation.<sup>20</sup> Interestingly, our results indicated that the HCA-treated group mitigated anthropometrical parameters and obesogenic effects, with the most compelling outcome observed in the HCA2 (30mg/kg) group. The actual mechanism by which HCA ameliorates obesity and anthropometrical parameters is not entirely clear. Studies postulate a few established mechanisms related to the inhibition of adipocyte deposition and differentiation, upregulation of lipolysis factors, or inhibition of pancreatic lipase.<sup>21,22</sup>

Measuring lipid profiles and deposition is crucial for determining metabolic health.<sup>23</sup> Our results demonstrated high serum TC and LDL levels in the MD group, which were also associated with a high TC/HDL ratio. This lipidaemic effect is also associated with significant fat accumulation across multiple areas in the MD group. Fructose contains lipogenic nutrients, and its consumption has been associated with metabolic dyslipidaemia resulting from the mass production of hepatic and intestinal lipoprotein particles. Fructose can be further metabolised into glycerol, which is a precursor for TG and lipid accumulation.<sup>24</sup> This could justify the presence of fat vacuolation in the liver, a primary target of metabolic organs, in rats administered with the HFHF diet. The obesogenic and lipidemic effects of HFHF-diet render other morphological alterations, including dilatation of the renal tubules, hypertrophy of the myocardium, and rough endothelial surface of the aorta, which was demonstrated in the MD group. Simultaneously, treatment with HCA1 and HCA2 was particularly effective in countering these increments, which were notably seen in abdominal, epididymal, and retroperitoneal fat pad regions. This could indicate anti-lipogenic or fat-reducing activity of HCA, potentially by modulating lipid metabolism and energy expenditure.

In terms of glucose metabolism, the AUC level in the MD group showed a higher trend compared to the Control group, indicating an impairment of insulin sensitivity. This may be related to the activity of lipid by-products, which mediate the insulin target cells, leading to blockage of the insulin signal.<sup>25</sup> Fructose consumption in clinical and experimental animal studies has been associated with alterations in metabolic parameters related to glucose tolerance, insulin, and obesity.<sup>26</sup> Few findings also postulate that products of glucosamine and other hexosamine derivatives could potentially induce glucose intolerance and insulin resistance.<sup>27</sup> In contrast, the MD+HCA1 and MD+HCA2 groups exhibited a decrement in glucose peak level, with glucose levels returning closer to baseline within 120 minutes, suggesting an improvement in glucose clearance capacity and enhancement of metabolic control. This effect was more pronounced in the MD+HCA1 group, indicating

its superior efficacy in mitigating glucose intolerance. These outcomes may be attributed to the role of HCA in enhancing glycogen synthesis and inhibiting gluconeogenesis, thereby reducing hepatic glucose output.<sup>28</sup>

In addition to metabolic outcomes, haematological and biochemical parameters were also assessed to determine the safety and toxicity of different dosages of HCA.<sup>29</sup> All haematological parameters revealed no significant abnormality, except for a significant decrease in RBC and MCV values in the MD+HCA3 group when compared to the Control group. Higher doses of HCA may elicit a suppressive effect on erythropoiesis or excessive destruction of the red cell, resulting from pro-oxidant effects.<sup>30</sup> We observed a biphasic effect of administered HCA on metabolic disturbance in rats. HCA at low to moderate doses may exert protective or compensatory hematopoietic effects, whereas HCA at high doses appears to exert a detrimental effect on erythrocytes. However, further mechanistic studies concentrating on oxidative stress biomarkers and red blood cell membrane stabilisation could clarify these haematological alterations. Prolonged high-fat diet intake is associated with an increase in ALT, AST, and ALP, indicating ongoing liver injury that could potentially lead to liver damage. This damaging effect is significantly observed in the MD group, with the reversal effect being more pronounced in the MD+HCA2 group compared to other treated groups, supporting its hepatoprotective role. HCA1 and HCA3 exert a minimal ameliorative effect with partial restoration of the metabolic balance. As shown in Table 3, albumin levels were lower than globulin levels in both control and experimental groups. This pattern is consistent with the physiological characteristics of rodents, which typically exhibit relatively higher globulin fractions<sup>31,32</sup>. The A/G ratios observed in the present study remained within the reported physiological reference ranges for male *Sprague-Dawley* rats, indicating preserved hepatic synthetic function. In addition, recent work highlighting analytical considerations and reference interval derivation for serum protein fractions supports the reliability of albumin and globulin measurements used

in this study, further strengthening interpretation of the observed protein profiles <sup>33,34</sup>.

Whereas all the electrolyte levels (sodium, potassium, and chloride) remained largely unchanged across groups, indicating stable electrolyte homeostasis. These results indicate that HCA at various doses following 12 weeks of the experimental period does not possess any toxic effect on the experimental animal.

A limitation of the present pilot study is the absence of direct measurements of oxidative stress and inflammatory markers, which are known contributors to the pathogenesis of metabolic disease. As this study was designed primarily for dose optimisation and histopathological assessment, sample availability precluded additional biochemical analyses. Future studies will incorporate oxidative stress and inflammatory parameters to further elucidate the mechanistic basis of *trans-3-HCA*'s protective effects.

## CONCLUSION

In summary, our data demonstrate that *trans-3-hydroxycinnamic acid* (HCA), a phenolic compound, has promising therapeutic potential for mitigating metabolic disturbances in rats fed the HFHF diet. Supplementation with HCA, particularly at 30 mg/kg, significantly improved body weight regulation, visceral fat accumulation, lipid profiles, and liver enzymes. It also ameliorated histopathological changes in key metabolic organs, including the liver, kidney, heart, and aorta, while demonstrating hepatoprotective and cardioprotective effects. Furthermore, HCA at various doses does not exhibit any toxic effect on the experimental animal. These findings suggest that HCA may serve as a valuable candidate for further development as a nutraceutical or adjunct therapy in managing metabolic syndrome and its associated complications.

## ACKNOWLEDGEMENT

The authors thank the School of Basic Medical Sciences, Faculty of Medicine, Universiti Sultan Zainal Abidin, for providing animal welfare and technical assistance during the experimental studies.

## AUTHORS CONTRIBUTION

Study concept and design: ZAO and MM; acquisition of data: ASA, BAI; analysis and interpretation of data: ASA, BAI; drafting of the manuscript: ASA, ZAO; critical revision of the manuscript: ZAO, ZZ, and MM; statistical analysis: ZZ, NAS, NAAB; obtained funding: ZAO; administrative, technical, or material support: NAS, NAAB; and study supervision: ZAO, NAS.

## FUNDING

This work was financially supported by the Fundamental Research Grant Scheme, Ministry of Higher Education, Unisza research project no FRGS/1/2023/SKK10/UNISZA/02/2.

## CONFLICTS OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be interpreted as a potential conflict of interest.

## REFERENCES

1. Yang M, Liu S, Zhang C. The related metabolic diseases and treatments of obesity. *Healthcare*. 2022;10(9):1616. doi:10.3390/healthcare10091616
2. Sarma S, Sockalingam S, Dash S. Obesity as a multisystem disease: Trends in obesity rates and obesity-related complications. *Diabetes Obes Metab*. 2021;23(Suppl 1):3-16. doi:10.1111/dom.14290
3. Preguiça I, Alves A, Nunes S, et al. Diet-induced rodent models of obesity-related metabolic disorders a guide to a translational perspective. *Obes Rev*. 2020;21(12):e13081. doi:10.1111/obr.13081
4. Kotzé-Hörstmann L, Cois A, Johnson R, et al. Characterization and comparison of the divergent metabolic consequences of high-sugar and high-fat diets in male Wistar rats. *Front Physiol*. 2022;13:904366. doi:10.3389/fphys.2022.904366
5. Dobrowolski P, Prejbisz A, Kuryłowicz A, et al. Metabolic syndrome-a new definition and management guidelines. *Arter Hypertens*. 2022;26(3):99-121. doi:10.5603/AH.a2022.0014
6. Kasprzak-Drozd K, Oniszczyk T, Stasiak M, et al. Beneficial effects of phenolic compounds on gut

- microbiota and metabolic syndrome. *Int J Mol Sci.* 2021;22(7):3715. doi:10.3390/ijms22073715
7. Salau VF, Erukainure OL, Ijomone OM, et al. Caffeic acid regulates glucose homeostasis and inhibits purinergic and cholinergic activities while abating oxidative stress and dyslipidaemia in fructose-streptozotocin-induced diabetic rats. *J Pharm Pharmacol.* 2022;74(7):973-984. doi:10.1093/jpp/rgac007
  8. Zakaria Z, Othman ZA, Suleiman JB, et al. Therapeutic effects of *Heterotrigena itama* (stingless bee) bee bread in improving hepatic lipid metabolism through the activation of the Keap1/Nrf2 signaling pathway in an obese rat model. *Antioxidants.* 2022;11(11):2190. doi:10.3390/antiox11112190
  9. Khan R, Naseem I. Antiglycation and antioxidant potential of coumaric acid isomers: A comparative in-vitro study. *J Biomol Struct Dyn.* 2024;42(22):12090-12104. doi:10.1080/07391102.2023.2280755
  10. Coman V, Vodnar DC. Hydroxycinnamic acids and human health: Recent advances. *J Sci Food Agric.* 2020;100(2):483-499. doi:10.1002/jsfa.10072
  11. Alam MA, Subhan N, Hossain H, et al. Hydroxycinnamic acid derivatives: A potential class of natural compounds for the management of lipid metabolism and obesity. *Nutr Metab.* 2016;13:27. doi:10.1186/s12986-016-0087-0
  12. Othman, Z.A., Wan Ghazali, W.S., Noordin, L., Mohd. Yusof, N.A. and Mohamed, M., 2019. Phenolic compounds and the anti-atherogenic effect of bee bread in high-fat diet-induced obese rats. *Antioxidants*, 9(1), p.33.
  13. Mnafigui K, Derbali A, Sayadi S, et al. Anti-obesity and cardioprotective effects of cinnamic acid in high fat diet-induced obese rats. *J Food Sci Technol.* 2015;52:4369-4377. doi:10.1007/s13197-014-1522-7
  14. Novelli ELB, Diniz YS, Galhardi CM, et al. Anthropometrical parameters and markers of obesity in rats. *Lab Anim.* 2007;41(1):111-119. doi:10.1258/00236770779399518
  15. Allison DB, Paultre F, Maggio C, et al. The use of areas under curves in diabetes research. *Diabetes Care.* 1995;18(2):245-250. doi:10.2337/diacare.18.2.245
  16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18:499-502.
  17. Chew NW, Ng CH, Tan DJH, et al. The global burden of metabolic disease: Data from 2000 to 2019. *Cell Metab.* 2023;35(3):414-428. doi:10.1016/j.cmet.2023.02.001
  18. Nahra R, Wang T, Gadde KM, et al. Effects of cotadutide on metabolic and hepatic parameters in adults with overweight or obesity and type 2 diabetes: A 54-week randomized phase 2b study. *Diabetes Care.* 2021;44(6):1433-1442. doi:10.2337/dc20-2447
  19. Meneses MJ, Sousa-Lima I, Jarak I, et al. Distinct impacts of fat and fructose on the liver, muscle, and adipose tissue metabolome: An integrated view. *Front Endocrinol.* 2022;13:898471. doi:10.3389/fendo.2022.898471
  20. Lu Y, Zhang C, Song Y, et al. Gallic acid impairs fructose-driven de novo lipogenesis and ameliorates hepatic steatosis via AMPK-dependent suppression of SREBP-1/ACC/FASN cascade. *Eur J Pharmacol.* 2023;940:175457. doi:10.1016/j.ejphar.2022.175457
  21. Kuppusamy P, Ilavenil S, Hwang IH, et al. Ferulic acid stimulates adipocyte-specific secretory proteins to regulate adipose homeostasis in 3T3-L1 adipocytes. *Molecules.* 2021;26(7):1984. doi:10.3390/molecules26071984
  22. Rybak M, Wojdyło A. Inhibition of  $\alpha$ -amylase,  $\alpha$ -glucosidase, pancreatic lipase, 15-lipoxygenase and acetylcholinesterase modulated by polyphenolic compounds, organic acids, and carbohydrates of *Prunus domestica* fruit. *Antioxidants.* 2023;12(7):1380. doi:10.3390/antiox12071380
  23. Aron-Wisniewsky J, Warmbrunn MV, Nieuwdorp M, et al. Metabolism and metabolic disorders and the microbiome: The intestinal microbiota associated with obesity, lipid metabolism, and metabolic health—pathophysiology and therapeutic strategies. *Gastroenterology.* 2021;160(2):573-599. doi:10.1053/j.gastro.2020.07.056

24. Gungor A, Balamtekin N, Ozkececi CF, et al. The relationship between daily fructose consumption and oxidized low-density lipoprotein and low-density lipoprotein particle size in children with obesity. *Pediatr Gastroenterol Hepatol Nutr.* 2021;24(5):483-490. doi:10.5223/pghn.2021.24.5.483
25. Zhou YJ, Xu N, Zhang XC, et al. Chrysin improves glucose and lipid metabolism disorders by regulating the AMPK/PI3K/AKT signaling pathway in insulin-resistant HepG2 cells and HFD/STZ-induced C57BL/6J mice. *J Agric Food Chem.* 2021;69(20):5618-5627. doi:10.1021/acs.jafc.1c01214
26. Azevedo-Martins AK, Santos MP, Abayomi J, et al. The impact of excessive fructose intake on adipose tissue and the development of childhood obesity. *Nutrients.* 2024;16(7):939. doi:10.3390/nu16070939
27. Rabbani N, Thornalley PJ. Hexokinase-linked glycolytic overload and unscheduled glycolysis in hyperglycemia-induced pathogenesis of insulin resistance, beta-cell glucotoxicity, and diabetic vascular complications. *Front Endocrinol.* 2024;14:1268308. doi:10.3389/fendo.2023.1268308
28. Tylutka A, Morawin B, Walas Ł, et al. Assessment of metabolic syndrome predictors in relation to inflammation and visceral fat tissue in older adults. *Sci Rep.* 2023;13(1):89. doi:10.1038/s41598-022-27213-w
29. Rakshit S, Shukla P, Verma A, et al. Protective role of rutin against combined exposure to lipopolysaccharide and D-galactosamine-induced dysfunctions in liver, kidney, and brain: Hematological, biochemical, and histological evidence. *J Food Biochem.* 2021;45(2):e13605. doi:10.1111/jfbc.13605
30. Mahrous, D. A., Shehata, A. E., Sahgat, M. K., Hamada, R. A., Samak, D. H., Goda, W. M., & Hassan, S. M. (2025). Aluminum oxide nanoparticles (Al<sub>2</sub>O<sub>3</sub>-NPs) toxicity in Sprague-Dawley rats: Clinico-Pathological & Biochemical Investigations. *Damanhour Journal of Veterinary Sciences*, 13(1), 11–26. <https://doi.org/10.21608/djvs.2025.343949.1144>
31. Weller, D., Azar, S., Johnson, M. A., & Azar, M. (1978). Albumin/globulin ratios, colloid osmotic pressures and Lowry protein microanalysis in rat plasma. *Clinical Science and Molecular Medicine*, 55(6), 529-532.
32. Zaias, J., Mineau, M., Cray, C., Yoon, D., & Altman, N. H. (2009). Reference values for serum proteins of common laboratory rodent strains. *Journal of the American Association for Laboratory Animal Science*, 48(4), 387-390.
33. Militello, L., El-Khoury, J., & Durant, T. (2024). Differences Between Serum and Plasma: An Indirect Approach to Derive a Combined Reference Interval for Total Protein, Albumin, and Globulin. *American Journal of Clinical Pathology*, 162 (Supplement\_1), S170–S170. <https://doi.org/10.1093/ajcp/aaqae129.373>
34. Kyselova Z. Toxicological aspects of the use of phenolic compounds in disease prevention. *Interdiscip Toxicol.* 2011;4(4):173-183. doi:10.2478/v10102-011-0030-6