



Effect of syringic acid on steroid and gonadotropic hormones, hematological indices, sperm characteristics and morphologies, and markers of tissue damage in methyl cellosolve-administered rats

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ABSTRACT

Methyl cellosolve (MTC) is an established gonadotoxic and hematotoxic compound that is commonly and universally utilized in herbicide, liquid soap, stain, dye, paint, and brake fluid manufacturing industries as a solvent. Due to its wide range usage, this study therefore investigated the effect of syringic acid (SYAC) on hematological indices, sperm characteristics and morphologies, and markers of tissue damage in MTC administered male Wistar rats. Thirty (30) rats divided into six groups were used. Rats in group 1 served as control, those in group 2 were administered MTC for 30 consecutive days, those in groups 3, 4, and 5 were treated with 25, 50, and 75 mg/kg body weight of SYAC respectively also for 30 consecutive days immediately after each day MTC administrations, while rats in group 6 received 75 mg/kg body weight of SYAC only throughout. Compared with control, administrations of MTC resulted in a significant decrease in spermatozoa count, number of normal and live spermatozoa, Hb count, MCH, MCHC, serum TC, and LH, while number of abnormal spermatozoa, RBC and WBC counts, activities of serum AST, ALT, GGT, LDH, and ADH were significantly increased. Treatments with 25 mg/kg of SYAC significantly reduced the RBC and WBC counts, serum activities of AST, ALT, GGT, and increased TC concentration. Treatments with 50 mg/kg SYAC significantly lowered the number of abnormal spermatozoa, RBC count, activities of serum ALT, AST, LDH, ADH, and increased the number of normal spermatozoa, MCV, MCH, and MCHC, while 75 mg/kg of SYAC significantly decreased the serum activities of AST, ALT, GGT, LDH, ADH, and increased serum TC concentration. Findings from this study have revealed the hepatoprotective effect of SYAC at all doses investigated but did not confer spermatoprotection and hematoprotection against MTC-induced toxicities, and looking at the 3 doses investigated, 50 mg/kg of SYAC yielded the best effect.

1. Introduction

Glycol ethers in which MTC belongs are universally utilized in the industries that produce dyes for textiles, inks for printing, jet fuel anti-icing additives, and varnishes [1]. These glycol ethers disrupt the endocrine signaling, causing toxicities in humans and various species of animals [2]. MTC and its derivatives have deleterious effect on organs (bone marrow, testis, fetus, and thymus) leading to elevated respiratory rates and energy metabolism [3]. MTC toxicity has been reported to

cause diminished WBC count, thymocyte and testicular destruction, and developmental problems [4].

Embryotoxicity and teratogenicity of MTC results when it is oxidatively metabolized to 2-methoxyacetate (2-MAC) [5]. ADH initially metabolize (oxidation) MTC to 2-methoxyacetaldehyde, a short-lived product that is further oxidized to 2-MAC by aldehyde dehydrogenase [6]. 2-MAC can be readily located in the body fluids including blood of persons exposed to it and its elimination is through urine [7]. Within the cells, 2-MAC is activated by combining with coenzyme A, forming 2-methoxyacetyl-CoA, a substrate for the tricarboxylic acid cycle [6].

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Abbreviations

MTC	methyl cellosolve
2-MAc	2-methoxyacetic acid
SYAC	syringic acid
Hb	hemoglobin
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
TC	total cholesterol
LH	luteinizing hormone
Hb	hemoglobin
RBC	red blood cell

WBC	white blood cell
AST	aspartate aminotransferase
ALT	alanine aminotransferase
GGT	gamma glutamyl transferase
LDH	lactate dehydrogenase
ADH	alcohol dehydrogenase
MCV	mean corpuscular volume
FSH	follicle stimulating hormone
2-MAc	2-methoxyacetate
EDTA	ethylenediaminetetraacetic acid
HMG CoA	β -hydroxy- β -methylglutaryl CoA

Thus, 2-MAc toxicity can be mitigated by intermediates of tricarboxylic acid cycle, acetate, D-glucose and serine co-treatments [6].

MTC is a teratogen that is toxic to the reproductive and hematopoietic systems in humans and diverse kinds of animal species [8]. MTC attacks and destroys the testis, which is the hallmark of its acute toxicity [2,9]. Testes relative weight was significantly reduced when 0.5 g/kg of MTC was administered per day for 2 days or more, while 0.1 g/kg/day caused a degeneration of pachytene spermatocytes within a day [10]. A continuous deterioration of the spermatocytes [10] as a result of initiated testicular apoptosis [11] was reported during a repeated MTC exposure. Two inhibitors of calcium channel namely diltiazem and verapamil were reported to stop in vivo MTC-induced toxicity in rats, indicating the involvement of the two drugs in the deregulation of calcium homeostasis in MTC toxicity [12]. Reduced sperm count per ejaculate, and elevated incidence of azoospermia and oligospermia were recorded in shipyard painters that were exposed to MTC at concentration of 2.6 mg/m³ [13]. LOAEL of 167 mg/m³ for testicular toxicity was reported in an MTC dose-response experiment in rats [14], unlike 2.6 mg/m³ recorded for humans, an indication of a greater sensitivity in humans. The seminiferous tubules of human and rat respond in a similar pattern to equal dosage of 2-MAc when exposed to it in vitro, but in these two species, there was a difference in the appearance of the dying spermatocytes [2].

SYAC is a phenolic compound and antioxidant abundantly present in grapes, olives, honey, and *Alpinia calcarata* leaves [15]. SYAC is a potent free radical scavenger already documented to be efficient in treating heart diseases, inflammatory disorders, neuronal damage, diabetes, cancer, and microbial infection [16,17]. The efficacy of SYAC as a therapeutic agent is conferred by the methoxy groups flanking the phenol structure. SYAC can eradicate reactive species and possesses modulatory function on various nuclear transcription factors and certain enzyme activities associated with angiogenesis, cancer, diabetes, and inflammation [17]. SYAC can protect the sciatic nerves of the brain from oxidative damage-induced axonal degeneration in rodent model of ischemic injury [18]. Its kidney and liver complications improving capabilities have also been reported [19,20]. MTC-induced tissue (liver, testis, kidney, and lung) oxidative stress, inflammation, and apoptosis have been reported [1,21–23], this study therefore investigated the effect of SYAC on hematological indices, sperm characteristics and morphologies, markers of tissue damage, and reproductive hormones in MTC-administered rats.

2. Materials and methods

2.1. Test substances, diagnostic kits, and chemicals

MTC (C₃H₈O₂, 99.5%) was procured from BDH Laboratory Supplies, Poole, England, while SYAC (C₉H₁₀O₅, 98%) was procured from AK Scientific, USA. Testosterone, FSH, and LH kits used were produced by DiaSino Laboratories Co., Zhengzhou, China. TC, AST, and ALT kits were

product of Randox Laboratories Ltd, United Kingdom, GGT kit was produced by Agappe Diagnostic Ltd, Kerala, India, while LDH kit was produced by BioSystems, Barcelona, Spain. Reagents/chemicals used were all bought from recognized companies and were pure and of analytical grade.

2.2. Experimental animals

Thirty (30) male albino Wistar rats (average weight of 220 g) were used in this study. They were purchased from the breeding unit of Animal Physiology Department, University of Ibadan, Ibadan. They were housed in well ventilated transparent plastic cages where they underwent the normal 12 h light-dark cycle. They were fed rat chow and portable drinking water throughout the study unrestricted.

2.3. Administrations of test substances

Rats were divided into six groups that contained five animals each, and after 4 weeks of acclimatization, rats in group one that served as control were only served food and water throughout, rats in group two were administered 100 mg/kg body weight of MTC [21] everyday for thirty days, rats in group three, four, and five were administered MTC everyday for thirty days but respectively, they were treated with 25, 50, and 75 mg/kg body weight of SYAC [24] for thirty days, and finally rats in group six were administered 75 mg/kg body weight of SYAC only for thirty days. MTC and SYAC were administered orally.

2.4. Collection and preparation of samples

A day after the last administration, blood samples were collected from the retroorbital sinus into 10 mL clean plain bottles and 5 mL EDTA bottles. Following the collection of blood, all rats were sacrificed by cervical dislocation, pinned down and the abdominal region was cut opened. Experimental animals were handled by following the international guidelines for the care and use of laboratory animals [25]. The collected blood samples in 10 mL plain tubes were centrifuged for 10 min at 3000 rpm, the supernatant which is the serum was decanted and aliquoted in 1 mL Eppendorf tubes and was used for the biochemical assays, while the blood samples collected in EDTA bottles were used for the estimations of various hematological indices. The caudal epididymis was located from which the spermatozoa were taken and analyzed for morphologies and characteristics.

2.5. Determination of sperm characteristics and morphologies

2.5.1. Determination of sperm motility

Method described by Cheesbrough [25] was followed to analyze the spermatozoa for motility. Briefly in that method, 10 μ L of semen was placed on warm and clean slides, where 2 drops of sodium citrate buffer were added, and the slides were covered using cover slips. Slides were

later examined and read under x40 magnification using a light microscope (Bio-microscope XSZ-170BN).

2.5.2. Determination of sperm viability

Live and dead sperm cells (sperm viability) were estimated by utilizing the Eosin-Nigrosin stain using the method described by Zemjanis [26]. Sperm cells were stained using Eosin-Nigrosin (made up of 5% Nigrosin, 1% Eosin, and 3 g of sodium citrate dihydrate dissolved in 100 mL of distilled water), and sperm cells that were not stained were considered alive, while those that were stained (picked the color of the stain) were considered dead.

2.5.3. Determination of sperm concentration or count

Rats' sperm concentrations were estimated by using an improved Neubauer (LABART, Germany) hemocytometer, according to the method described by Zemjanis [26].

2.5.4. Determination of sperm morphologies

Morphological abnormalities were examined in the spermatozoa extracted from each rat. This was facilitated using Wells and Awa stains on four hundred spermatozoa counts smeared on microscope slides as described by Wells and Awa [27].

2.6. Hematological analyses

2.6.1. Determination of PCV

Dacie and Lewis [28] method was followed. About 75% of a plain capillary tube was filled with blood. To prevent blood loss, the capillary tube was sealed, spined at 12000 rpm for 5 min using a Hawksley microhematocrit centrifuge (Hawksley, London). The capillary tube was inserted in a microhematocrit reader for the quantification of PCV and expressed in percentage.

2.6.2. Determination of Hb

Hb concentration was estimated spectrophotometrically by following the method described in Cypress Diagnostic kit. Briefly, 5 mL of the working reagent (made up of 0.6 mM potassium ferricyanide, 0.77 mM potassium cyanide, buffers, and stabilizers) was added into a test tube, followed by the addition of 20 μ L of the whole blood, and were mixed, allowed to stand for 180 s at 25°C. Thereafter, the absorbance at 540 nm was read, and the hemoglobin level was expressed in g/dL.

2.6.3. Determination of RBC

RBC count was estimated by following the protocol of Dacie and Lewis [28]. A dilution (1:200) of the blood was made using red blood cell diluting fluid. The mixture was left for 120 s and thereafter, RBC was counted with a light microscope (Bio-microscope XSZ-170BN) using x40 objectives. RBC count was expressed in L.

2.6.4. Determination of WBC

Also, the protocol of Dacie and Lewis [28] was followed. Blood was diluted with a diluting fluid (1:20) and mixed gently. After that, the total WBC was estimated under x10 objective of a light microscope (Bio-microscope XSZ-170BN) and expressed in L.

2.6.5. Determination of WBC differential count

The differentials were estimated by following the described method of Dacie and Lewis [28]. On grease-free slides, were placed evenly spread blood in other to form a blood film that is thin. Acetone-free methyl alcohol was then used to fix the blood film for about 5 min until it was dried. Blood films were thereafter stained with Field A and B stains, which enabled 100 WBCs to be differentiated with the use of the oil immersion objectives of a microscope (Bio-microscope XSZ-170BN).

2.7. Biochemical analyses in serum

2.7.1. Estimation of serum TC concentration, and activities of ALT, AST, GGT, and LDH

Serum total cholesterol concentration was determined according to the procedure described in Randox kit (Randox Laboratories Ltd, United Kingdom), based on the principle that involves the enzymatic hydrolysis and oxidation. The quinoneimine indicator formed from the reaction of hydrogen peroxide, 4-aminoantipyrine, and phenol, in the presence of peroxidase, is measured spectrophotometrically at 546 nm. Activity of serum ALT was determined by following the method described in Randox kit (Randox Laboratories Ltd, United Kingdom), which is based on the ability to monitor the level of pyruvate hydrazone formed with 2,4-dinitrophenyl-hydrazine. The developed color was measured at 546 nm. Activity of serum AST was determined by following the method described in Randox kit (Randox Laboratories Ltd, United Kingdom), which is based on the ability to monitor the level of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine. The developed color was measured colorimetrically at 546 nm. GGT activity in the serum was estimated according to the procedure described in Agappe kit (Agappe Diagnostic Ltd, Ernakulam, Kerala, India). In that method, 1 mL of the working reagent made up of Tris buffer (pH 8.25) and glycyl-glycine was added to 0.1 mL of sample. Thereafter, the change in optical density per minute for 3 min was monitored at 405 nm, while LDH activity in the serum was estimated according to the procedure described in BioSystems kit (BioSystems S.A., Barcelona, Spain), which is based on the ability of the enzyme to catalyze the reduction of pyruvic acid by NADH to form lactic acid and NAD⁺. The activity of the enzyme, which corresponds with the decreasing rate of NADH was measured at 340 nm at a minute interval for 3 min.

2.7.2. Estimation of serum activity of ADH

ADH activity was estimated according to the method of Walker [29], based on the ability of ADH to catalyze the oxidation of ethanol by NAD⁺. The increasing NADH formed was monitored for 3 min at 340 nm.

2.7.3. Estimation of serum testosterone, LH, and FSH levels

Serum LH and FSH levels were determined by following the method described in their respective DiaSino ELISA kit (DiaSino Laboratories Co., Ltd, Zhengzhou, China), which is based on sandwich principle where the serum sample, enzyme labeled anti-LH or enzyme labeled anti-FSH, and anti-LH or anti-FSH coated microwells are combined. LH or FSH in the serum sample is allowed to react with the antibodies, resulting to a sandwiched LH or FSH molecule in between the enzyme-linked antibodies and the solid phase, leading to the formation of a complex between the antibodies that are enzyme-linked, LH or FSH within the serum sample, and solid phase by immunological reactions. Substrate solution was added, which resulted into a chromogenic reaction, catalyzed by the complex. The absorbance of the resulting product, which is proportional to the amount of serum LH or FSH was measured at 450 nm. Serum testosterone was determined by following the method described in its DiaSino ELISA kit (DiaSino Laboratories Co., Ltd, Zhengzhou, China), which is based on-step competitive principle. The serum sample, coated microwells, and testosterone derivant were combined and during incubation, the testosterone presents in the sample derivant coated on microwells compete for binding to the antibodies that are enzyme labeled. The formed complex catalyzes the chromogenic reaction with the substrate solution, and the color intensity that is inversely proportional to the concentration of testosterone in the serum sample, is measured at 450 nm.

2.8. Statistical analysis

Gathered data were subjected to One-Way Analysis of Variance (ANOVA) and Tukey test to identify the values that are significantly

Table 1
Effect of SYAC treatments on morphology of sperm cells in MTC administered rats.

	AD	TAILLESS	HEADLESS	MPB	BT	CT	SMP	RT	RH	CTAH	CMP
CONTROL	-	++	+	-	-	++	-	-	-	-	-
MTC	-	+++	+++	++	-	++	-	-	+	-	-
MTC + 25 SYAC	-	+++	+	++	+	+	-	-	+	+	-
MTC + 50 SYAC	-	++	++	++	+	+	-	-	+	+	+
MTC + 75 SYAC	+	+++	+++	++	-	+	-	-	-	+	+
75 SYAC	-	+	-	+	-	++	-	-	-	-	-

Key: +++ = Appreciable amount (>20%), ++ = Moderate amount (6–20%), + = Minute amount (1–5%), BT = Bent tail, MPB = Mid piece bent, CT = Coiled tail, RT = Reversed tail, SMP = Swollen mid piece, RH = Reversed head, CMP = Coiled mid piece, AD = Acrosomal defect, CTAH = Coiled tail around head, MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

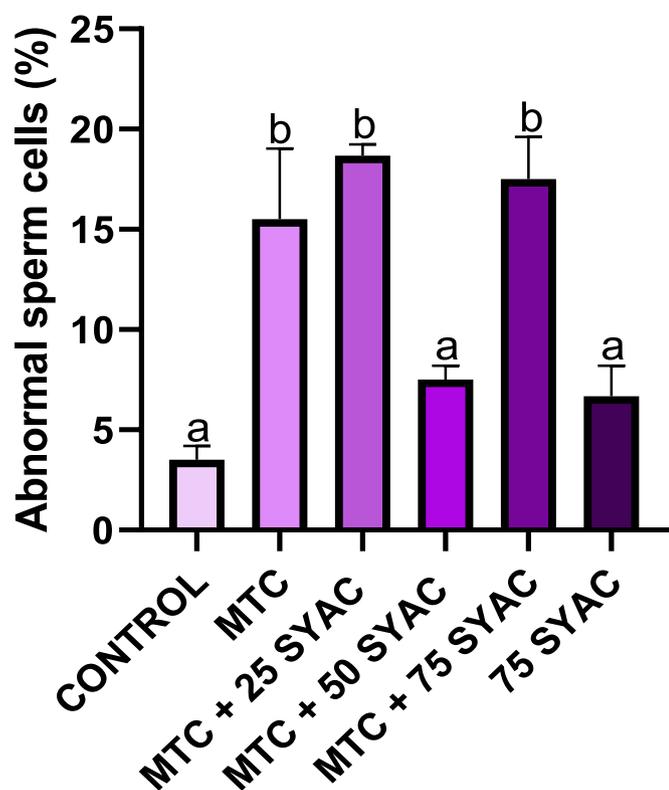


Fig. 1. Total abnormal sperm cells in SYAC-treated MTC administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

different among the six groups ($n = 5$) using version 8.0 of Graph Pad Prism. Results were reported as mean \pm standard deviation (SD). P value < 0.05 was taken to be statistically significant.

3. Results

3.1. Effect of SYAC treatments on sperm morphology of MTC administered rats

In [Table 1](#), qualitative results of different sperm cell morphologies were presented. These MTC-induced morphological abnormalities were seen in moderate to appreciable quantities. These abnormalities are headless sperm, tailless sperm, sperm with mid piece bent, reversed head, and coiled tail ([Table 1](#)). Treatments with different doses of SYAC yielded no significant results, except SYAC at 50 mg/kg body weight that yielded a better effect compared with the other doses (25 and 75 mg/kg body weights).

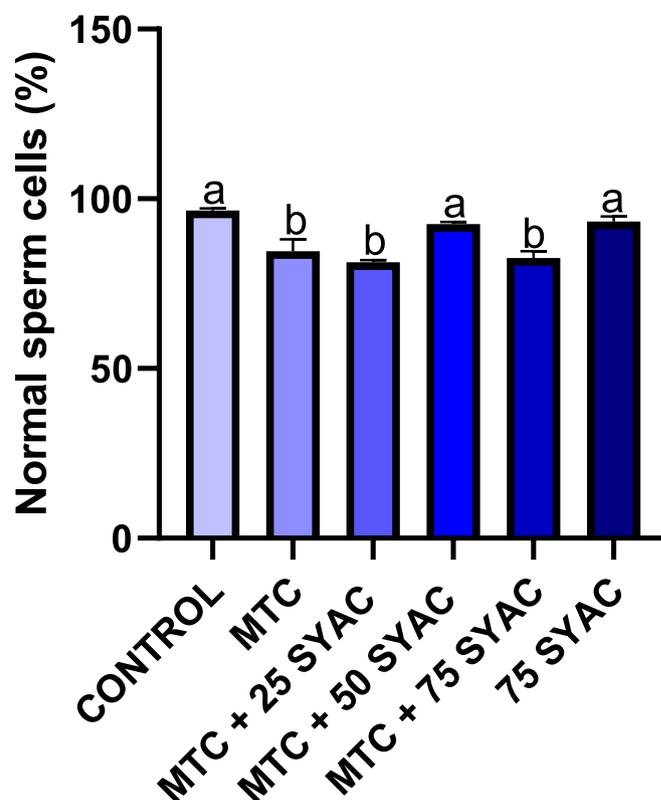


Fig. 2. Normal sperm cells in SYAC-treated MTC administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

3.2. Effect of SYAC treatments on abnormal and normal sperm cells of MTC administered rats

In [Figs. 1 and 2](#), the quantitative results of abnormal and normal sperm cells respectively, were presented. Abnormalities in sperm cells were significantly increased ($p < 0.05$) by 342.86% due to MTC administrations compared with control ([Fig. 1](#)). These abnormalities were significantly lowered ($p < 0.05$) by only 50 mg/kg body weight of SYAC (by 51.61%) ([Fig. 1](#)). For normal sperm cells, there was a significant decrease ($p < 0.05$) by 12.44% when MTC was administered compared with the control ([Fig. 2](#)), but compared with MTC only, only 50 mg/kg body weight of SYAC significantly increased ($p < 0.05$) the normal sperm by 9.47% ([Fig. 2](#)).

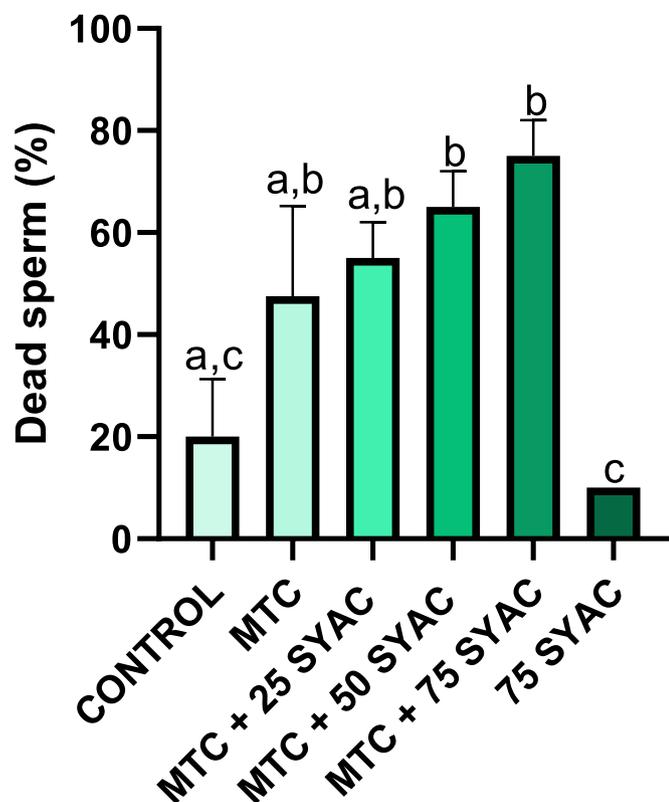


Fig. 3. Dead sperm cells in SYAC-treated MTC administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

3.3. Effect of SYAC treatments on dead and live sperm cells of MTC administered rats

Figs. 3 and 4 depict the results of the percentage of dead and live sperm cells (sperm viability) respectively. A non-significant ($p > 0.05$) increase in the percentage number of dead spermatozoa was recorded compared with control when MTC only was administered (Fig. 3). Administrations of 50 and 75 mg/kg body weight of SYAC did not decrease the dead sperm cells caused as a result of MTC administrations (Fig. 3), but a significant ($p < 0.05$) increase by 36.84% and 57.89% respectively was seen (Fig. 3). On the other hand, percentage of live spermatozoa (Fig. 4), was non-significantly decrease ($p > 0.05$) compared with control after MTC administrations. Neither of the treatment with 25, 50, and 75 mg/kg body weight of SYAC was effective compared with MTC only (Fig. 4).

3.4. Effect of SYAC treatments on sperm cell concentration of MTC administered rats

Fig. 5 depicts the result of sperm concentration in rats administered MTC and treated with SYAC. Compared with control, concentration of sperm was significantly decreased ($p < 0.05$) by 60.61% when MTC was administered to rats. Following treatments with SYAC, sperm concentration was further significantly reduced ($p < 0.05$) by 43.26%, 87.56%, and 72.02%, when 25, 50, and 75 mg/kg body weight respectively of SYAC were used as treatments compared with rats administered MTC only (Fig. 5).

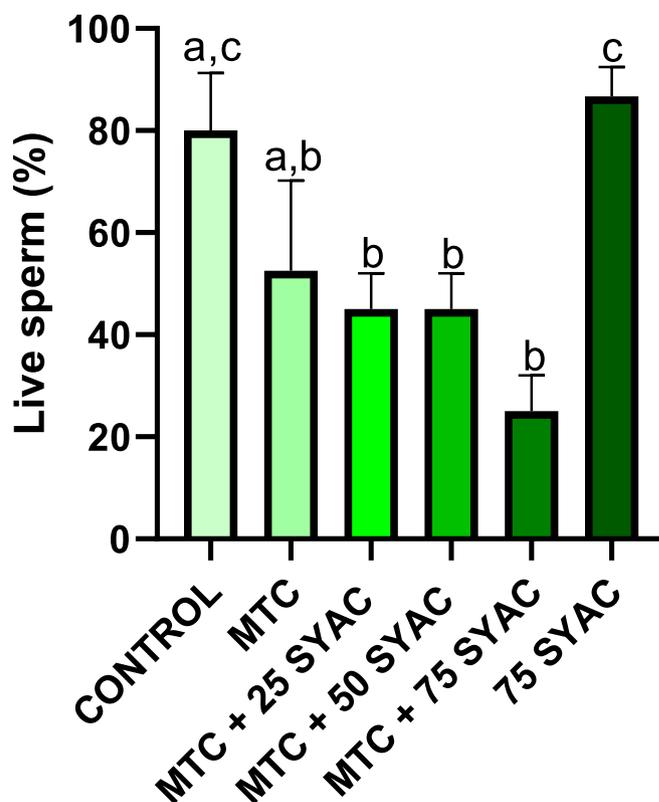


Fig. 4. Live sperm cells in SYAC-treated MTC administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

3.5. Effect of SYAC treatments on sperm cell motility of MTC administered rats

In Fig. 6, sperm motility result was presented. Percentage number of sperm motility was non-significantly ($p > 0.05$) decreased when rats were administered MTC only compared with control. All doses investigated did not increase the sperm motility of rats, instead sperm motility was further reduced significantly ($p < 0.05$) by 64.60% when 50 mg/kg body weight of SYAC was administered compared with MTC only (Fig. 6).

3.6. Effect of SYAC treatments on hematological indices of MTC administered rats

Table 2 depicts the results of hematological parameters in SYAC-treated MTC administered rats. Administrations of MTC and SYAC at all treatment doses did not have any significant ($p > 0.05$) effect on PCV (Table 2). Compared with control, Hb was significantly decreased ($p < 0.05$) by MTC (13.23%) administrations, but SYAC at all doses administered were not effective ($p > 0.05$) compared with MTC only (Table 2). Also in Table 2, there was a significant increase ($p < 0.05$) by 72.39% after MTC administrations compared with control and was significantly ($p < 0.05$) lowered to 38.10% by 25 mg/kg and 53.25% by 50 mg/kg body weight of SYAC compared with MTC only. Similarly, WBC was significantly increased ($p < 0.05$) by 28.24% when MTC was administered compared with control but was significantly ($p < 0.05$) brought down by 26.19% compared with MTC only (Table 2).

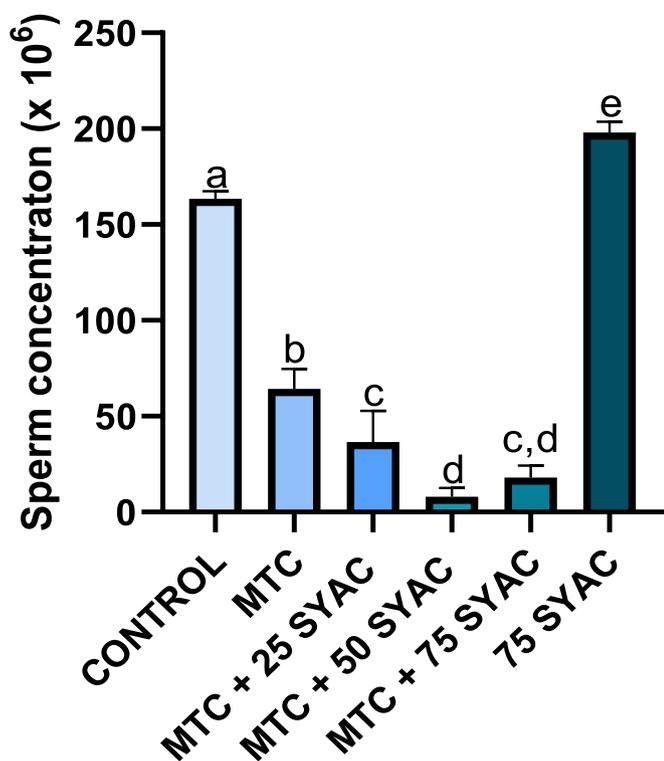


Fig. 5. Sperm concentration in SYAC-treated MTC administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

3.7. Effect of SYAC treatments on WBC differentials of MTC administered rats

Presented in Table 3 are the results of WBC differentials. Compared with MTC only, 50 mg/kg body weight of SYAC increased significantly ($p < 0.05$) the MCV count by 104.49%, while MCHC and MCH (Table 3) were significantly decreased ($p < 0.05$) by 12.39% and 41.33% respectively following MTC administrations compared with control. Like MCV, MCHC and MCH were significantly increased ($p < 0.05$) by 50 mg/kg body weight of SYAC compared with control (Table 3). The increments were 12.56% and 108.14% respectively. For eosinophils, basophils, monocytes, neutrophils, and lymphocytes counts, administrations of MTC and treatments with SYAC showed no effect (Table 3).

3.8. Effect of SYAC treatments on concentration of serum TC of MTC administered rats

Serum concentration of TC is depicted in Fig. 7. In MTC only administered rats, TC was significantly reduced ($p < 0.05$) by 90.03% compared with rats in control group. SYAC treatments (25 and 75 mg/kg body weight) significantly increased ($p < 0.05$) TC concentration by 587.5% and 741.67% respectively, compared with rats administered MTC only.

3.9. Effect of SYAC treatments on activities of serum ALT, AST, and GGT of MTC administered rats

Depicted in Figs. 8–10 are results of the activities of ALT, AST, and GGT respectively, in the serum. Compared with control rats, administrations of MTC to rats led to a significant increase ($p < 0.05$) in activities of serum ALT (by 81.22%) and AST (by 53.87%). After treatments with 25, 50, and 75 mg/kg body weights of SYAC, significant decreases

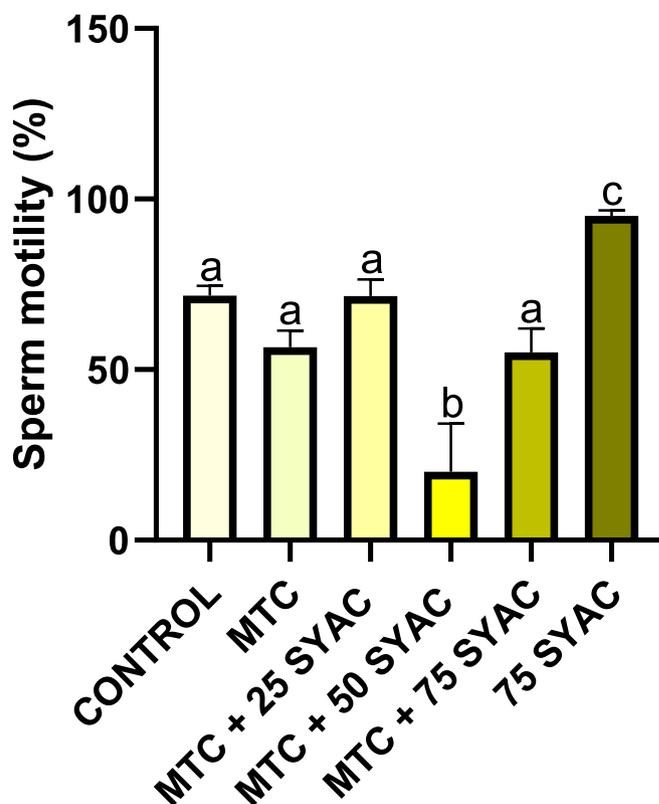


Fig. 6. Sperm motility in SYAC-treated METCEL administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

Table 2
Hematological indices in SYAC-treated MTC administered rats.

	PCV (%)	Hb (g/dL)	RBC ($\times 10^{12}L^{-1}$)	WBC ($\times 10^9L^{-1}$)
Control	47.33 \pm 0.58 ^a	15.50 \pm 0.72 ^a	6.70 \pm 1.08 ^a	6.55 \pm 0.35 ^a
MTC	44.67 \pm 1.53 ^a	13.45 \pm 0.07 ^b	11.55 \pm 0.21 ^b	8.40 \pm 0.14 ^{bc}
MTC + 25 SYAC	46.00 \pm 1.41 ^a	15.30 \pm 0.28 ^{ab}	7.15 \pm 0.07 ^{ac}	6.20 \pm 0.00 ^a
MTC + 50 SYAC	43.00 \pm 2.00 ^a	13.50 \pm 0.57 ^b	5.40 \pm 0.28 ^a	8.45 \pm 0.50 ^c
MTC + 75 SYAC	46.50 \pm 4.95 ^a	15.45 \pm 0.92 ^{ab}	9.25 \pm 0.64 ^{bc}	7.20 \pm 0.42 ^{ab}
75 SYAC	43.67 \pm 1.16 ^a	14.43 \pm 0.40 ^{ab}	5.40 \pm 0.57 ^a	13.60 \pm 0.14 ^d

Each result represents mean \pm standard deviation (SD) ($n = 5$). Results carrying different letters entirely along the same column are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid, PCV = packed cell volume, Hb = hemoglobin, RBC = red blood cell, WBC = white blood cell.

($p < 0.05$) in the activity of ALT (Fig. 8) by 67.93%, 68.43%, and 69.26% respectively were recorded compared with MTC only. Again, after SYAC treatments, AST (Fig. 9) activity was decreased significantly ($p < 0.05$) by 25 mg/kg (56.40%), 50 mg/kg (51.97%), and 75 mg/kg (34.77%) compared with METCEL only. Compared with control, activity of serum GGT (Fig. 10) was increased significantly ($p < 0.05$) by 100% due to MTC administrations and was significantly lowered ($p < 0.05$) by 40% and 60% after treatments with 25 and 75 mg/kg body weights of SYAC compared with rats administered MTC only.

Table 3
Differential of WBC and indices of RBC in SYAC-treated MTC administered rats.

	LYMP (%)	NEUT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	EOS (%)	BAS (%)	MONO (%)
Control	68.80 ± 1.64 ^a	29.60 ± 2.07 ^a	53.53 ± 5.19 ^{ac}	21.58 ± 2.73 ^{ac}	34.54 ± 0.20 ^a	0.00 ± 0.00 ^a	1.00 ± 0.00 ^a	1.00 ± 0.00 ^a
MTC	69.00 ± 2.58 ^a	29.50 ± 1.92 ^a	39.87 ± 4.41 ^a	12.66 ± 1.70 ^b	30.26 ± 1.28 ^b	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	1.00 ± 0.00 ^a
MTC + 25 SYAC	70.00 ± 2.00 ^a	30.00 ± 2.00 ^a	53.23 ± 0.41 ^{ac}	18.89 ± 1.84 ^{ab}	33.07 ± 1.22 ^{ab}	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
MTC + 50 SYAC	70.00 ± 1.00 ^a	29.00 ± 1.00 ^a	81.53 ± 1.65 ^b	26.35 ± 1.63 ^c	34.06 ± 0.23 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
MTC + 75 SYAC	70.33 ± 2.52 ^a	28.67 ± 2.31 ^a	42.83 ± 2.13 ^{ac}	12.45 ± 1.22 ^b	33.17 ± 1.14 ^{ab}	0.00 ± 0.00 ^a	1.00 ± 0.00 ^a	0.00 ± 0.00 ^a
75 SYAC	69.25 ± 2.22 ^a	29.50 ± 2.89 ^a	59.64 ± 8.32 ^c	20.63 ± 3.01 ^{ac}	34.58 ± 0.23 ^a	0.00 ± 0.00 ^a	1.50 ± 0.71 ^a	0.00 ± 0.00 ^a

Each result represents mean ± standard deviation (SD) (n = 5). Results carrying different letters entirely along the same column are statistically significant (p < 0.05). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid, LYMP = lymphocyte, NEUT = neutrophil, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, EOS = eosinophil, BAS = basophil, MONO = monocyte.

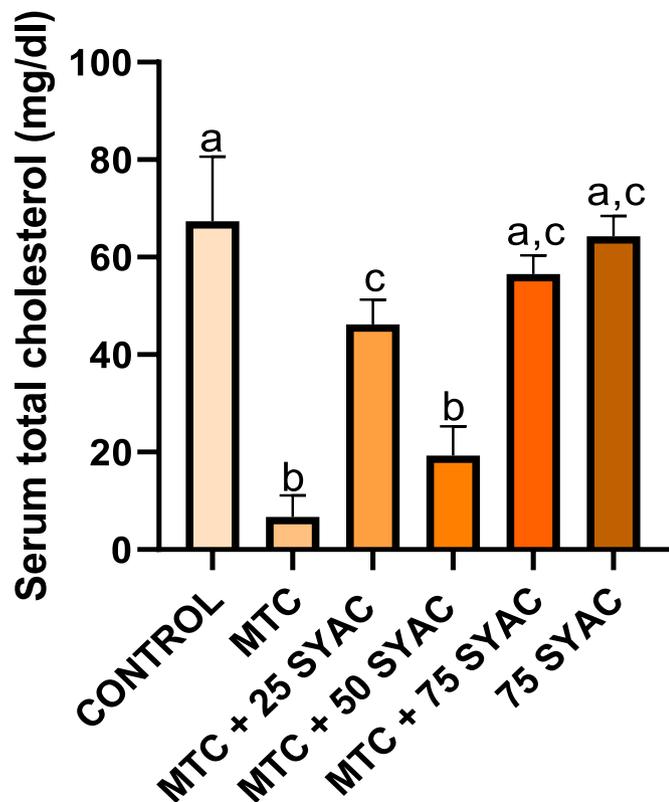


Fig. 7. Serum cholesterol concentration in SYAC-treated METCEL administered rats. Each bar represents mean ± standard deviation (SD). Bars carrying different letters entirely are statistically significant (p < 0.05). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

3.10. Effect of SYAC treatments on activity of serum LDH of MTC administered rats

Serum LDH result is presented in Fig. 11. Compared with control, a significant increase (p < 0.05) by 274.69% was recorded by MTC administrations. Compared with MTC only, treatments with SYAC (50 and 75 mg/kg) lowered significantly (p < 0.05) the activity of LDH by 70.35% each.

3.11. Effect of SYAC treatments on activity of serum ADH of MTC administered rats

Result of ADH activity in the serum is shown in Fig. 12. The result indicates a significant increase (p < 0.05) in serum activity of ADH by 105.76% compared with control, in METCEL administered rats. At 25 mg/kg body weight, SYRA yielded no significant (p > 0.05) effect, while

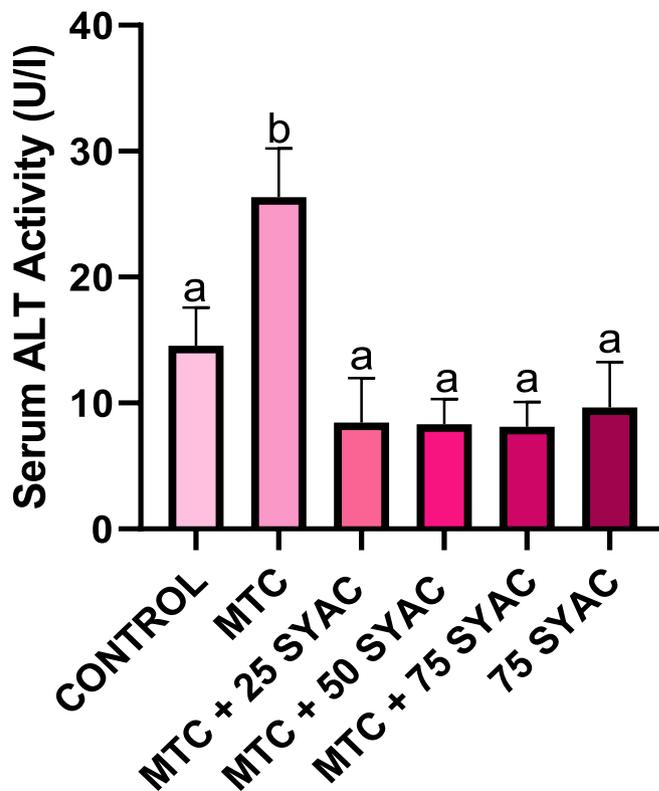


Fig. 8. Serum ALT activity in SYAC-treated METCEL administered rats. Each bar represents mean ± standard deviation (SD). Bars carrying different letters entirely are statistically significant (p < 0.05). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

at 50 and 75 mg/kg body weight, SYRA significantly reduced (p < 0.05) the activity of ADH by 41.79% and 36.57% respectively compared with METCEL only.

3.12. Effect of SYAC treatments on levels of serum reproductive hormones of MTC administered rats

Figs. 13–15 depict the results of testosterone, LH, and FSH respectively. Administrations of MTC and treatments by all doses studied showed no significant effect (p > 0.05) on the levels of serum testosterone and FSH. For serum concentration of LH, a significant reduction (p < 0.05) by 19.35% was recorded following the administrations of MTC compared with rats in control group. All SYAC treatments did not yield any significant effect (p > 0.05).

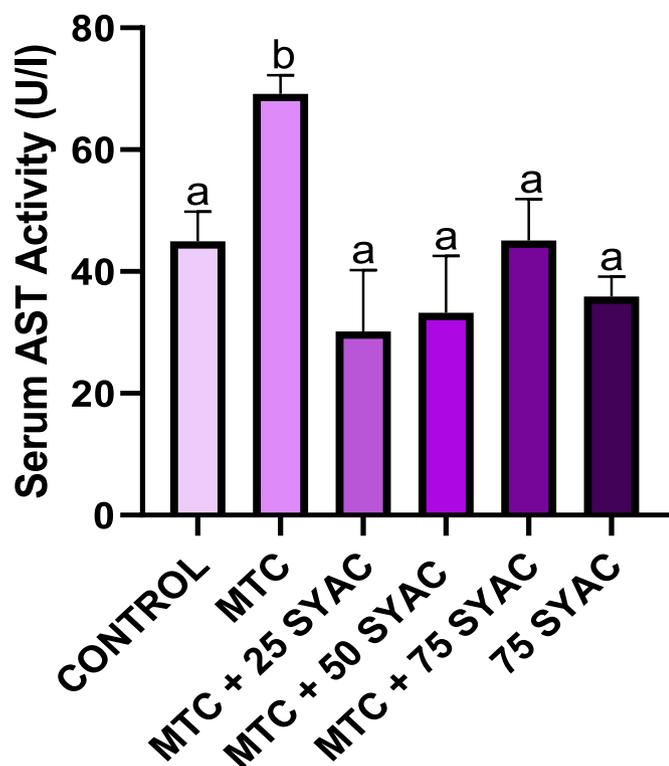


Fig. 9. Serum AST activity in SYAC-treated METCEL administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

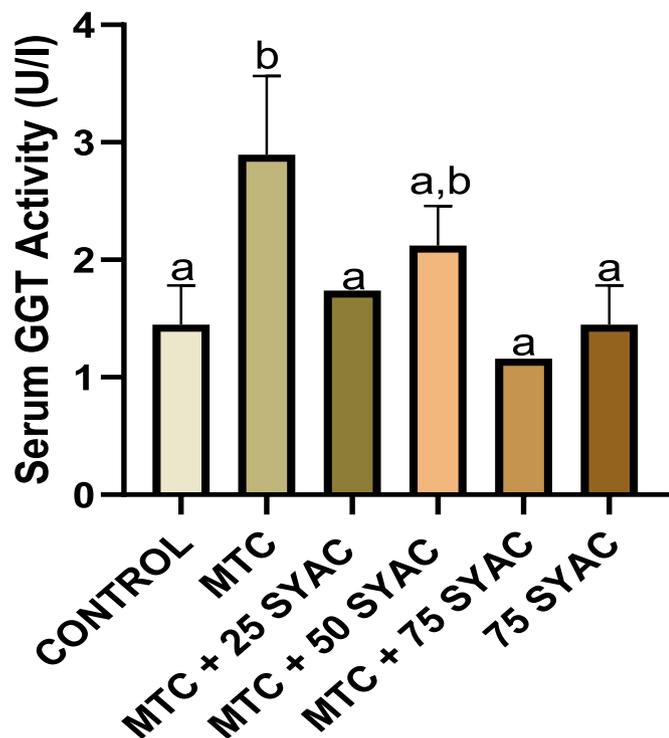


Fig. 10. Serum GGT activity in SYAC-treated METCEL administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

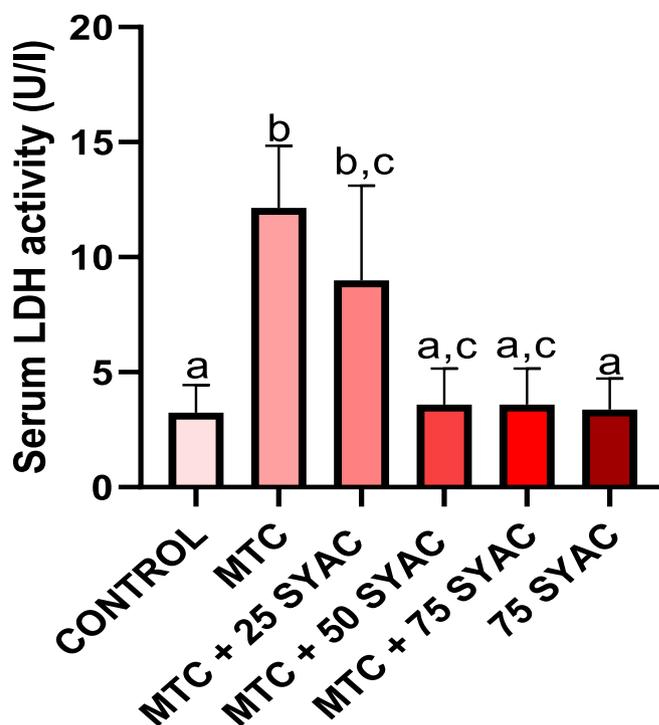


Fig. 11. Serum LDH activity in SYAC-treated METCEL administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

4. Discussion

MTC is a ubiquitous substance known to be hematotoxic, gonadotoxic, teratogenic and spermatotoxic [30]. It is found in industrial and household products including dyes, liquid soaps, lacquers, hydraulic fluids, and pesticides. Because of that, exposures to MTC environmentally and industrially can be inevitable. Therefore, this study looked at the effects of SYAC treatments on MTC-induced spermatotoxicity, hematotoxicity, and tissue damage in rats.

Andrological parameters such as sperm cell motility, concentration, viability (live/dead), and morphology are usually used to know the wellbeing and status of the reproductive system and fertility in males [31]. Such subjects are considered to be infertile if there are high appearances of spermatozoa abnormalities in semen [32]. Administrations of MTC to rats in this study had adverse effect on sperm cells as marked by significant decrease in sperm concentration, live spermatozoa, sperm motility, and increase in sperm abnormalities such as sperm with mid piece bent, headless sperm, reversed head, coiled tail, and tailless sperm. These results are confirmation of previously reported findings by Panchal et al. [33] and Hamdi et al. [34]. MTC may have elicited gonadotoxic and spermatotoxic (alterations in spermatogenesis) effects by attacking the seminiferous tubules where spermatozoa are formed, leading to decrease sperm cell concentration and production of abnormal spermatozoa. Perturbations in spermatogenesis by MTC administration that led to various morphologies may also be responsible for decrease in motility or total immotility recorded in this study. All the doses of SYAC investigated in this study were unable to protect the rats against MTC-induced alterations in spermatogenesis and spermatotoxicity. We further observed that treatment with 75 mg/kg only of SYAC showed no toxicity in the animals but could not treat or exert a positive change following MTC-induced spermatotoxicity. This may have resulted due to the potency of MTC to elicit gonadotoxicity and spermatotoxicity in the rats. In this study, MTC was administered for 30

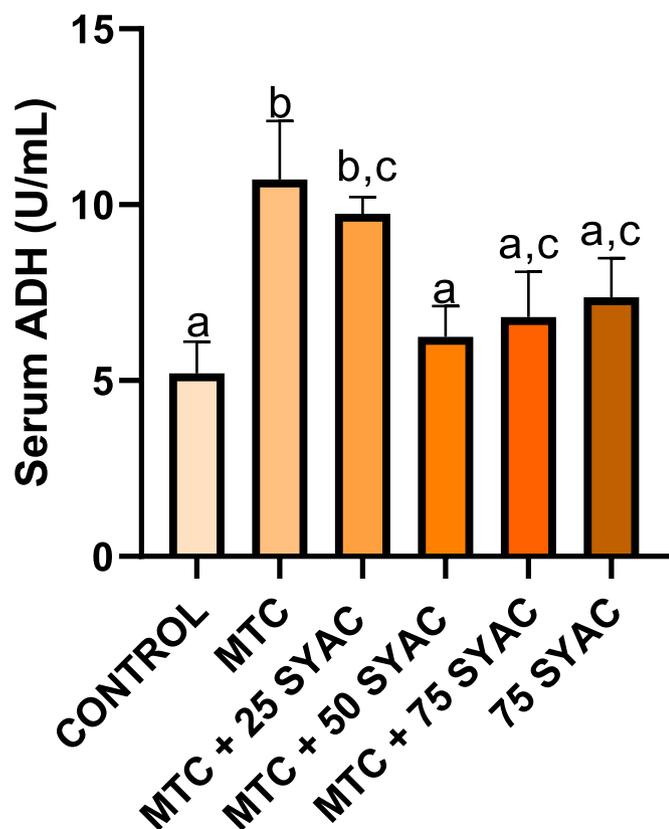


Fig. 12. Serum ADH activity in SYAC-treated METCEL administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

consecutive days, therefore, the proposed reason for the observed effects may be due to the severe testicular cell damage induced by MTC in the rats. The severity of the testicular damage may have been so enormous that SYAC treatments were ineffective, and instead of suppressing the toxicity, it exacerbated the effect of MTC. In other words, SYAC may not be a protective therapeutic agent against MTC-induced gonadotoxicity and spermatotoxicity when administered simultaneously.

The blood is a vital fluid in the body that serves as pathological and physiological indicator of wellbeing in animals [35]. Hb, a component of blood, is the pigment that confers red color of the blood and helps in the transport of oxygen to cells. In this study, MTC-induced decrease in Hb level was recorded. This low level of Hb may indicate altered or inadequate pigmentation and formation of blood in the animals as previously reported by Starek et al. [36] and Adeyemo-Salami and Farombi [37]. PCV (otherwise called hematocrit) indicates the volume of erythrocytes or RBCs in the blood, which is a useful tool to diagnose polycythemia (high red cells) and anemia (low red cells) [38]. Our findings revealed a non-significant decrease in PCV following MTC administrations, suggesting the anemic potential of MTC in the rats, as previously documented by Adeyemo-Salami and Farombi [37] and Starek et al. [36]. WBC and its differentials, made up of the monocytes, eosinophils, neutrophils, basophils, and lymphocytes are involved in the body defense mechanism and are also associated with inflammation [39]. In this study, MTC-induced increase in total WBC and RBC were recorded. Elevated WBC count is an indication that administrations of MTC may have elicited inflammatory reactions in the animals that caused the release and migration of the WBCs to the site of injury, while elevated RBC count (polycythemia) may have resulted in other to meet the oxygen demand of the animals. MCH, MCHC, and MCV are RBC indices. MCH denotes the hemoglobin average mass per RBC in the blood

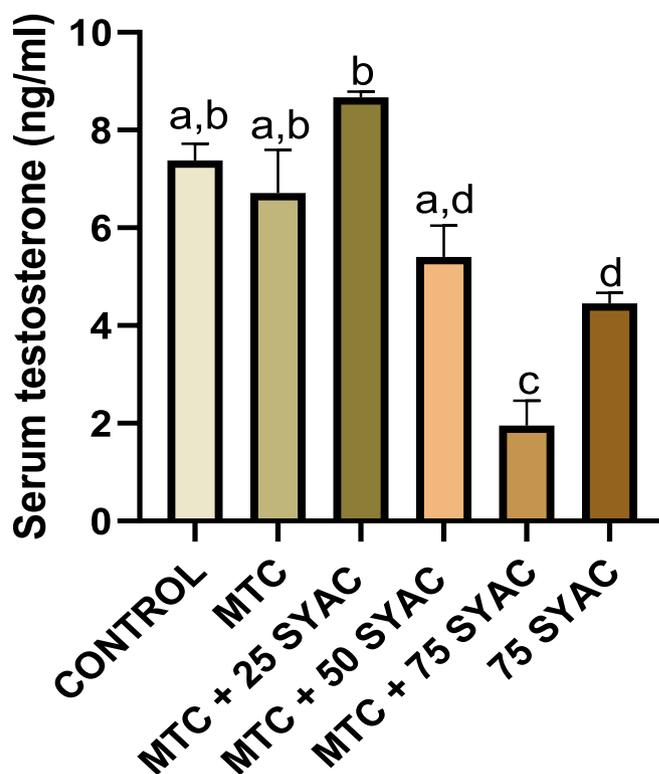


Fig. 13. Serum testosterone concentration in SYAC-treated METCEL administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

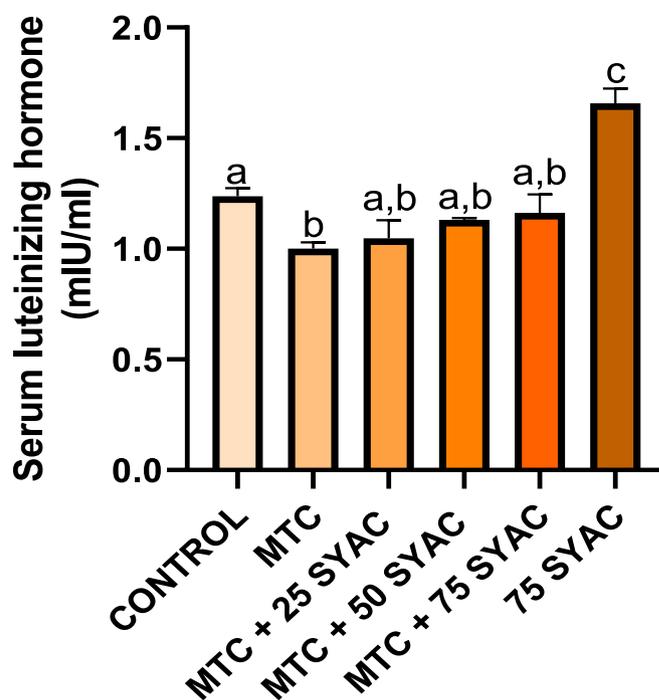


Fig. 14. Serum LH concentration in SYAC-treated METCEL administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

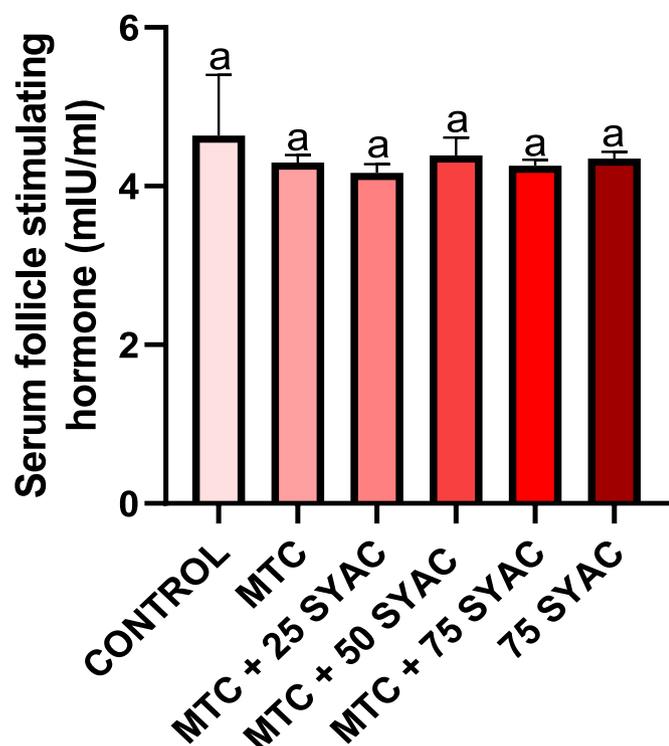


Fig. 15. Serum FSH concentration in SYAC-treated METCEL administered rats. Each bar represents mean \pm standard deviation (SD). Bars carrying different letters entirely are statistically significant ($p < 0.05$). MTC = methyl cellosolve, 25 SYAC = 25 mg/kg syringic acid, 50 SYAC = 50 mg/kg syringic acid, 75 SYAC = 75 mg/kg syringic acid.

sample, MCHC denotes the hemoglobin level in a packed red blood cell volume [39], while MCV denotes the average volume of RBC and gives an idea of the size of each RBC in an animal. In this study, MTC administrations led to the decrease in MCH, MCHC, and MCV in rats. Low MCH and MCHC in the MTC administered rats is an indication of low mass and concentration of Hb respectively in the packed RBC, a condition known as hypochromic anemia, while low MCV in the MTC administered rats is an indication of low RBC size, a condition known as microcytic anemia (microcytosis) that can all occur due to iron deficiency and perturbed process of erythropoiesis in animals. The results obtained in this study agree with the report of Adeyemo-Salami and Farombi [37] that documented similar findings. Also from our findings, we found that SYAC at the dose of 50 mg/kg increased the RBC indices (MCH, MCHC, and MCV) significantly, while 25 mg/kg of SYAC decreased the RBC and WBC counts significantly. These are indications that SYAC may have the ability to protect the blood cells and regulate the normal process of erythropoiesis in the animals.

Cholesterol is a lipid that serves many roles including precursor functions in the biosynthesis of steroid hormones and bile acids. Cholesterol is gotten from fat containing foods where it exists as cholesterol esters and can also be biosynthesized majorly in the liver and other parts of the body like the reproductive tissues, adrenal cortex, and intestine [40]. Cholesterol biosynthesis starts with the formation of mevalonate from HMG CoA, catalyzed by HMG CoA reductase, the rate limiting enzyme that is involved in the regulation of cholesterol biosynthesis [41]. Effect of MTC exposures on level of serum total cholesterol is very sparse, in this study serum total cholesterol level was significantly reduced by MTC administrations. This may be attributed to an altered process of cholesterologenesis either by the inhibition of the rate limiting enzyme (HMG CoA reductase) or the substrate for the rate limiting enzyme (mevalonic acid) is not available for cholesterol synthesis due to MTC-induced hepatotoxicity in the animals. SYAC treatments at 25 and 75 mg/kg body weight significantly increased the serum

total cholesterol, suggesting its hepatoprotective role, as well as hepatocytes and enterocyte's stimulatory role to biosynthesize cholesterol.

Hepatotoxicity which is simply an injury to the liver cells as a result of exposures to hepatotoxic substances such as alcohol e.g., ethanol [42], drugs e.g., acetaminophen [43], environmental toxicants e.g., dimethyl nitrosamine [44], and some food additives. The injury results in an impairment in the hepatic function and destruction of hepatic cell membrane that lead to the release and elevated level of some markers of hepatic function like alkaline phosphatase (ALP), AST, ALT, and GGT into the blood [44]. In this study, MTC-induced hepatic damage was recorded as marked by the significant increase in the activities of serum GGT, AST, and ALT. Again, in this study, SYAC at all doses tested significantly decreased the serum activities of these enzymes, suggesting its hepatoprotective role against MTC-induced hepatotoxicity in rats. Our findings agree with previously published studies by Okkay et al. [45] who reported the hepatoprotective effect of SYAC against thioacetamide-induced hepatic encephalopathy and Gheena et al. [46] who reported the hepatoprotective effect of concurrent administrations of SYAC and silymarin against sodium valproate-induced liver injury in rats.

LDH is an enzyme localized in the cytoplasm of almost every tissue. It can be found in the kidney, heart, liver, and muscle at high levels. Also moderately, LDH can be seen in the erythrocytes. LDH is an oxidoreductase enzyme that catalyzes the formation of lactate from pyruvate using NADH, in a reversible reaction. Basically, LDH comes to play during the anaerobic metabolism of glucose when there a short supply or complete absent of oxygen [47]. In this study, MTC-induced elevation in serum activity of LDH was recorded, an indication that the administrations of MTC may have elicited tissue damage in the animals that caused the release of the enzyme from the damaged tissues to the blood stream, as reported by Feng et al. [48]. Also, in two separate studies, Adedara and Farombi [49,50], reported an increase in the activity of testicular LDH following exposure of rats to ethyl cellosolve. Our study also revealed a significant reduction in the serum activity of the enzyme after SYAC (50 and 75 mg/kg) treatments, which suggests the cytoprotective ability of the phenolic acid. Our result agrees with the reports of Manjunatha et al. [51] who found that SYAC at 50 mg/kg significantly reduced the serum LDH activity in isoproterenol induced cardiotoxicity in rats and Zhao et al. [52] who also found that SYAC at 25 and 50 mg/kg protected the hippocampus against $AlCl_3$ -induced elevation in LDH activity in rat model of Alzheimer's disease.

ADH is a zinc containing enzyme that is abundantly seen in the liver. ADH catalyzes alcohol oxidation to form aldehyde. Similarly, MTC otherwise known as 2-methoxyethanol is oxidized by ADH to form 2-methoxyacetaldehyde and then to 2-MAC by aldehyde dehydrogenase [53]. MTC which is the parent compound is not on its own toxic, but its metabolite 2-MAC. Therefore, ADH is actively involved in the propagation of MTC-induced toxicity. Exposure to MTC repeatedly increases tissue toxicities due to high induction of ADH activity that produces 2-MAC from MTC [53]. Serum ADH activity was significantly increased in this study, which as explained above may be due to MTC exposures in the animals, being the primary enzyme in the metabolism of the glycol ether. The reduction in activity of ADH following SYAC treatments is another confirmation of its cytoprotective property. The mechanism could be that the phenolic acid has metal chelating potentials that prevent the availability of zinc, an element needed by ADH as cofactor to perform its catalytic role. Past studies have documented the metal chelating properties of polyphenols including the study of Li et al. [54] who reported a high Fe^{2+} and Cu^{2+} -chelating activities for rambutan peel phenolics.

FSH and LH formed in the anterior pituitary (AP) are gonadotropins that have stimulatory functions on the gonads (testes in males and ovaries in females), helping them to perform their reproductive duties. LH and FSH are pituitary factors associated with female and male fertility. 2-MAC, a metabolite of MTC acts on specific receptors for these hormones in the AP, causing setbacks in the production of pituitary FSH

and LH [55]. In the Leydig cells, 2-MAC can also block receptors for LH, resulting in the inhibition of testosterone (a steroid reproductive hormone) production [55]. Also, this metabolite of MTC can affect the Sertoli cell functions by decreasing the testosterone transporter proteins levels [56]. Inability of the circulating testosterone to find its way to the seminiferous tubules disturbs the division of germ cell and spermatozoa production. In this study, the serum level of testosterone was insignificantly lowered, while LH level was significantly reduced in the MTC-administered rats. Testosterone is a steroid hormone biosynthesized from cholesterol, and so the decrease in the testosterone level may be due to the significant reduction in serum cholesterol level (its precursor). MTC-induced significant reduction in serum level of LH may be due to the direct attack of MTC toxic metabolite on pituitary gland where the gonadotropin is produced, and as a consequence, this may also be responsible for the reduction of testosterone level, knowing that a major function of LH is to stimulate the Leydig cells of the testis to produce testosterone. Our findings agree with the report of Djabali et al. [55] who also recorded a significant reduction in LH and testosterone following administrations of 50 and 150 ppm of MTC. The ineffectiveness of SYAC treatments at all the doses investigated is an indication that the phenolic acid may not possess hypothalamic-pituitary-gonadal axis protecting properties. This inference may also be justified by the inability of the phenolic acid to protect the testes against the MTC-induced sperm abnormalities recorded in this study.

In conclusion, our findings in this study corroborate the already reported MTC-induced hematotoxicity and spermatotoxicity, but SYAC treatments at all doses investigated did not confer spermatoprotection and hematoprotection against these MTC-induced toxicities but confers hepatoprotection against MTC-induced hepatic damage. Finally, considering the effect of SYAC treatments on all the parameters checked in this study, 50 mg/kg of SYAC (the medium dose) yielded the best effect.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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