Original article

Evaluation of testicular torsion management in Ogbomoso, South-Western Nigeria and surgical detorsion-augmented treatment with phytochemical fractions of *Corchorus olitorius* leaf in experimental rats

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**Abstract**

Background: There is need to investigate whether phytochemicals along with surgical detorsion could serve as better management options in TT patients rather than surgical detorsion (SD) alone.

Methods: The descriptive cross-sectional part of this study is questionnaire-based addressing sociodemographic characteristics of participants and their experience in management of TT. In the experimental part, male rats (n = 32) were grouped into: sham, Ischemia-reperfusion injury (IRI), dichloromethane (DCM) and ethanol fraction (100 mg/kg) of CO. Evaluation of tissue GPx, total thiol, SOD, MDA and H2O2 was done. Serum estimations of nitrite, TNF-α and IL-6, MPO, sperm motility, count and viability was also carried out. Tissue expression of bax and caspase 3 was assessed.

Results: 68.9 % respondents agreed that SD alone is non-effective in the management of TT while 83.6 % reported a need to augment surgery with medications. Oxidative stress markers like H2O2, MDA and nitrite increased by IRI were decreased in post-treatment groups, along with a significant increase in the tissue level of GSH, GST, SOD, GPx, and total thiol. Inflammatory mediators were elevated in IRI while post-treatment rats showed significant decrease. IRI decreased sperm count significantly this was reversed by post-treatment. Bax and caspase 3 was increased in IRI rats and post-treatment with CO fractions reduced them.

Conclusions: Quantitative cross-sectional study has revealed through experience of clinicians that surgical detorsion alone is not effective in managing TT. Augmented treatment with CO leaf fractions suppressed testicular IRI through inhibition of pro-apoptotic proteins expression, oxidative stress and inflammation.

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**Abbreviations:**
ABP, Androgen binding protein; CO, *Corchorus olitorius*; ETC, Electron transport chain; DCM, Dichloromethane; GC-MS, Gas chromatography-mass spectroscopy; GPx, Glutathione peroxidase; GSH, Reduced glutathione; GST, Glutathione-S-Transfase; H2O2, Hydrogen peroxide; IL-6, Interleukine 6; IRI, Ischemia reperfusion injury; MDA, Malondialdehyde; MPO, Myeloperoxidase; NSAID, Non-steroidal anti-inflammatory drug; PCT, Percentage; ROS, Reactive oxygen species; SOD, Superoxide dismutase; TNF-α, Tumor necrotic factor-α; TTDT, Testicular torsion/detorsion; SD, Surgical detorsion.

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1. Introduction

The main goal of urgent and timely management of testicular torsion (TT) is to improve the testicular salvage rate (TSR), prevent the risk of future infertility and reserve reproductive capacity among adolescents. TT is a urologic emergency, that arises from the twisting or rotation of the spermatic cord that supplies the testes (Ellati et al., 2009; Da Justa et al., 2013) which must be diagnosed and treated early to prevent testicular injury (Da Justa et al., 2013). It is common in new born, adolescents and young men (Kapoor, 2008; Eltzschig and Ecke, 2011; George and Nick, 2018), with an overall incidence rate of one in 4,000 males globally (Ellati et al., 2009). In Sub-Saharan African community, the annual incidence rate was reported 2.7 per 100,000 men below the age of 40 (Bello, 2018), and other studies have also reported that TT contributed to infertility in about 1.8 % of sample population in Africa (Ekwere et al., 2007; Kapoor, 2008).

Timely intervention is highly crucial in the management of TT because late diagnosis and delayed management might result in total loss of testicular cells and function (Davol and Simmons, 2005; Drilik and Kocvara, 2013). Currently in the clinical settings, occurrence of TT requires that surgical detorsion is done, to reverse blood flow impedance and ease the transient pain experienced (Patoulias et al., 2017), however, surgical detorsion within the time frame prescribed for the avoidance of necrosis invariably results in testicular ischemia–reperfusion injury (T-IR) (Saito et al., 2012). Testicular reperfusion further exacerbates testicular damage and causes late organ damage through oxidative stress, and activation of inflammatory and apoptotic pathways (Afolabi et al., 2021; Minutoli et al., 2015). In order to reduce testicular damage and increase testicular salvage rate, several experimental studies have been conducted by pretreatments with various agents (Ranae et al., 2011; Ozbal et al., 2012). Given sporadic and unpredictable nature of the occurrence of testicular torsion (Ringdahl and Teague, 2006), the pretreatment regimen may not be practicable in the management of TT in human subjects. However, limited experimental data are available on post treatments mode of intervention, a more realistic management strategy that is realistic for the management human subjects.

Previous studies have highlighted the complexity in management of testicular ischemia–reperfusion injury. Firstly, it has two phases that contributed to testicular damage independently (Granger and Kviety, 2015). The first stage is a brief period that correlates with reperfusion of testicular tissue, oxidative stress is brief and cellular injury can be corrected. Once the oxidative stress lasts for several days, second phase begins and testicular damage has gone berserk (Shayan et al., 2021; Raisi et al., 2020). Secondly, cascades of pathophysiological pathways are activated after TT onset and continue during reperfusion. Thirdly, a need for combination of intervention and timely administration of intervention targeted at each phases separately. Unfortunately, studies conducted have shown that surgical detorsion only may not be efficient in the management of testicular torsion (Sharp et al., 2013). The reason could be that from the point of onset of torsion several pathophysiological processes were activated which serve as a precept to aggravate testicular damage during reperfusion. Hence, there is a need to investigate whether administration of phytochemicals targeted at blocking major pathways and crosstalk of the testicular ischemia–reperfusion injury along with surgical detorsion could serve as better management options in TT patients rather than surgical detorsion only. This study was therefore conducted with the following objectives: to explore the knowledge of medical doctors on the current clinical management of testicular torsion and to assess if surgical detorsion only is effective in managing testicular torsion patients or there is a need to augment surgical detorsion with pathway inhibitors to increase the testicular salvage rate after torsion repair. Phytochemicals obtained from dichloromethane and ethanol fractions of Corchorus olitorius leaf were chosen based on previous reports on role against oxidative stress, inflammation and apoptosis against ischemic-related diseases (Alabi et al., 2020).

2. Materials and methods

2.1. Descriptive cross-sectional study

Descriptive quantitative study was carried out among medical doctors from different area of specialization in different hospital settings (health centers, private, general and teaching hospitals) in Ogbomoso, Oyo State, South-Western Nigeria. The least sample size for the study was estimated through the formula of Cochran for simple proportion:

\[
N = \left(\frac{(1.96)^2 \times P \times (1-P)}{d^2}\right)
\]

Where, \(N\) = Sample size, \(P\) = expected prevalence, \(Q = 1-P\), \(d = \) margin of error. \(P = 1\) in 4,000 males (Ringdahl and Teague, 2006; Kapoor, 2008).

\[
P = \frac{1}{4000} = 0.00025\times 100 = 0.025\%\quad Q = 1–0.025 = 0.975, \quad D = 0.05\% = 0.5%\quad 1.96^2 = 0.025x0.975/0.05^2 = 38.
\]

The minimum sample size was 38, with an addition of 8 (non-response rate of 20 %), 46 was obtained as the sample size. 61 questionnaires were used for the survey.

The stratified random sampling technique was utilized for this study. Medical doctors who gave consent to participate in the study were included after obtaining prior permission from the hospital authorities. They were assured of anonymity and confidentiality. Exclusion criteria were medical doctors who are unwilling to participate in this study. Questionnaire was administered in English and collected back after completion.

Prior to the commencement of this study, ethical was obtained from the Bowen University Teaching Hospital Research and Ethics Committee.

2.2. Solvent extraction of plants

Fresh leaves of C. olitorius were authenticated at the forest research institute of Nigeria Jericho, Ibadan with voucher specimen FHI number (112603). C. olitorius leaves were air dried for 3 weeks and ground into powder and soaked with ethanol 70 % (1:10; w/v ratio) for 72 h. The extract was filtered and evaporated under reduced pressure. The remaining aqueous layer was dried with rotary evaporator. Applying (1:10; w/v ratio), 5 g crude extract was dissolved in 90 % ethanol. The solution was then partitioned with solvents of increasing polarity. The solvents include dichloromethane (% yield was 2 g), and ethanol (% yield was 64 g).

2.3. Laboratory rats

Sprague-Dawley male rats of weight range 150–200 g and average age of 8 weeks, were allowed to feed liberally on commercial rat pellets and water. The research in relation to use of animal has complied with all necessary national regulations and institutional policies for the use and care of animals. The ethical approval number assigned for this study was BUTH-REC –068.

2.4. Reagents

Enzyme-linked immunosorbent assay kit for the evaluation of inflammatory mediators, male sexual hormone, androgen binding protein and inhibin were purchased from Elabscience Biotechnology, Elabscience Biotechnology (USA) also supplied the polyclonal and secondary antibodies for the assessment of pro-apoptotic bax and caspase 3 expression.
2.5. Experimental protocol

Thirty-two male Wistar rats were randomly divided into four groups (n = 8) as follows: sham (Group I), ischemia reperfusion injury (Group II), dichloromethane fraction 100 mg/kg post-treatment (Group III), and ethanol fraction 100 mg/kg post-treatment (Group IV). Animals were administered anesthetic agents (50 mg/kg ketamine and 10 mg/kg xylazine) and all surgical procedures were carried out under sterile condition. For the sham control rats, scrotal region was shaved, dis-infected with 88 % isopropyl alcohol and incision performed at mid-scrotal area. The spermatic cord of left testis was mobilized and returned into the scrotal sac, after which the scrotum was closed with 2–0 chromic catgut suture Ischemic-reperfusion and treatment groups; mid-scrotal incision carried out and spermatic cord mobilized. Counter-clockwise rotation of the testicle with its cord (720°) was done and testis was fixed to the scrotal wall with 6/0 propylene suture. After 90 min of torsion and ischemia, reperfusion through detorsion was effected. Animals were post-treated with 100 mg/kg DCM and ethanol fractions of CO leaf (orally) for seven days. After seven days of detorsion and treatment, the rats were euthanized. All samples including testis and blood were collected for evaluation of biochemical parameters and tissue histopathology. The dosage choice of 100 mg/kg DCM and ethanol fractions of CO were administered via oral route, based on previous study of Alabi et al., 2020.

2.6. Sample collection

Blood was collected through retro-orbital plexus. Serum collection was done through centrifugation at 800 x g for 15 min. The testis was sectioned longitudinally, weighed and a section was stored in bouin’s fluid for immunohistological study. Also, caudal epididymis of left testis was extracted for sperm analysis.

2.7. Biochemical analysis

The testicular tissue level of superoxide dismutase (SOD) was evaluated through the method described by Misra and Fridovich, (1972). The glutathione peroxidase (GPx) level was evaluated by using the method of Beutler et al, (1963). The level of reduced glutathione (GSH) in the testis was assessed at 412 nm using the method of Jollow et al, (1974) and glutathione-s-transferase (GST) activity evaluated by the method of Habig et al, (1974). Tissue total thiol was evaluated through the method described by Ellman, (1959). Serum hydrogen peroxide (H₂O₂) was determined by method of Wolff, (1994). Lipid peroxidation (MDA) was assessed by the protocol described by Vasishnue and Kale, (1990). The level of nitric oxide in the serum was evaluated through estimating the nitrite level as described by Crespo et al, (1999). Myeloperoxidase (MPO) was evaluated by the protocol described by Xia and Zweier, (1997).

2.8. Analysis of sperm

After surgical excision of epididymis, sperm sample was then extracted and diluted with normal physiological saline. The sperm motility of the epididymis was evaluated by estimating motile spermatozoa per unit area and expressed as percentage motility. Hemocytometer was used to estimate the sperm count, which was expressed as million per ml of suspension. The sperm viability was assessed using Eosin/Nigrosin stain (Raji et al., 2003).

2.9. Histology of testis

The testis obtained from all experimental rats were preserved with Bouin’s fluid.

Grossing.

The tissues were observed and cut into small pieces of not more than 4 mm thick into pre-labeled cassettes. These were further immersed in 10 % formal saline for 24 h to fix.

Tissue Processing: this is done automatically using automatic tissue processor (Leica TP 1020). The tissue were allowed to pass through various reagents including; stations 1 & 2 containing 10 % formal saline, station 3 to station 7; alcohol (70 %, 80 %, 90 %, 95 %, absolute 1 & absolute 11) for the purpose of dehydration. The tissues continued to pass through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 h, tissues stayed in each station for 1 h.

Embedding: each processed tissue was given a solid support medium (paraffin wax) and this is done using a semi-automatic tissue embedding center. The molten paraffin wax was dispensed into a metal mold and the tissue was buried and oriented in it, a pre labeled cassette was placed on this and was transferred to a cold plate to solidify. The tissue block formed was separated from the mold.

Sectioning using Microtomy: the blocks were trimmed to expose the tissue surface using a rotary microtome at 6μmicerometer. The surfaces were allowed to cool on ice before sectioning. The tissues were sectioned at 4 μm (ribbon section).

Floating: the sections were floated on water bath (Raymond lamb) set at 55 °C and these were picked using clean slides. The slides were labeled.

Drying: the slides were dried on a hotplate (Raymond lamb) set at 60 °C for 1 h and stained using Haematoxylin and Eosin.

2.10. Evaluation of tissue bax and caspase 3

Testicular expressions of pro-apoptotic proteins (caspase 3 and bax) were estimated through immunohistochemistry as described by Alabi et al., (2020). Briefly, paraffin sections were melted at 60 °C in the oven. Dewaxing of the samples in xylene was followed by passage through ethanol of decreasing concentrations (100 %–80 %). Peroxidase quenching with 1 % H₂O₂/methanol was followed by antigen retrieval performed by microwave heating in 0.01 mol/L citrate buffer (pH 6.0) to boil. All the sections were blocked in normal goat serum (10 %, HistoMark, KPL, Gaithersburg, Maryland, USA) and probed with anti-bax and anti-caspase 3 antibodies, as appropriate (Elabscience Biotechnology, USA), 1:200 overnight at room temperature. Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0 g/mL) secondary antibody and subsequently, streptavidin peroxidase (horseradish peroxidase–streptavidin) according to manufacturer’s protocol (Elabscience Biotechnology, USA). The reaction product was enhanced with diaminobenzidine (DAB, Amresco, USA) for 2–3 min and counterstained with high definition hematoxylin (Enzo, New York, USA), with subsequent dehydration in ethanol. The slides were covered with coverslips and sealed with resinous solution. The immune-reactive positive expression of bax and caspase 3-intensive regions were viewed starting from low magnification on each slide then with 400 × magnifications using a photo microscope (Olympus) and a digital camera (Toupacam; Touptek Photonics, Zhejiang, China). The measurement of immune-reactive positive expression of bax and caspase 3– were assessed digitally with the aid of a quantification software (ImageJ 1.48 v; National Institutes of Health, Bethesda, MD, USA).

2.11. Gas chromatography mass spectroscopy (GC–MS)

The detection of active components in polar ethanol solvent was carried out using GC–MS. An Agilent instrument with 7890
GC technology was used for analysis, and an Agilent detector model with 5975 MSD technology (Mass Spectroscopy Detector) was used. In the methods of chromatographic separation, there are two phases that include mobile and stationary phases. The mobile phase was the carrier gas (helium, 99.99% purity), and the stationary phase was the column (model HP5 MS column with a length of 30 m, an internal diameter of 0.320 mm and a thickness of 0.25microns). The oven temperature program is an initial temperature of 80°C, which must be maintained for 1 min. It increases by 10°C per minute to a final temperature of 240°C for 6 min. The injection volume was 1 µL, and the temperature of heater or detector was 250°C. Operation: The sample extracted was placed in a vial bottle, and the vial bottle was placed in an auto-injector sample compartment. The automatic injector introduced the sample into the liner. The mobile phase pushed the sample of the liner into the column, where the separation into a different component occurs at different retention times. The MS interprets the MZ spectrum (mass-to-charge ratio) with molar mass and structures.

2.12. Statistical analysis

All values were expressed as the mean ± standard error of mean. The difference between two groups were compared using t-test (paired), and analysis of variance (ANOVA) was used for comparison within all groups along with Tukey’s post hoc test. Statistical analysis were carried out with GraphPad Prism 5.0 version. All statistical significance was set as p < 0.05.

3. Results

The results were divided into three parts: the sociodemographic characteristics of the study participants.

Research questions addressing the participants’ experiences in the management of their TT patients.

Experimental induction of reperfusion injury in rats and sequential post-treatment with COLF.

3.1. Sociodemographic characteristics of the study participant

From the frequency distribution Tables 1-3, medical doctors from teaching hospitals represent the largest study participant while those from general hospital are the least on the frequency table in Ogbomoso community. Medical school graduates of 2016–2021 represent the highest participants, followed by 2010–2015 graduates and the least participant is 2004–2009 set. Pediatricians are the least respondents while the surgeons are the highest respondents in this study.

3.2. Assessments of research questions

**Table 1**

| Category of hospital | Frequency | %  |
|----------------------|-----------|---|
| Private              | 5         | 8.2|
| General              | 3         | 4.9|
| Teaching             | 46        | 75.4|
| Non-response rate     | 7         | 11.5|

None of the respondent utilized Medical treatment (M.T) only, 21.4 % (13) utilized medical treatment plus referral only, and 55.7 % (34) utilized surgery while 22.9 % (14) does not specify their treatment approach for the management of TT (Fig. 1B).

Results obtained from Fig. 1C shows, 11.5 % (7) reported that orchiectomy should be performed in<6 h of severe testicular torsion, and 44.3 % (27) reported that orchiectomy should be performed at exactly-six hours of severe testicular torsion. 21.3 % (13) reported that orchiectomy should be performed after six hours of severe testicular torsion, while 9.8 % (6) reported that orchiectomy should be performed after 12 h of severe testicular torsion while non-response rate of 13.1 % (8) was reported.

From Fig. 1D, 8 % (6) of the respondents’ reported infarction only as the outcome of delayed surgical intervention, 14.8 % (9) reported only infertility, 8.2 % (5) reported testicular atrophy only, 1.6 % (1) reported infarction and testicular atrophy, 4.9 % (3) reported infertility and testicular atrophy, 8.2 % (5) reported infarction and infertility while 52.5 % (32) reported infarction, infertility and testicular atrophy as the consequences of delayed surgical intervention.

Based on participants knowledge on the effectiveness of surgical detorsion only in the management of testicular torsion, 68.9 % (42) of the participants faulted the effectiveness of surgical detorsion only in the management of TT while 29.5 % (18) supported the effectiveness of surgical detorsion in the management of TT (Fig. 2A). 83.6 % suggested a need to augment surgery with medication in the management of testicular torsion while 16.4 % (10) of the participants disagree on the need to augment surgery with medication (Fig. 2B).

Augmenting surgical detorsion with medications as reported by the participants; 54.1 % (33) reported infection prevention, 9.8 % (6) reported for prevention of future infertility, 1.6 % (1) reported pain management, 11.5 % (7) reported infection and future infertility prevention, 4.92 % (3) reported infection prevention pain management, none of the respondents reported pain management and prevention of future infertility, 1.6 % (1) of the respondents reported to prevent infection, future infertility and management of pain while 16.4 % (10) non-response rates were received from the respondents (Fig. 1E).

Respondents’ records of number of misdiagnosed cases of TT patients in their years of practice; 6.56 % (4) of the respondents reported previously misdiagnosed cases of TT while 81.9 % (50) reported no misdiagnosed cases of torsion of the testes before. 11.5 % non– response rate was reported from the respondents (Fig. 2C). In addition, 75. % (3) have previously misdiagnosed Epididymoorchitis instead of TT while only 25 % (1) of the respondents misdiagnosed hydrocele in place of TT (Fig. 2D).
3.3. Experimental reperfusion injury and post-treatment

The results obtained from reperfusion injury and treatment study showed that testicular torsion/detorsion-induced reperfusion injury enhanced the tissue level of oxidative stress bio-markers ($\text{H}_2\text{O}_2$, MDA and nitrite) ($p < 0.05$), (Fig. 3A, B and C) in the testis. This was reversed, when rats were treated with dichloromethane DCM and ethanol fractions of *Corchorus olitorius* (CO) leaf (Fig. 3A, B and C). Similar to this result, testicular tissue antioxidants (GSH, total thiol, SOD, GPx, and GST) were...
significantly reduced in TT-IRI group compared with sham (Fig. 4A-D and Fig. 5A-B). This was reversed when rats were post-treated with DCM and ethanol fractions of CO leaf significantly (Fig. 4A-D and Fig. 5A-B).

Significant elevation of MPO and TNF-α in serum was observed when compared with sham. Serum IL-6 level was increased insignificantly. Post-treatment of rats with DCM and ethanol fractions experienced a significant decrease (p < 0.05) in the tissue level of MPO. DCM fraction significantly reduced the TNF-alpha while ethanol fraction reduced the serum level of IL-6 significantly compared with reperfusion injury group (Fig. 6A, B and C).

Reperfusion injury reduce the serum testosterone level (p < 0.05). Testosterone level was significantly increased in treatment rats compared with sham and IRI. Serum ABP was significantly increased in the DCM post-treated rats only. IRI and treatment did not alter serum level of inhibin across (Fig. 7A, B and C).

The result obtained from reperfusion injury rats showed a slight but insignificant reduction in sperm count and sperm viability compared with sham. Post-treatment increased sperm count and could not exert any effect on viability. Sperm motility was significantly reduced in TT/IRI rats when compared with sham (p < 0.05). Post-treatment of rats with DCM and ethanol leaf fraction of CO significantly improved the sperm motility (p < 0.05) (Fig. 8A-B and C).

Reperfusion injury increased the expression of bax and caspase 3 in testis (p < 0.05) sham control rats and treatment rats showed a significant reduction in tissue expression of caspase 3 and bax (Fig. 9A-B, Fig. 10A1-D2).

The GCMS analysis of ethanol fraction of CO leaf suggest the presence of 6 polar soluble compounds. 2-isopropylethylamine (area pct 55 %), and vanadocene 1,2,3,4,5-pentamethyl (area pct 15 %) are the 2 major compounds suspected to be present inside the ethanol fraction (Fig. 11A, B and C).

4. Discussion

The socio-demographic result showed that the participants were recruited from varying years of experiences across different specializations in various hospital settings. This study’s findings may represent the baseline knowledge of large number of medical personnel in the management of TT which may further help to improve its management strategies. It further corroborates the findings of Kitami, (2017) that accurate knowledge and sufficient experience about different management techniques of TT are essential for medical practitioners to make correct diagnosis and proper management of TT. The second research question investigated different management strategies the respondents have previously employed in the management of TT. As reported by previous studies, proper management strategies are essential for both the survival of the testes and maintenance of reproductive capacity (Baruga and Munabi, 2013; Patoulias et al., 2017). From this survey, 55.7 % (34) of the respondents utilized surgery only as their main line of management of TT. This is because S.D is the baseline treatment to re-establish blood flow and ease the transient pain associated with TT as reported by (Shergill, 2003; Kapoor, 2008). Also, the area of specialization of the respondents determines the management strategy as well. Since, TT is a prevalent surgical and urological emergency in adolescents and men, surgeons and pediatricians form the major parts of the respondents that fall into the category of those that utilized surgery (surgical detorsion) in the management of TT and this is in line with findings from Baruga and Munabi, (2013) and Patoulias et al., (2017).

The next research question sought to investigate the time of torsion onset that surgical detorsion would no longer be necessary rather than orchiectomy. From this study, 86.9 % (53) of the respondents altogether, agreed that orchiectomy should be performed with severe testicular pain that spanned within and beyond six hours of testicular torsion. This finding is similar to
the report of Kapoor (2008) that severe testicular damage occurs within six hours of testicular torsion. The outcome of this study also agree with previous report of Visser and Heyns, (2003) which shows that orchiectomy should be performed after six hours of TT onset. Ayman and Victor, (2006) therefore reported that early pre-presentation and proper management of TT as early as possible before six hours of TT onset may help to increase the chance of saving the testes.

As part of the research question addressed in this study, the respondents reported various complications associated with delayed management of TT. These complications are infarction, testicular atrophy and infertility. Some of the respondents also reported double complications such as infarction and testicular atrophy, infertility and testicular atrophy, and infarction and infertility. Also, some of the respondents reported testicular atrophy, infertility and infarction altogether as the main consequence of delayed management of TT. This findings correlate with the study of Sharp et al. (2013) that revealed infertility and testicular atrophy as part of the consequences of delayed management of TT.

Furthermore, the limitation of successful surgical detorsion (SD) only in the management of TT has been recognized in previous studies (Sharp et al., 2013). Then, the question raised among the respondents is how effective is surgical detorsion only in the management of TT? 68.9 % (42) of the respondents reported that SD only is not effective in the management of TT while 29.5 % (18) reported that SD only is effective in the management of TT. The reason majority of the respondents faulted the effectiveness of surgical detorsion only may be that they are aware that SD causes reperfusion injury which further exacerbate testicular damage. This agrees with findings of literatures (Kalogeris et al., 2014; Minutoli et al., 2015). The outcome of this finding therefore led to the generation of another question on whether or not there is need to augment SD with medications.
to improve the testicular salvage rate (TSR). 83.6% (51) agreed on the need to augment SD with medications while 16.4% disapproved on the need to augment surgery with medications. Since majority of the respondents agreed on augmenting surgery with medication, the purpose for augmentation was investigated among the respondents. From this study, the respondents’ purposes include; to prevent infection, future infertility and for pain management.

**Fig. 6.** The role of IRI and post-treatment on tissue level of MPO, TNF alpha and IL-6 represented on bar chart. Data was expressed as mean ± SEM. *Data represent significant difference from sham control (*p < 0.05, **p < 0.01, *** p < 0.001). **Data represent significant difference from reperfusion injury (*p < 0.05, **p < 0.01, *** p < 0.001).

**Fig. 7.** The role of IRI and post-treatment on serum level of testosterone, androgen binding protein and inhibin represented on bar chart. Data was expressed as mean ± SEM. *Data represent significant difference from sham control (*p < 0.05, **p < 0.01, *** p < 0.001). **Data represent significant difference from reperfusion injury (*p < 0.05, **p < 0.01, *** p < 0.001).
From the experimental study design, depletion of antioxidants like SOD, GSH, GPx, GST and total thiol concomitantly with increased level of hydrogen peroxide, nitrite and MDA are indicators of oxidative stress in testicular torsion/detorsion (TTDT)-induced reperfusion injury (Weidinger and Kozlov, 2015). The reversal of the indices of oxidative stress when rats were post-treated with DCM and ethanol fraction of CO further confirm the role of CO leaf fractions against oxidative stress thereby suggesting the antioxidant role of the CO leaf fractions in the management of detorsion-induced injury in testis. GC–MS analysis of DCM fraction in our previous study suggest the presence of active compounds like cystamine related structure, benzenthiol, vanadocene 1,2,3,4,5-pentamethyl, 3-methoxyflavone and 2-methoxy-4-vinylphenol. The result obtained from GC–MS analysis of the ethanol fraction from this study suggested the presence of 6 compounds. From the 6 compounds suggested to be present in the ethanol fraction, 2-isopropylethylamine (area pct 55 %), and vanadocene 1,2,3,4,5-pentamethyl (area pct 15 %) are the suspected active compounds. 2-isopropylethylamine was shown to possess the largest percentage composition of about 55 %. 2-isopropylethylamine is a known organic compound with amine and a pair of electron on its nitrogen atom. The presence of a lone of electrons enable this compound to react with electrophiles (Sorgi, 2001) and thus referred to as proton scavenger. The presence of this compound in abundance can act synergistically with other active compounds of the ethanol fraction to elicit the observed mitigating effects against testicular reperfusion injury in this study.

The observed elevation of MPO and TNF-α in testicular IRI confirms inflammatory response as one of the pathophysiological mechanisms that lead to the adverse effects of reperfusion injury as documented in other studies like that of Beutler, (1999). Both DCM and ethanol leaf fractions of CO reduced MPO activity thus displaying their anti-inflammatory activities. However the
differential effect of the two fractions whereby the DCM fraction but not the ethanol fraction reduced the TNF-α in TTDT rats may indicate specific anti-inflammatory actions of the different components of the fractions. The DCM fraction may possibly inhibit the early production of membrane bound cytokines from monocytes, macrophage and T-lymphocyte, thereby reducing the serum TNF-α level. The ethanol fraction seems to exert its protective effect on the sertoli cells and seminiferous peritubular cells through a strong inhibition of IL-6 production from these testicular cells along with its late production from macrophages. The TNF-α is potent chemoattractant of neutrophil, a strong producer of ROS and MPO. Serum MPO was decreased by DCM and ethanol fractions but more greatly reduced in the DCM fraction treatment rats. The effect of DCM fraction on MPO further confirm the correlation between DCM fraction and TNF-α. This result also suggests that DCM fraction of CO leaf can be a strong inhibitor of early pro-inflammatory response.

The observed increase in bax and caspase 3 (which are pro-apoptotic proteins) expression in the testes of TTDT rats is similar to result of other Al-Maghrebi et al., (2012), which demonstrated increased apoptosis in IRI and correlation with testicular atrophy. Activation of ROS, inflammation and other pathways in response to ischemic insult and reperfusion injury is known to eventually trigger the death of germ cells, sustentacular cells, and seminiferous peritubular cells (Kumar et al., 2015). This death can either be by apoptosis or necrosis with apoptosis usually the early and most common form of death triggered by reperfusion injury. The reduced level of bax and caspase 3 protein expression in the DCM and ethanol fraction treated rats suggest that CO leaf fractions can protect the testicular tissue against detorsion-induced injury through the inhibition of apoptosis. Inhibition of testicular cell apoptosis by DCM and ethanol fractions may be cause of enhanced testosterone and androgen binding protein level in treatment rats compared with reperfusion injury non-treated rats.

Sperm cell parameters such as number, motility, morphology and viability are major determinants of ability to achieve conception and as such defects or deficiency in them account for many cases of infertility (Harris et al., 2011). TTDT could not exert a significant effect on sperm count and sperm viability. Similar to the study of Abbas et al., 2021 and Fashid et al., 2021, the sperm motility was drastically reduced by TTDT compared with the control. The reduced motility could be due to protein damage induced by excessive ROS production, leading to electron transport chain (ETC) proteins damage, lipid peroxidation and DNA impairment which can impair mitochondrial function (Costantini et al., 1996) and ATP production. Since the high energy in form of ATP is required by the flagella to propel the sperm, disruption of ATP production would reduce their motility. From the present study, improved sperm motility by DCM and ethanol fraction treatment could be as a result of the presence of anti-oxidant compounds in the two fractions, thereby preserving the mitochondrial function (Adel et al., 2009).

That the sperm count and viability were unaffected by TTDT in the present study could be due to the short duration of the experiment, especially if we consider the fact that semen samples extracted from epididymis are mature spermatozoa. The sperm count and viability of epididymis semen could not have been altered in the acute reperfusion period of 7 days.

In conclusion, quantitative cross sectional study has shown through the experiences of the medical doctors from the research questions that surgical detorsion only is not effective in the management/treatment of testicular torsion patients and there is need to augment surgery with medications to prevent post-surgical complications following testicular torsion repair in the future. Ameliorative effect of DCM and ethanol fractions of CO in the testis of experimental rats through attenuation of oxidative stress, inflammatory response and suppression of proteins like bax and caspase 3 further confirm the need to augment surgery with treatment. Aside chemical agents or drugs, phytochemicals targeted at blocking major pathways of the testicular ischemia–reperfusion injury along with surgical detorsion (SD) could serve as better managements options.
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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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