Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2020.72.78

Research Article
Therapeutic Potential of Methanolic Extract of Fruits of *Physalis angulata* against *Salmonella typhi* Infection

[1]Tolulope O. Oladosu, [2]Tinuola T. Adebolu and [2]Muftau K. Oladunmoye

[1]Department of Microbiology Federal University of Agriculture, Makurdi, Benue State, Nigeria
[2]Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria

Abstract

Background and Objective: Drug resistant *Salmonella typhi* is an alarming issue in the health sector which has drawn attention of researchers to search for alternative therapeutic sources. Due to increase in resistance of *Salmonella typhi* to antibiotics, the efficacy of methanolic extract of fruits of *Physalis angulata* (PFM) in treating *S. typhi* infection was investigated in present study. **Materials and Methods:** Apparently healthy Wistar rats were orogastrically challenged with ID of *S. typhi* multi-antibiotic resistant (MAR) isolate. At onset of signs of illness, the infected rats were divided into three groups-the first group was treated with the minimum inhibitory concentration (MIC) of the extract (400 mg kg^{-1} b.wt.) the second group with minimum bactericidal concentration (MBC) of the extract (500 mg kg^{-1} b.wt.) while the third group was left untreated. Haematological and histopathological studies were carried out on the blood and the vital internal organs of the rats respectively at the termination of treatment. Liver function tests were also carried out on the rats. **Results:** Treatment of *S. typhi* infected rats with PFM (400, 500 mg kg^{-1} b.wt.) caused the recovery of the rats between 2 and 3 days and also caused the packed cell volume (PCV) of the infected rats to increase from 30.00±0.10% to 39.00±0.50% and 41.00± 0.20%, respectively. A similar trend was observed for haemoglobin, Red Blood Cells (RBCs) and neutrophil counts. On the other hand, it caused the counts of total White Blood Cells (WBC) and lymphocyte of the *S. typhi* infected rats which shot up as a result of the infection to reduce considerably. **Conclusion:** Hence, concluded that PFM exerted therapeutic, haematinic and immunomodulatory effects on the *S. typhi* infected rats treated with the extract.

Key words: *Salmonella typhi*, *Physalis angulata,* therapeutic effects, immunomodulation, haemoglobin, biochemical analysis

Citation: Tolulope O. Oladosu, Tinuola T. Adebolu and Muftau K. Oladunmoye, 2020. Therapeutic potential of methanolic extract of fruits of *Physalis angulata* against *Salmonella typhi* infection. Res. J. Med. Plants, 14: 72-78.

Corresponding Author: Tinuola T. Adebolu, Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salmonella typhi is a member of a large group of bacteria which are of prominent public health impact globally[1]. This bacterium belongs to a family called Enterobacteriaceae, whose members are usually Gram negative bacilli resident in the gastrointestinal tract. *Salmonella typhi* is the aetiology of typhoid fever which is normally treated with conventional antibiotics however because of the emergence of antibiotic resistant strains, treatment of this infection has become a serious task therefore the need for alternative therapy.

Resistance displayed by microorganisms to most commercially available conventional antibiotics is not a new phenomenon. The surge in antibiotic resistance however has led to the empirical search into the exploitation of plants for medicinal use[2]. Most of the plants being researched on have a history of being used in folklore medicine in different parts of the world.

Physalis angulata L. is an herbaceous annual plant belonging to the family Solanaceae. It is used in folklore medicine to treat many diseases in the western part of Nigeria. It is reported to have antibacterial activity against many bacterial species[3,4]. The *in vitro* research carried out by our team also reported the antibacterial activity of the methanolic extract of the fruits of *Physalis angulata* L. against multiple antibiotic resistant strain of *S. typhi*[5,6]. It therefore becomes worthwhile to investigate whether the extract can be exploited as an alternative to conventional antibiotic in the treatment of induced *S. typhi* infection in Wistar rats as part of the search for a more effective therapy. In this study therefore, assays such as histopathology, haematology and biochemistry were used in investigating the therapeutic and immunological potentialities of the methanolic extract of the fruit of *P. angulata* against infections caused by *S. typhi in vivo.*

MATERIALS AND METHODS

Study area: This research was carried out between February-April, 2017, at the Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria. The animal bioassay was conducted in the Animal House of the Department of Anatomy, College of Medicine, Afe-Babalola University, Ado-Ekiti, Ekiti State, Nigeria

The *Physalis angulata* plant used was authenticated at the University of Lagos Herbarium with the authentication number LUH: 7660. The *S. typhi* used in this investigation was isolated from HIV patients in a tertiary healthcare institution in Nigeria using the method of Cheesbrough[7]. The isolate *S. typhi* (MAR) was a multi-antibiotic resistant strain but which was susceptible to PFM using agar well diffusion assay carried out during preliminary studies. The MIC and MBC of the extract were determined adopting the method of EUCAST[8] and Buller *et al.*[9] respectively. The method of Adebolu *et al.*[10] was adopted for the animal bioassay. Acclimatization was carried out for a period of 14 days. The Infectivity dose (ID) of *S. typhi* was determined using fifteen Wistar rats. The rats were infected orogastrically. The determined ID (1.1×10^6 CFU mL^{-1}) was administered to the rats and they were observed for a period of five days for signs of infection. The Wistar rats were divided into three therapeutic groups, the 1st group was treated with the MIC (400 mg kg^{-1} b.wt.), the sec group was treated with the MBC of PFM (500 mg kg^{-1} b.wt.) while the 3rd group was not untreated. The treatment was initiated at the establishment of infection and terminated at 120 h of administration of extract. Haematological analyses were carried out to determine the total WBC, differential WBC, RBC counts, PCV and haemoglobin, using a standard reference[11]. Biochemical markers were analysed by adopting the methods of Shakya *et al.*[12] with little modifications while histopathological studies were carried out following the procedures of Slaoui and Fiette[13].

Statistical analysis: Data generated were subjected to ANOVA using XL-Sat.2016 version.

RESULTS

Effect of PFM on recovery duration: The *S. typhi* infected rats treated with PFM (500 mg kg^{-1} b.wt.) recovered within two days of the commencement of treatment, while the group treated with PFM (400 mg kg^{-1} b.wt.) recovered within 3 day of commencement of treatment (Table 1).

Effect of PFM on PCV and other blood parameters: Treatment of *S. typhi* infected rats with PFM caused the PCV which reduced from 50.00±0.33% in uninfected rats to 30.00±0.10% as a result of the infection to increase to 41.00±0.20% for the rats administered the MBC of PFM (500 mg kg^{-1} b.wt.) and 39.00±0.50% for the rats treated with the MIC (400 mg kg^{-1} b.wt.) of PFM. Similar trends were observed in the haemoglobin and RBC counts (Fig. 1).

Effect of PFM on total and differential WBC counts: Treatment of *S. typhi* infected rats with PFM caused the WBC and lymphocyte counts which increased as a result of the

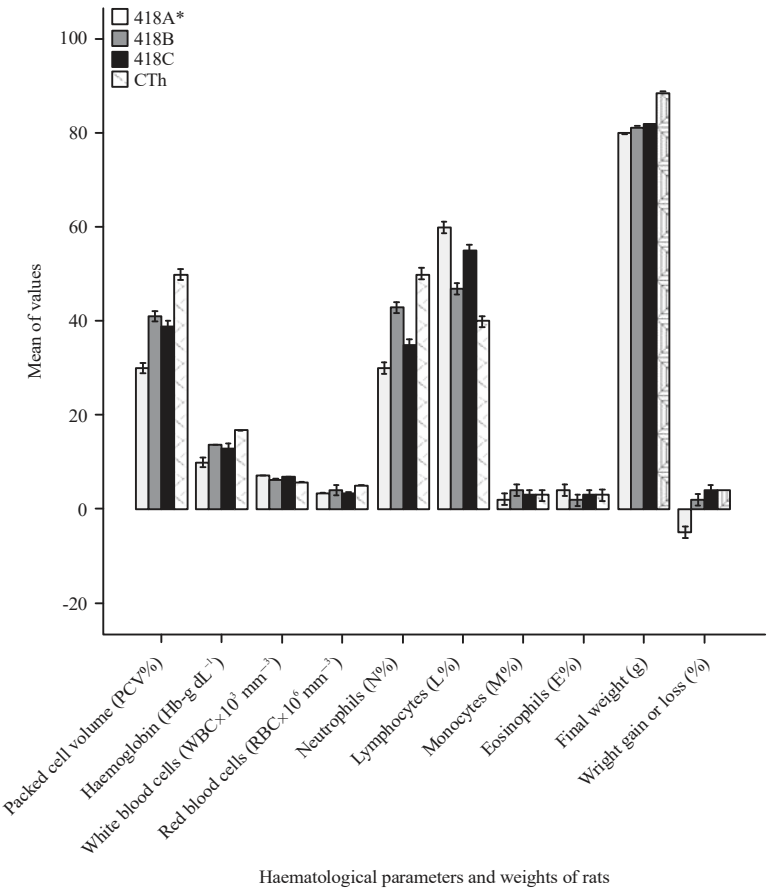

Fig. 1: Effects of administration of PFM on the haematological parameters and weights of rats infected with *S. typhi*, 418A*: Subgroup infected with *S. typhi* but not treated (Negative control), 418B: Subgroup infected with *S. typhi* and treated with 500 mg kg^{-1} of PFM extract, 418C: Subgroup infected with *S. typhi* and treated with 400 mg kg^{-1} of PFM extract, CTh: Therapeutic control

Table 1: Effect of administration of different doses of PFM on the recovery of rats infected with multi-antibiotic resistant *S. typhi*

Groups	Infectivity dose (CFU mL^{-1})	Onset of symptoms (days)	Dosage administered	No. of days for treatment	Recovery period (days)	Control (rats used)	Recovery period for control
S. typhi (418B)	1.1×10^6	2	500 mg kg^{-1}	5	2	-	0
S. typhi (418C)	-	-	400 mg kg^{-1}	5	3	3	-

418B: Group infected with *S. typhi* and treated with 500 mg kg^{-1} of PFM extract, 418C: Group infected with *S. typhi* and treated with 400 mg kg^{-1} of PFM extract

infection ($7.20\pm0.12\times10^3$ mm^{-3} and 60.00 ± 0.38%, respectively) from $5.80\pm0.11\times10^3$ mm^{-3} and 40.00 ± 0.46%, respectively on the other hand reduce to $6.40\pm0.35\times10^3$ mm^{-3} and 47.00 ± 0.45%, respectively for those administered with PFM at 500 mg kg^{-1} b.wt. These values were a little lower than the values obtained from the groups treated with 400 mg kg^{-1} of PFM. On the other hand, treatment with PFM caused the neutrophil counts which decreased from 50.00 ± 0.41% to 30 ± 0.21% as a result of the infection to increase to 43.00 ± 0.11% for the group treated with 500 mg kg^{-1} of PFM and 35.00 ± 0.20% for those treated with 400 mg kg^{-1} b.wt. (Fig. 1).

Effect of treatment of *S. typhi* infected rats with PFM on weight: Treatment with PFM also caused weight gained in the *S. typhi* infected rats treated with PFM (500 mg $kg^{-1}\pm2$%, 400 mg $kg^{-1}\pm4$%) as against the infected rats that were not administered the PFM (Fig. 1).

Effect of treatment of *S. typhi* infected rats with PFM on liver enzymes: Infection of rats with *S. typhi* caused an increase in alkaline phosphatase (ALP) enzyme levels which further increased with treatment with PFM (Fig. 2). On the other hand, treatment of *S. typhi* infected rats with PFM caused a decrease in aspartate transaminase (AST) levels

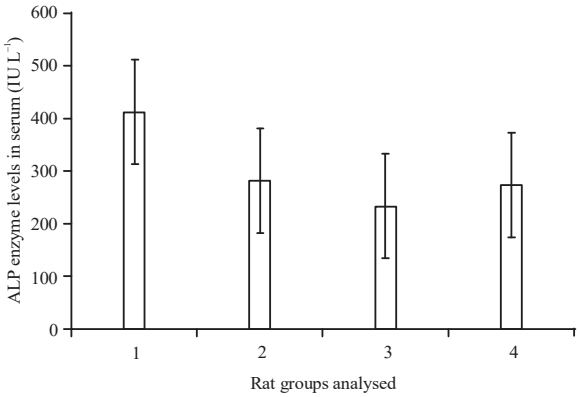

Fig. 2: Effects of administration of methanolic extract of fruits of *Physalis angulata* (PFM) on alkaline phosphatase levels of rats infected with *S. typhi*

1: Infected with *S. typhi* and treated with 400 mg kg^{-1} b.wt., of PFM, 2: Infected with *S. typhi* and treated with 500 mg kg^{-1} b.wt., of PFM, 3: Rat group not infected and not treated with PFM, 4: Infected with *S. typhi* and treated with PFM

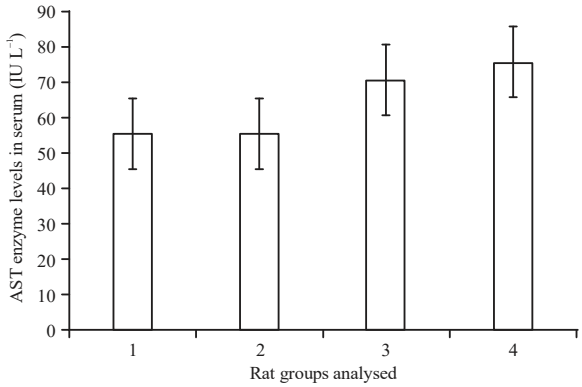

Fig. 3: Effects of administration of methanolic extract of fruits of Physalis angulata(PFM) on Aspartate transaminase levels of rats infected with *S. typhi*

1: Infected with *S. typhi* and treated with 400 mg kg^{-1} b.wt., of PFM, 2: Infected with *S. typhi* and treated with 500 mg kg^{-1} b.wt., of PFM, 3: Rat group not infected and not treated with PFM (Control group 1), 4: Infected with *S. typhi* and not treated with PFM (Control group 2)

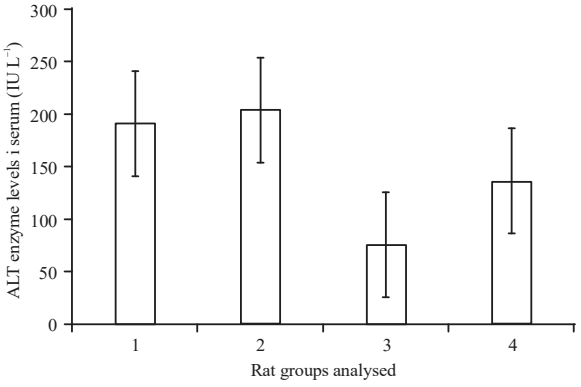

Fig. 4: Effects of administration of methanolic extract of fruits of *Physalis angulata* (PFM) on Alanine Transaminase levels of rats infected with *S. typhi*

1: Infected with *S. typhi* and treated with 400 mg g^{-1} b.w.t., of PFM, 2: Infected with *S. typhi* and treated with 500 mg kg^{-1} b.w.t., of PFM, 3: Rat group not infected and not treated with PFM (Control group 1), 4: Infected with *S. typhi* and not treated with PFM (Control group 2)

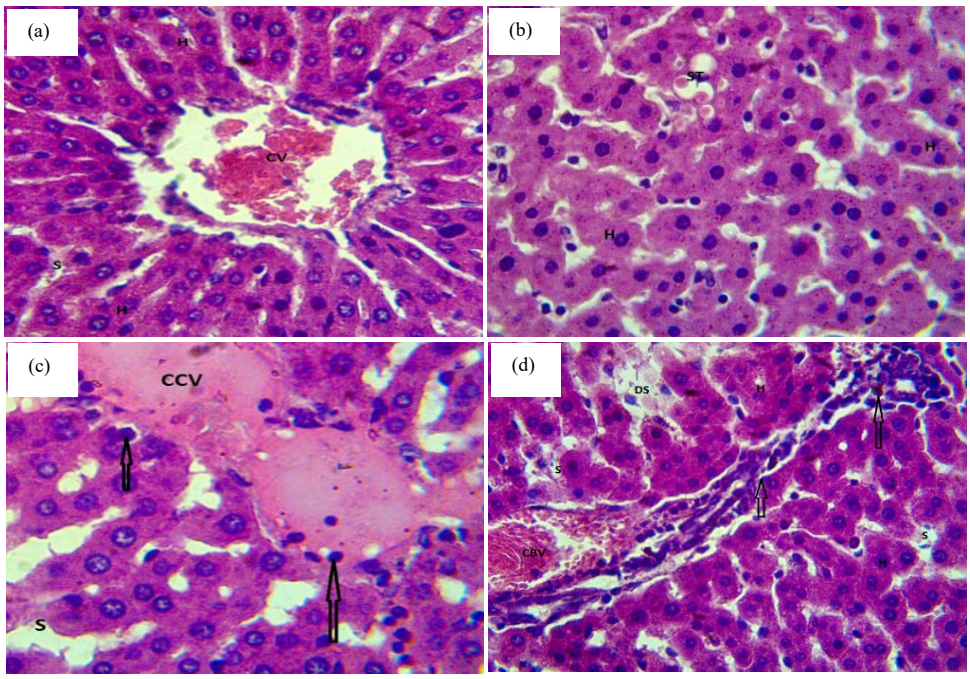

Fig. 5(a-d): Photomicrograph of the (a) Liver of a rat infected with *S. typhi* but not treated with PFM. Haemorrhage occurs at the central vein (CV), (b) Liver tissue of control rat (Not infected and not treated), (c) Section of a liver from a rat infected with *S. typhi* that is treated with 400 mg kg^{-1} of PFM. Congested central vein (CCV) and lymphocytosis (arrow) were observed. The hepatocytes (H) appear normal and (d) Liver sample from a rat infected with *S. typhi* and treated with 500 mg kg^{-1} of PFM. Hepatocellular drainage is notable revealing recovery (arrow), though with a little congested blood vessel (CBV), dilated sinusoids (DS) and hepatocytes appear normal.
(X400 Haematoxylin and Eosin staining technique)

which shot up as a result of the infection in the rats (Fig. 3). Treatment of the infection with PFM however also caused an increase in the alanine transaminase (ALT) concentration levels which was way higher than that of the healthy control rats (75.8 U $L^{-1} \pm 49.2$) (Fig. 4).

Effect of treatment of *S. typhi* infected rats with PFM on internal organs: Histopathological studies of the liver of one of the *S. typhi* infected rats not treated with PFM revealed haemorrhage at the central vein (Fig. 5a). However, with treatment of the infected rats with PFM, the liver cells appeared normal though there was congestion in the central vein and lymphocytosis suggesting the stimulation of lymphocytes at the site of infection for administered PFM dose of 400 mg kg^{-1} of PFM but the liver of the group treated with 500 mg kg^{-1} of PFM showed a better recovery because there was notable hepatocellular drainage and no lymphocytosis (Fig. 5b-d). Acute tubular necrosis was observed in the glomerulus of the kidney of the *S. typhi* infected rats not administered PFM (Fig. 6a). Treatment with PFM (400 mg kg^{-1} b.wt.) resulted in mild interstitial inflammatory cells infiltration in the kidney while treatment with 500 mg kg^{-1} of PFM revealed the appearance of new cells and clearing off of dot necrosis (Fig. 6b-d).

DISCUSSION

The *S. typhi* infected rats treated with the higher concentration of PFM (500 mg kg^{-1} b.wt.) recovered faster (2 days) than their counterparts that were treated with 400 mg kg^{-1} b.wt. (3 days). This points to the fact that the MBC of PFM achieved the elimination of the infection faster than the MIC of the extract. The haematological parameters also attest to the dose dependent recovery pattern recorded in this study. This is in agreement to the findings of Anisuzzman *et al.*[14].

Lymphocytosis and neutropenia that were observed in the *S. typhi* infected rats showed that the rats actually became sick however the administration of PFM to the infected rats caused a reversal of these effects showing that PFM has an immunomodulatory effect with a superior effect with the higher dose of PFM at 500 mg kg^{-1} b.wt. The increase

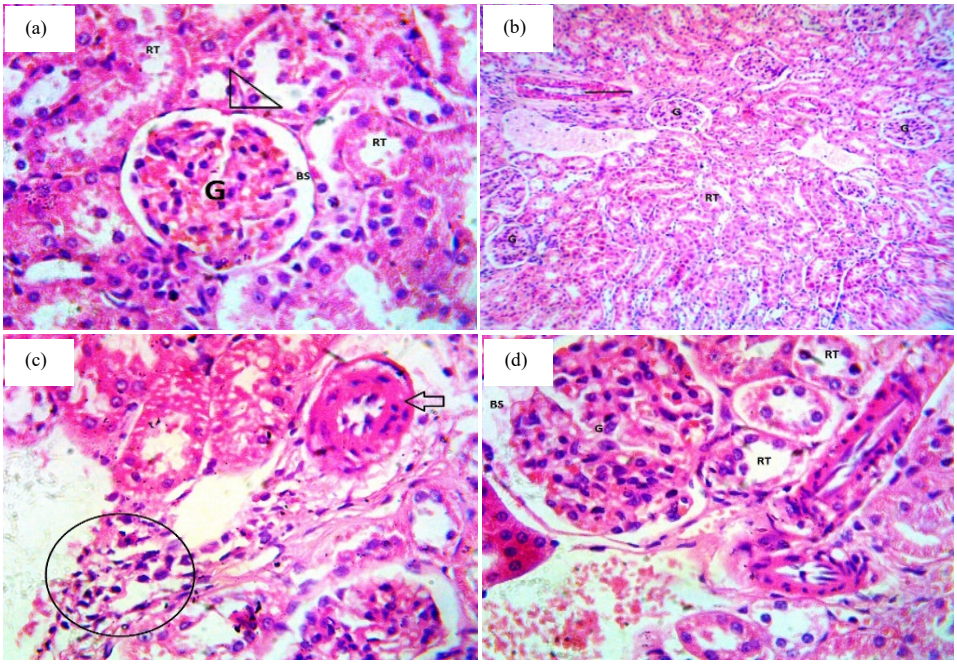

Fig. 6(a-d): Photomicrograph of the (a) Kidney of rat infected with *S. typhi* without treatment with PFM. Renal tissue composed of the renal corpuscle reveals nephrotoxic acute tubular necrosis in the glomerulus, (b) Kidney tissue of control rat (Not infected and not treated with PFM), (c) Kidney of rat infected with *S. typhi* and treated with 400 mg kg^{-1} PFM. Symmetric medial hypertrophy (arrow), mild interstitial inflammatory cells infiltration (circle) and (d) Kidney of a rat infected with *S. typhi* and treated with 500 mg kg^{-1} PFM. New cells appear and there is reduction in the appearance of dot necrosis.

a, c and d: X400 Haematoxylin and Eosin staining technique, b: X100 Haematoxylin and Eosin staining technique

in PCV observed in the blood of *S. typhi* infected rats that were administered PFM shows that PFM also possess haematopoietic properties which hastened the production of RBCs which took care of the reduction which occurred during active infection of rats with *S. typhi*. This is in line with a report from Munshi and Montgomerry[15]. The weight gain at the end of the study signifies recovery in the rats administered PFMs. This is in line with findings from Kumar and Samaras[16].

The observation that the administration of PFM caused an increase in the level of the enzyme ALP in the serum of *S. typhi* infected rats treated with the extract is indicative of the fact that PFM exerts cytotoxic effect as with the infection with *S. typhi* on the liver of the rats leading to high production of this enzyme. High levels of ALP are indicative of liver disease[17]. On the other hand, the AST values of rats infected with *S. typhi* and treated with PFM were observed to be almost the same as for the apparently healthy control rats which shows that PFM had little or no negative effects on the elevated AST enzyme in the serum of the rats. AST is reputed to rise upon damage to liver tissues, whereas it reverts quickly after the damage is being corrected. This corroborates the report of Shakya *et al.*[12].

Haemorrhage occurred at the central vein in the examined section of the liver sample from one of the rats infected with *S. typhi* but not treated with PFM. Lesions in the liver are peculiar to the advanced state of infection with *S. typhi*. This was however reversed after treatment with PFM in this study. The rat group treated with 500 mg kg^{-1} b. wt., of PFM after infection with *S. typhi* experienced a significant improvement with regards to the hepatocellular drainage of the venous blood to return into circulation compared to the representative from the group treated with 400 mg kg^{-1} b.wt., of PFM. This also points to the dose-dependent hepatoprotective and therapeutic efficacy of the PFM against *S. typhi* in the liver of the rats and indicative of recovery. This tallies with the finding of Germano *et al.*[18]. For rats treated with 500 mg kg^{-1} b.wt. of PFM extract, new cells were developing with marked disappearance of necrosis. This shows that PFM has a stimulatory effect leading to the activation of M2 macrophages towards the recovery and stability of the renal tissues[19].

CONCLUSION

This research has logically investigated the efficacy of *Physalis angulata* L. as a medicinal plant against *S. typhi* infection *in vivo* using the animal model. The study revealed that PFM at the dose of 400-500 mg kg^{-1} b.wt., exerted therapeutic activity in rats infected with *S. typhi* reducing the duration of infection to between 2 and 3 days with the higher dose exerting a greater effect. PFM also has haematopoietic potentials which can be exploited in treating anaemia.

SIGNIFICANCE STATEMENT

This study reports the therapeutic activity of methanolic extract of fruits of *Physalis angulata* based on the MIC and MBC of the extract in *S. typhi* infected Wistar albino rats. This study has also evaluated an aspect of the biosafety of the extract which is dose-dependent. More research works however are required before the extract could be adopted for human use. Especially in terms of dosaging, drug kinetics and biosafety.

REFERENCES

1. Jajere, S.M., 2019. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. Vet. World, 12: 504-521.
2. Gupta, P.D. and T.J. Birdi, 2017. Development of botanicals to combat antibiotic resistance. J. Ayurveda Integr. Med., 8: 266-275.
3. Silva, M.T.G., S.M. Simas, T.G.F.M. Batista, P. Cardarelli and T.C.B. Tomassini, 2005. Studies on antimicrobial activity, *in vitro*, of *Physalis angulata* L. (Solanaceae) fraction and physalin B bringing out the importance of assay determination. Mem. Inst. Oswaldo Cruz, 100: 779-782.
4. Donkor, A.M., R.L.K. Glover, J.K. Boateng and V.Y. Gakpo, 2012. Antibacterial activity of the fruit extract of *Physalis angulata* and its formulation. J. Med. Biomed. Sci., 1: 21-26.
5. Oladosu, T.O., T.T. Adebolu and M.K. Oladunmoye, 2016. Evaluation of the types of bacteria in the blood of HIV-1 patients attending ART clinic at the FMC Owo, Nigeria and their antibiogram profile. HIV Curr. Res., Vol. 1. 10.4172/2572-0805.1000103.
6. Oladosu, T.O., T.T. Adebolu and M.K. Oladunmoye, 2016. *In vitro* antibacterial testing of fruit and leaf extracts of *Physalis angulata* (L) on multidrug resistant bacterial isolates from HIV patients. Int. J. Innov. Res. Adv. Stud., 3: 118-122.
7. Cheesbrough, M., 1995. Medical Laboratory Manual for Tropical Countries, Volume 2: Microbiology. University of Cambridge Press, Cambridge, UK., ISBN-13: 9780750603782, pp: 479.
8. EUCAST., 2003. Determination of Minimum Inhibitory Concentrations (MICs) of antibacterial agents by broth dilution. Clin. Microbiol. Infect., 9: 1-7.
9. Buller, N., A. Thomas and M. Barton, 2014. Antimicrobial susceptibility testing. Australian and New Zealand Standard Diagnostic Procedures, Department of Agriculture, Water and the Environment, Canberra City, Australia, July 2014.
10. Adebolu, T.T., O.O. Adeoye and V.O. Oyetayo, 2011. Effect of garlic (*Allium sativum*) on *Salmonella typhi* infection, gastrointestinal flora and hematological parameters of albino rats. Afr. J. Biotechnol., 10: 6804-6808.
11. Oladunmoye, M.K., 2007. Comparative evaluation of the effects of leaf extract from *Spondias mombin* on rats with induced infections from *Bacillus cereus* and *Clostridium sporogenes*. Res. J. Phytochem., 1: 68-73.
12. Shakya, R., M.K. Hoque, A.S. Sapkota and P.K. Gupta, 2012. Histological changes in liver of albino rats treated with different doses of sodium valproate. Proceedings of the 2nd International Anatomical Sciences and Cell Biology Conference in Conjunction with 36th Annual Conference of the Anatomy Association of Thailand, December 6-8, 2012, Chiang Mai, Thailand, pp: 248-254.
13. Slaoui, M. and L. Fiette, 2011. Histopathology Procedures: From Tissue Sampling to Histopathological Evaluation. In: Drug Safety Evaluation: Methods and Protocols, Gautier, J.C. (Ed.). Humana Press, Totowa, NJ., USA., ISBN: 978-1-60761-849-2, pp: 69-82.
14. Anisuzzman, M., M. Hasan, A.K. Acharzo, A.K. Das and S. Rahman, 2017. *In vivo* and *in vitro* evaluation of pharmacological potentials of secondary bioactive metabolites of *Dalbergia candenatensis* leaves. Evidence-Based Complement. Altern. Med., Vol. 2017. 10.1155/2017/5034827.
15. Munshi, H.G. and R.B. Montgomery, 2000. Severe neutropenia: A diagnostic approach. Western J. Med., 172: 248-252.
16. Kumar, S. and K. Samaras, 2018. The impact of weight gain during HIV treatment on risk of pre-diabetes, diabetes mellitus, cardiovascular disease and mortality. Front. Endocrinol., Vol. 9. 10.3389/fendo.2018.00705.
17. Martinez, M.N., 2011. Factors influencing the use and interpretation of animal models in the development of parenteral drug delivery systems. AAPS J., 13: 632-649.
18. Germano, M.P., V. D'Angelo, R. Sanogo, A. Morabito, S. Pergolizzi and R. de Pasquale, 2001. Hepatoprotective activity of *Trichilia roka* on carbon tetrachloride-induced liver damage in rats. J. Pharm. Pharmacol., 53: 1569-1574.
19. Dong, X., S. Swaminathan, L.A. Bachman, A.J. Croatt, K.A. Nath and M.D. Griffin, 2007. Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. Kidney Int., 71: 619-628.