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Evaluation of Anti-inflammatory, Anti-pyretic, Analgesic, and Hepatoprotective Properties of *Terminalia macroptera*



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Key words

Terminalia macroptera, Combretaceae, Médicaments Traditionnels Améliorés/improved traditional medicines, medicinal plant antipyretic, anti-inflammatory, hepatoprotective

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ABSTRACT

In Mali, improved traditional medicines [“Médicaments Traditionnels Améliorés”] are prepared from traditionally used medicinal plants. Recently, the Department of Traditional Medicine has identified *Terminalia macroptera* Guill. & Perr. (Combretaceae) as a potential candidate for an improved traditional medicine. *T. macroptera* is a West African medicinal plant used in Mali against various health disorders, with more than 30 different indications mentioned by traditional healers, including hepatitis, gonorrhea, fever, pain relief, and various infectious diseases (*Helicobacter pylori*-associated diseases). To date, validation of most of the biological activities of has been mainly carried out *in vitro*, except for antimalarial activities. In this study, the potential anti-inflammatory, antipyretic, analgesic, and hepatoprotective properties of *T. macroptera* were investigated in different murine models. Administration of *T. macroptera* ethanolic root and leaf extracts in rats significantly reduced pyrexia, pain, inflammation, and hepatic marker enzymes such as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in the different murine models used ($p < 0.05$). A phytochemical screening of *T. macroptera* revealed the presence of tannins, flavonoids, saponins, anthracene derivatives, sterols, triterpenes, and sugars in both leaf and root extracts as the main phytochemical compounds. This was confirmed by qualitative analysis, liquid chromatography coupled with high-resolution mass spectrometry. *T. macroptera* extracts demonstrated interesting *in vivo* antipyretic, analgesic, anti-inflammatory, and hepatoprotective activities. Therefore, *T. macroptera* should be proposed and further evaluated as a potential improved traditional medicine for the treatment of liver-related disorders and for the relief of pain and fever.

* These authors contributed equally to this work.

Introduction

Research on medicinal plants in Mali has been dynamic since political independence in 1960. In 1968, the Department of Traditional Medicine (DMT) was created under the authority of the Ministry of Health. One of the objectives of the DMT is to assess the biological activities of medicinal plants used in traditional medicine, and to formulate and produce phytomedicines based on improved traditional medicines called MTAs “Médicaments Traditionnels Améliorés”. MTAs are categorized into four types. The requirements to get an official marketing authorization vary according to the category (► **Table 1**). The basic requirement is documenting the traditional uses of the remedy, its safety and efficacy, indicating its standardized dosage, and providing quality control data. Today, the majority of registered MTAs are classified as Category 2, for which clinical trial data is not mandatory [1]. Recently, the DMT identified *Terminalia macroptera* Guill. & Perr. (Combretaceae) as a potential candidate for an MTA.

This plant is widely used in West African countries to treat many health disorders, although research that proves biological activity remains scarce. Eleven publications reported the traditional use of different parts of the plant in the treatment of disorders such as liver disease, malaria, urinary tract infection, diarrhea, pain, fever, and wounds (► **Table 2**). *In vitro* antibacterial, antifungal, antiplasmodial, antitrypanosomal, leishmanicidal, and antiviral activities have been previously reported in the roots, leaves, and bark of this species. Reported *in vitro* biological activities include antioxidant, enzyme inhibition, antiproliferative, hemolytic, and immunomod-

ulatory effects (► **Table 3**). Only two *in vivo* studies have been carried out [2]. In a recent study, our team demonstrated the ability of *T. macroptera* roots (TMR) and leaves (TML) to limit *Plasmodium* parasitemia and to increase survival in two murine models of uncomplicated and cerebral malaria, respectively. In addition, we demonstrated that according to the Organization for Economic Cooperation and Development’s (OECD) Globally Harmonized System of Classification, both extracts were non-toxic orally [3]. The same batch of *T. macroptera* was used in both studies.

In order to complete these data and to investigate some of the ethnopharmacological claims, especially the use of *T. macroptera* as an antipyretic, anti-inflammatory, hepatoprotective, and analgesic agent, our team conducted the first recorded *in vivo* studies to evaluate these properties. The study aims to provide a stronger research basis for the potential future use of *T. macroptera* as an MTA in Mali.

Results and Discussion

At present, the following chemical labelling and classification of acute systemic toxicity, based on oral LD₅₀ values, are from the recommendations of the Globally Harmonized System of Classification [OECD, 2008], and ranked as: very toxic, ≤ 5 mg/kg; toxic, > 5 ≤ 50 mg/kg; harmful, > 50 ≤ 500 mg/kg; and no label, > 500 ≤ 2000 mg/kg [4]. In our previous work [3], oral administration of TML and TMR at a dose of 2000 mg/kg did not cause mortality among experimental animals. This indicates that the LD₅₀s of TML and TMR are

► **Table 1** MTA classes according to the Ministry of Health Regulation in Mali and requested items for marketing authorization delivery [1].

	Description	MTA class			
		1 Traditional medicine prepared by a traditional health practitioner for an individual patient with fresh or dried raw materials, with a short shelf life	2 Traditional medicine currently used in the community, prepared in advance, and composed of crude raw materials	3 Standardized extracts prepared in advance following scientific research	4 Molecules purified from traditional medicines following scientific research
Requested items for marketing authorization delivery	Covering letter ¹	X	X	X	X
	Samples ²	X	X	X	X
	Administrative dossier ³	X	X	X	X
	Pharmaceutical dossier ⁴		X	X	
	Expert analytical report ⁵		X	X	X
	Pharmacology and toxicology dossier ⁶			X	X
	Clinical dossier ⁷			X	X
	Expert report on traditional use ⁸	X	X	X	X
Fees ⁹	X	X	X	X	

¹Addressed to the Ministry of Health, including the name and address of the manufacturer. ²Ten samples as sold. ³Registration form of the manufacturer and memoranda of understanding between the manufacturer and a research institution. ⁴Complete monograph(s) of the plant’s component(s). Method and stages of preparation and production and Expert report on Good Manufacturing Practices. ⁵Quality control method for raw materials. Results of stability and quality control tests of raw materials and excipients. Method and results of quality control during production. Quality control results of the finished product. Stability tests results of the finished product. ⁶Pharmacodynamic data. Results of acute and subchronic toxicity tests. Literature review of pharmacology and toxicology. Expert report on the tests carried out. ⁷Ethical approval for clinical trials. Clinical trial protocol following standard methods (phase I and II). Results of clinical trials. Expert report on clinical trials carried out. ⁸Evidence of the long history of use of the medicine in its current or traditional form (minimum 20 years). Detailed presentation of known toxicological risks. Risks of incorrect use of the medicine. Risks of physical or psychological dependence. ⁹Registration fees receipt.

greater than 2000 mg/kg in albino Swiss mice. Therefore, according to this classification, these fractions can be classed as Category 5 and considered to have relatively low acute toxicity. To work under the most favorable experimental conditions, *T. macroptera* extracts were used in the experiments at 100, 200, and 400 mg/kg for antipyretic, analgesic, anti-inflammatory, and hepatoprotective

activity, corresponding to doses 20, 10, and 5 times lower than the safe dose, respectively.

Leaves and roots of *T. macroptera* are traditionally used for the treatment of inflammatory conditions like pain, fever, and liver diseases [5]. However, according to the literature review, the analgesic, antipyretic, and anti-inflammatory properties of *T. macroptera* have never been investigated before. Other species of *Terminalia* have, however, demonstrated analgesic, antipyretic, and anti-inflammatory properties in studies. The methanolic extract of the leaves of *Terminalia arjuna* (Roxb, ex DC.) Wight & Arn. (100 mg/kg, po) demonstrated *in vivo* analgesic and anti-inflammatory activities with a 51 % inhibition of acetic acid-induced pain and a 75 % inhibition of edema from the third hour of carrageenan injection [6], respectively. Analgesic and antipyretic activities were also shown in the ethanolic extract of the fruit of *Terminalia bellirica* (Gaertn.) Roxb. (200 mg/kg, p.o.) with a 62 % inhibition of acetic acid-induced pain and a reduction of yeast-induced pyrexia from the first hour after administration of the treatment [7].

The effect of ethanolic crude extracts on yeast pyrexia in rats is shown in ► **Table 4**. Pyrexia was significantly reduced by TMR and TML treatments when compared to gavage with distilled water. Interestingly, TML at 400 mg/kg was able to lower body temperature 1 h after treatment (T25) ($p < 0.0001$). At the same dose (400 mg/kg), TMR was active against pyrexia 2 h after treatment (T26). Three hours after treatment (T27, T28), the three doses (100, 200, and 400 mg/kg) showed similar efficacy, efficiently reducing pyrexia ($p < 0.05$). Paracetamol (100 mg/kg), which was used as a reference drug, also significantly reduced pyrexia from T25 to T28 when compared to the water-treated group ($p < 0.05$), similarly to TMR and TML from T27 ($p > 0.05$). In addition, TMR and TML ethanolic crude extracts were highly effective in reducing the number of contortions induced by acetic acid compared to distilled water (► **Fig. 1a**). Efficacy using the three doses (100, 200, and 400 mg/kg) ($p < 0.0001$ for all) was similar to the efficacy observed after treatment with paracetamol. The maximum analgesic effect was obtained after using 400 mg/kg of TMR, with a pain inhibition of $78.3 \pm 0.8\%$.

► **Table 2** Traditional uses of *T. macroptera* described in different countries of West Africa.

Indications	Number of quotes	Country of quote	Reference
Liver diseases	6	Burkina Faso, Guinea-Bissau, Mali, Senegal	[5, 30–34]
Malaria	5	Burkina Faso, Guinea, Mali, Senegal	[5, 32, 33, 35, 36]
Urinary tract infection	5	Burkina Faso, Guinea-Bissau, Mali, Senegal	[5, 31–34]
Diarrhea	5	Burkina Faso, Mali, Nigeria	[2, 32–34, 37]
Pain	3	Mali, Senegal	[5, 32, 34]
Fever	3	Mali	[5, 30, 34]
Wound	3	Mali, Senegal	[5, 32, 34]
Asthenia	2	Mali, Senegal	[5, 32]
Snake bite	2	Mali, Senegal	[32, 38]
Cough	2	Mali	[34, 37]
Skin diseases and boils	2	Burkina Faso, Mali	[33, 38]
Aphrodisiac	1	Senegal	[32]
Conjunctivitis	1	Senegal	[32]
Gastric ulcer	1	Burkina Faso	[33]
Absence or delay of menstruation	1	Mali	[37]

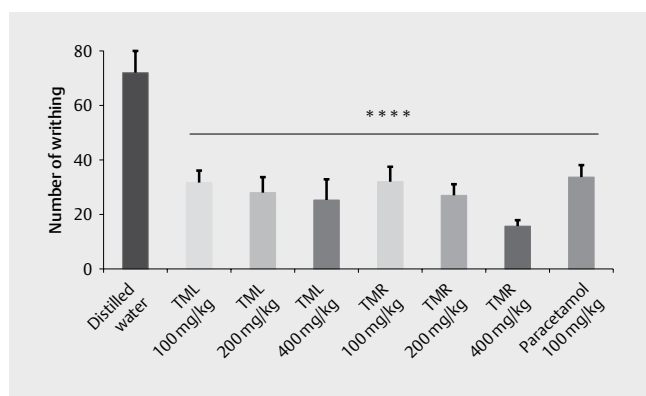
► **Table 3** Pharmacological activities of *T. macroptera* reported in the literature.

Plant parts used	Type of test	Pharmacological activities	Model/test used	Reference
Leaf, stem bark, and root bark	<i>In vitro</i>	Antibacterial	<i>In vitro</i> microdilution, disk diffusion, and direct bioautographic assay	[11, 39–42]
Leaf, stem bark, and root bark	<i>In vitro</i>	Antifungal	Disk diffusion assay	[43]
Root bark	<i>In vitro</i>	Antiplasmodial	Fluorometric assay	[3, 36, 44]
Root bark	<i>In vitro</i>	Antitrypanosomal	Fluorometric assay	[44]
Root bark	<i>In vitro</i>	Leishmanicidal	Fluorometric assay	[44]
Root	<i>In vitro</i>	Antiviral	<i>In vitro</i> antiviral assay by titration	[45]
Leaf, stem bark, and root bark	<i>In vitro</i>	Antioxidant	DPPH radical scavenging	[11–13]
Root	<i>In vitro</i>	Antiproliferative	Trypan blue assay	[12]
Leaf, stem bark, and root bark	<i>In vitro</i>	Enzymatic inhibitor	<i>In vitro</i> inhibition of α -glucosidase, 15-lipoxygenase, and xanthine oxidase assay	[13]
Leaf	<i>In vitro</i>	Hemolytic	Colorimetric assay	[11]
Leaf, stem bark, and root bark	<i>In vitro</i>	Immunomodulatory	Complement fixation assay	[13, 20–21]
Root and leaf	<i>In vivo</i>	Antimalarial	Parasitemia and survival evaluation in <i>P. Plasmodium chabaudi</i> and <i>P. Plasmodium bergeri</i> ANKA-infected mice models	[3]
Barks	<i>In vivo</i>	Antidiarrheal	Castor oil-induced diarrhea in rats	[11]

► **Table 4** Antipyretic activity of *T. macroptera* extracts on yeast-induced pyrexia in Wistar rats.

Group	Temperature (°C)					
	Basal temperature (T0)	T24	T25	T26	T27	T28
Distilled water	37.3 ± 0.4	38.8 ± 0.2	38.9 ± 0.04	38.8 ± 0.1	38.7 ± 0.2	38.6 ± 0.1
TML 100 mg/kg	37.3 ± 0.3	38.2 ± 0.3	39.0 ± 0.3	38.7 ± 0.2	37.7 ± 0.3 ^d	37.7 ± 0.1 ^d
TML 200 mg/kg	37.0 ± 0.1	38.3 ± 0.3	38.7 ± 0.4	38.5 ± 0.3	37.6 ± 0.2 ^c	37.3 ± 0.1 ^d
TML 400 mg/kg	37.8 ± 0.3	38.9 ± 0.5	38.2 ± 0.4 ^b	38.1 ± 0.3 ^a	37.8 ± 0.4 ^c	37.7 ± 0.5 ^c
TMR 100 mg/kg	37.3 ± 0.3	38.2 ± 0.3	39.0 ± 0.3	38.7 ± 0.2	37.7 ± 0.3 ^d	37.7 ± 0.1 ^d
TMR 200 mg/kg	37.1 ± 0.2	38.3 ± 0.3	38.8 ± 0.6	38.7 ± 0.4	37.9 ± 0.4 ^c	37.4 ± 0.2 ^d
TMR 400 mg/kg	37.2 ± 0.3	39.0 ± 0.6	38.7 ± 0.4	37.9 ± 0.6 ^c	37.7 ± 0.3 ^d	37.7 ± 0.4 ^c
Paracetamol 100 mg/kg	37.1 ± 0.1	38.3 ± 0.4	38.1 ± 0.5 ^b	37.6 ± 0.3 ^d	37.2 ± 0.2 ^d	37.2 ± 0.2 ^d

The basal rectal temperature of the rats was taken using a digital clinical thermometer (T0). At the end of the day (T8), each animal was given a subcutaneous injection of a 20% w/v aqueous suspension of yeast and then fasted overnight. The rectal temperature of the animals was taken 16 h after the yeast injection (T24). The rectal temperature of the rats was taken hourly during the 4 h following the administration of the treatments (T25 to T28). Data are expressed as the mean ± SD (n = 6 per group). Two-way ANOVA followed by Dunnett's multiple comparison tests were used for analysis. Statistical significance p < 0.05. ^ap < 0.05 compared to the distilled water group, ^bp < 0.01 compared to the distilled water group, ^cp < 0.001 compared to the distilled water group, ^dp < 0.0001 compared to the distilled water group.



► **Fig. 1** Analgesic activity of *T. macroptera* leaf (TML) and root (TMR) extracts on acetic acid-induced writhing in Swiss mice. One-way ANOVA followed by Dunnett's multiple comparison tests were used for analysis. Treatment efficacies were compared to distilled water; **** p < 0.0001.

Furthermore, TMR and TML ethanolic crude extracts at the three doses (100, 200, and 400 mg/kg) were able to significantly reduce edema 3 h after carrageenan injection when compared to the distilled water treatment. Indomethacin, the reference treatment, presented similar efficacy. The maximum anti-inflammatory effect (92.6 ± 7.6%) was obtained with TML treatment at 400 mg/kg 5 h after carrageenan injection (► **Table 5**).

Fever, pain, and inflammation are clinical manifestations associated with a wide range of diseases. Fever is one of the symptoms marking the onset of an infection or inflammation. The use of *T. macroptera* in feverish illnesses might be explained by its ability to interfere with the fever pathway, where exogenous pyrogens, such as microbes and/or their toxins, stimulate mononuclear phagocytes to release proinflammatory and pyrogenic cytokines such as TNF- α , IL1, IL-6, and IFN γ [8]. The release of such cytokines was also shown to be associated with pain in the murine model we used to measure the analgesic activity of *T. macroptera*, based on acetic acid-induced writhing [9]. These pyrogenic cytokines trigger arachidonic acid metabolism, including prostaglandin E2 (PGE2) production, a lipid

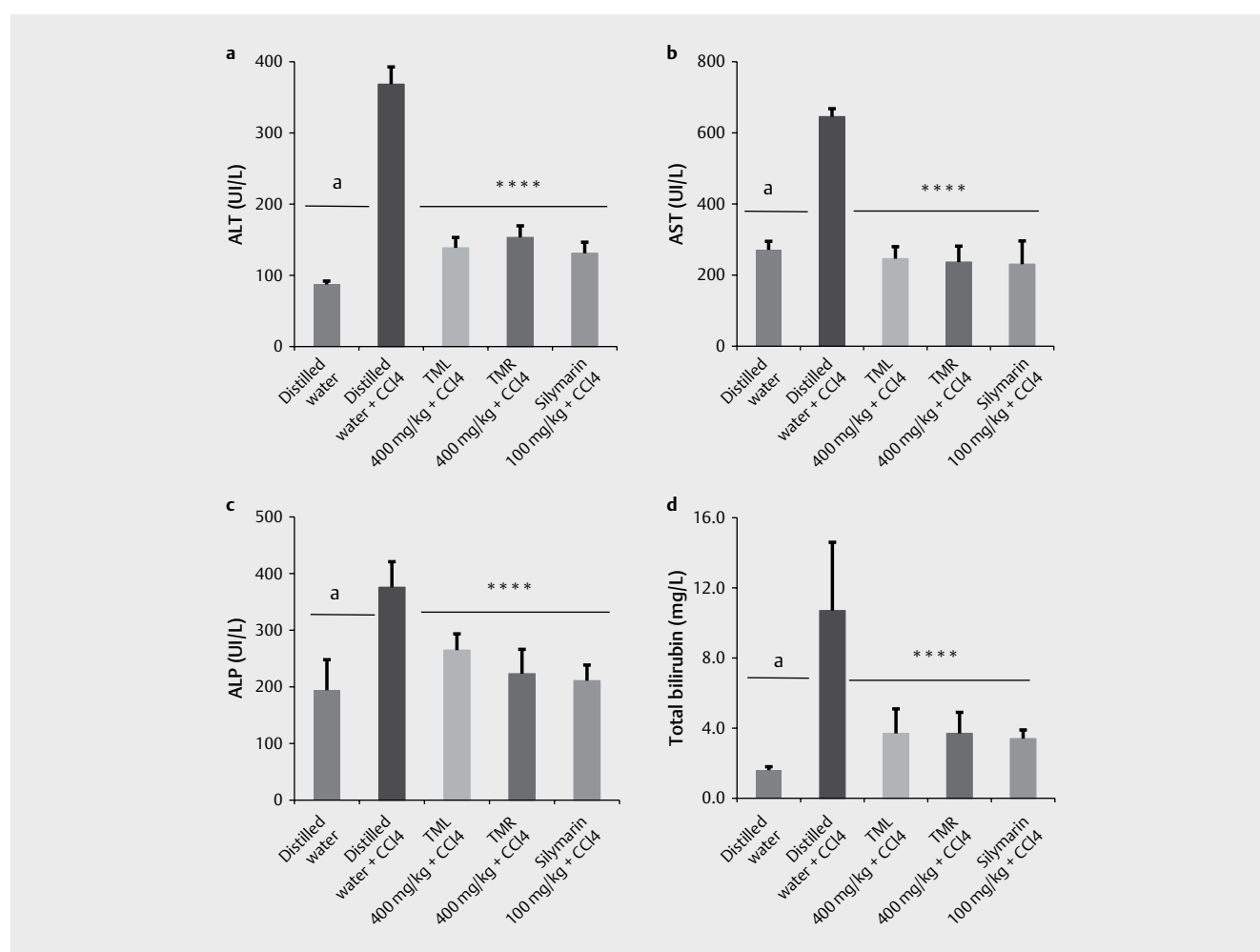
mediator largely associated with fever and inflammation [8]. Furthermore, in the present study, we screened the efficacy of extracts of *T. macroptera* in a mouse model of CCl₄-induced acute hepatic damage to test its potential as a potent hepatoprotective medicinal plant. CCl₄ administration induces a high hepatocyte injury, leading to the extensive formation of free radicals such as trichloromethyl and peroxytrichloromethyl, which are highly toxic for the liver [10]. The effect of TMR and TML treatments at 400 mg/kg on hepatic marker enzymes in CCl₄-induced hepatic injury in rats is shown in ► **Fig. 2**. The levels of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), and total bilirubin (TB) were significantly increased in the rat group intoxicated with the intraperitoneal injection of CCl₄, and treated with distilled water (CCl₄ model control) compared with the non-intoxicated rat group treated with distilled water (healthy control) (p < 0.0001). TMR and TML treatments significantly reduced the levels of ALT (► **Fig. 2a**), AST (► **Fig. 2b**), ALP (► **Fig. 2c**), and TB (► **Fig. 2d**) compared to distilled water in the CCl₄ model controls (p < 0.0001) (► **Fig. 2**). Several studies have demonstrated the anti-radical *in vitro* activity of leaf and root extracts of *T. macroptera* [11–13], suggesting that the hepatoprotective activity of our extracts could partly be due to the inhibition of the production of these free radicals. Furthermore, TMR and TML efficacy was similar to that of the reference drug silymarin. Interestingly, levels of hepatic enzymes obtained after TML, TMR, and silymarin treatments were similar to the healthy control group. These results are consistent with those from studies carried out on other species of *Terminalia*. These also demonstrated dose-dependent hepatoprotective properties by reducing transaminases and alkaline phosphatase such as *T. bellirica* fruits [14] and *Terminalia catappa* leaves [15].

In this study, a qualitative analysis by LC-HRMS of *T. macroptera* extracts was undertaken to compare their composition and biological activities. A total of 59 compounds were detected and identified through HRMS and MS/MS fragmentation patterns using MS-FINDER and the DNP database (► **Table 6**). The MS-FINDER dereplication method allowed us to annotate the corresponding peaks, mostly found in the Combretaceae family (► **Table 3**). We separat-

► **Table 5** Anti-inflammatory activity of *T. macroptera* extracts on carrageenan-induced edema in Swiss mice.

Group	Thickness of paw (mm)				% Inhibition		
	0 h	1 h	3 h	5 h	1 h	3 h	5 h
Distilled water	1.12 ± 0.02	2.04 ± 0.15	2.14 ± 0.30	1.90 ± 0.21			
TML 100 mg/kg	1.13 ± 0.05	1.99 ± 0.22	1.85 ± 0.22 ^b	1.62 ± 0.29 ^b	11.70 ± 3.90	33.00 ± 12.20	53.30 ± 4.90
TML 200 mg/kg	1.14 ± 0.09	1.96 ± 0.18	1.69 ± 0.17 ^d	1.55 ± 0.12 ^c	15.80 ± 7.90	45.30 ± 2.90	48.40 ± 9.10
TML 400 mg/kg	1.20 ± 0.09	1.55 ± 0.26 ^d	1.42 ± 0.05 ^d	1.30 ± 0.06 ^d	72.50 ± 15.90	82.40 ± 1.10	92.60 ± 7.60
TMR 100 mg/kg	1.19 ± 0.08	1.95 ± 0.08	1.84 ± 0.10 ^b	1.50 ± 0.12 ^d	17.40 ± 3.30	32.50 ± 18.70	63.50 ± 17.80
TMR 200 mg/kg	1.15 ± 0.04	1.80 ± 0.11 ^a	1.68 ± 0.10 ^d	1.47 ± 0.07 ^d	28.60 ± 3.70	46.40 ± 9.00	59.10 ± 0.80
TMR 400 mg/kg	1.13 ± 0.04	1.50 ± 0.06 ^d	1.38 ± 0.08 ^d	1.32 ± 0.04 ^d	60.70 ± 1.80	75.10 ± 0.50	78.50 ± 5.10
Indomethacin 8mg/kg	1.14 ± 0.04	1.88 ± 0.06	1.73 ± 0.24 ^d	1.45 ± 0.13 ^d	19.60 ± 8.60	55.60 ± 5.10	66.30 ± 2.90

Data are expressed as the mean ± SD (n=6 per group). Two-way ANOVA followed by Dunnett's multiple comparison tests were used for analysis. Statistical significance p < 0.05. ^aP < 0.05 compared to the distilled water group, ^bp < 0.01 compared to the distilled water group, ^cp < 0.001 compared to the distilled water group, ^dp < 0.0001 compared to the distilled water group.



► **Fig. 2** Hepatoprotective effects of *T. macroptera* extracts in CCl_4 -intoxicated rats. Rats were pretreated with distilled water, TMR, TML, or silymarin daily for 7 days (n=5 per group). Except for the healthy control group (white sticks), rats were intoxicated with CCl_4 (0.5 mL/kg i.p.) 1 h after the last treatment. **a** Serum levels of ALT in U/L. **b** Serum levels of AST in U/L. **c** Serum levels of ALP in U/L. **d** Total bilirubin in mg/L. Data are expressed as the mean ± SD. One-way ANOVA followed by Dunnett's multiple comparison tests were used for analysis. Statistical significance p < 0.05. ^aP < 0.0001 compared to the distilled water group; **** p < 0.0001 compared to the CCl_4 -treated group

ed the detected compounds into three categories: compounds detected only in the roots, compounds detected only in the leaves, and compounds detected in both roots and leaves.

Terminalia species have been shown to contain various secondary metabolites including cyclic triterpenes and their derivatives (flavonoids, tannins, and phenolic acids). In our study, the LC/MS

► **Table 6** Putative identified features in roots and leaves ($m/z \times RT$ pairs) using HRMS and MS/MS fragmentation patterns using ► **MS-finder** and the DNP database.

Detected only in the roots			
m/z	Rt	Molecular formula	Identity
681.3842 [M + H] ⁺	3.06	C ₃₆ H ₅₆ O ₁₂	Termiarjunoside II
600.9909 [M - H] ⁻	1.00	C ₂₈ H ₁₀ O ₁₆	Terminalin
521.3469 [M + H] ⁺	3.06	C ₃₀ H ₄₈ O ₇	Bellericagenin B
315.0852 [M + H] ⁺	3.25	C ₁₇ H ₁₄ O ₆	Combretastatin C1
471.3475 [M + H] ⁺	3.62	C ₃₀ H ₄₆ O ₄	2- α -Hydroxymicromeric acid
649.3951 [M + H] ⁺	3.36	C ₃₆ H ₅₆ O ₁₀	Quadranoside VIII
991.5133 [M + H] ⁺	3.36	C ₄₈ H ₇₈ O ₂₁	2,3,19,23-Tetrahydroxy-12-oleanen-28-oic acid-(2- α ,3 β ,19 β)-3-O- $[\beta$ -D-Galactopyranosyl-(1->3)- β -D-glucopyranoside], 28-O- β -D-glucopyranosyl ester
461.0721 [M - H] ⁻	2.52	C ₂₁ H ₁₈ O ₁₂	Ellagic acid-2,8-Di-Me ether, 3-O- β -D-xylopyranoside
463.3053 [M + H] ⁺	2.59	C ₂₇ H ₄₂ O ₆	Norquadrangularic acid A
495.0784 [M - H] ⁻	0.59	C ₂₁ H ₂₀ O ₁₄	1,5-Digalloylquinic acid
811.4467 [M - H] ⁻	4.26	C ₄₂ H ₆₈ O ₁₅	Arjunolitin
469.0059 [M - H] ⁻	0.98	C ₂₁ H ₁₀ O ₁₃	Flavogallonic acid
Detected only in the leaves			
m/z	Rt		Identity
583.1117 [M - H] ⁻	2.64	C ₂₈ H ₂₄ O ₁₄	2''-O-Galloylvitexin
611.1603 [M + H] ⁺	2.32	C ₂₇ H ₃₀ O ₁₆	Quercetin 3-(4-galactosylrhamnoside)
953.0919 [M - H] ⁻	1.00	C ₄₁ H ₃₀ O ₂₇	Terchebin
635.2003 [M - H] ⁻	3.38	C ₂₃ H ₄₀ O ₂₀	β -D-Galactopyranosyl-(1->6)- β -D-galactopyranosyl-(1->6)- β -D-galactopyranosyl-(1->3)-L-arabinose
277.0343 [M + H] ⁺	0.99	C ₁₃ H ₈ O ₇	3,4,8,9,10-Pentahydroxy-6H-dibenzo[b,d]pyran-6-one
321.0240 [M + H] ⁺	1.01	C ₁₄ H ₈ O ₉	Luteolic acid
765.0982 [M - H] ⁻	2.36	C ₃₅ H ₂₆ O ₂₀	Ellagic acid-3-Me ether, 7-O-[3,4,5-trihydroxybenzoyl-(1->3)-[3,4,5-trihydroxybenzoyl-(1->4)]- α -L-rhamnopyranoside]
699.3561 [M + H] ⁺	4.74	C ₃₅ H ₅₄ O ₁₄	3,14,19-Trihydroxycard-20(22)-enolide-(3 β ,5 β ,14 β)-form-3-O- $[\beta$ -D-galactopyranosyl-(1->4)- α -L-rhamnopyranoside]
473.1484 [M - H] ⁻	0.37	C ₁₇ H ₃₀ O ₁₅	β -D-Galactopyranosyl-(1->6)- β -D-galactopyranosyl-(1->3)-L-arabinose
895.0855 [M - H] ⁻	2.74	C ₃₉ H ₂₈ O ₂₅	Quisqualin A
295.1024 [M - H] ⁻	0.42	C ₁₈ H ₁₆ O ₄	Combretastatin D2
Detected in both the leaves and roots			
m/z	Rt		Identity
955.1099 [M - H] ⁻	2.50	C ₄₁ H ₃₂ O ₂₇	Chebulinic acid
635.0901 [M - H] ⁻	1.15	C ₂₇ H ₂₄ O ₁₈	1,3,6-Trigalloylglucose- β -D-Pyranose
785.0877 [M - H] ⁻	2.24	C ₃₄ H ₂₆ O ₂₂	Tercaatin
609.1478 [M - H] ⁻	2.27	C ₂₇ H ₃₀ O ₁₆	Quercetin 3-(4-galactosylrhamnoside)
331.0668 [M - H] ⁻	0.39	C ₁₃ H ₁₆ O ₁₀	3-Galloylglucose
503.3368 [M - H] ⁻	4.07	C ₃₀ H ₄₈ O ₆	Belleric acid
197.0460 [M - H] ⁻	1.33	C ₉ H ₁₀ O ₅	4-O-Ethylgallic acid
633.0734 [M - H] ⁻	0.68	C ₂₇ H ₂₂ O ₁₈	Corilagin
801.4097 [M - H] ⁻	3.54	C ₄₃ H ₆₂ O ₁₄	2,3,23-Trihydroxy-12-oleanen-28-oic acid-(2 α ,3 β)-23-O-(3,4,5-Trihydroxybenzoyl), 28-O- β -D-glucopyranosyl ester
447.0576 [M - H] ⁻	2.04	C ₂₀ H ₁₆ O ₁₂	Eschweilenol C
631.3835 [M - H] ⁻	4.11	C ₃₆ H ₅₆ O ₉	Jessic acid-3-O- α -L-arabinopyranoside
793.4412 [M - H] ⁻	3.50	C ₄₂ H ₆₆ O ₁₄	2,3,19-Trihydroxy-12-oleanen-28-oic acid-(2 α ,3 α ,19 α)-3-Ketone, 28-O- $[\alpha$ -L-rhamnopyranosyl-(1->4)- β -D-glucopyranosyl] ester
451.3208 [M + H] ⁺	3.37	C ₃₀ H ₄₂ O ₃	Erythrophyllic acid
1083.0579 [M - H] ⁻	0.34	C ₄₈ H ₂₈ O ₃₀	Isoterchebulin
639.3526 [M - H] ⁻	4.16	C ₃₇ H ₅₂ O ₉	23-Galloylarjunolic acid
933.0627 [M - H] ⁻	0.41	C ₄₁ H ₂₆ O ₂₆	Arjunin
781.0525 [M - H] ⁻	0.40	C ₃₄ H ₂₂ O ₂₂	Isoterchebuloylglucose

► Table 6 Continued

Detected in both the leaves and roots			
<i>m/z</i>	Rt		Identity
359.1492 [M + H] ⁺	3.70	C ₂₀ H ₂₂ O ₆	2',4',5,7-Tetrahydroxy-8-methylflavanone-Tetra-Me ether. 2',4',5,7-Tetramethoxy-8-methylflavanone
817.4045 [M - H] ⁻	3.32	C ₄₃ H ₆₂ O ₁₅	2,3,6,23-Tetrahydroxy-12-oleanen-28-oic acid-(2 α ,3 β ,6 β)-23-O-(3,4,5-Trihydroxybenzoyl), 28-O- β -D-glucopyranosyl ester
297.1523 [M - H] ⁻	4.65	C ₁₉ H ₂₂ O ₃	4-Hydroxy-4'-methoxy-7,7'-epoxyignan
603.3892 [M - H] ⁻	5.75	C ₃₅ H ₅₆ O ₈	1,3-Dihydroxycycloart-24-en-28-oic acid-(1 α ,3 β)-3-O- α -L-arabinopyranoside
521.0953 [M - H] ⁻	3.27	C ₂₃ H ₂₂ O ₁₄	Flavellagic acid-2,3,8-Tri-Me ether, 7-O- β -D-glucopyranoside
783.0685 [M - H] ⁻	0.62	C ₃₄ H ₂₄ O ₂₂	Terflavin B
1085.0729 [M - H] ⁻	0.62	C ₄₈ H ₃₀ O ₃₀	Terflavin A
487.3422 [M - H] ⁻	4.22	C ₃₀ H ₄₈ O ₅	2,6-Dihydroxybetulic acid
667.4056 [M + H] ⁺	3.37	C ₃₆ H ₅₈ O ₁₁	Chebuloide II
329.0313 [M - H] ⁻	3.23	C ₁₆ H ₁₀ O ₈	3,8-Di-O-methylellagic acid
973.1208 [M - H] ⁻	2.43	C ₄₁ H ₃₄ O ₂₈	Neochebulinic acid
311.1681 [M - H] ⁻	4.94	C ₂₀ H ₂₄ O ₃	4,4'-Dimethoxy-7,7'-epoxyignan
485.3257 [M - H] ⁻	4.30	C ₃₀ H ₄₆ O ₅	Lonchoterpene
631.0577 [M - H] ⁻	0.57	C ₂₇ H ₂₀ O ₁₈	Terflavin D
501.3215 [M - H] ⁻	3.90	C ₃₀ H ₄₆ O ₆	Ivorenigenin A
513.3162 [M - H] ⁻	3.82	C ₃₁ H ₄₆ O ₆	Methyl quadrangularate N
491.0825 [M - H] ⁻	2.39	C ₂₂ H ₂₀ O ₁₃	Ellagic acid-3,8-Di-Me ether, 2-O- β -D-glucopyranoside
681.3880 [M - H] ⁻	3.91	C ₃₆ H ₅₈ O ₁₂	Bellericaside B
505.3543 [M - H] ⁻	3.87	C ₃₀ H ₅₀ O ₆	Quadrangularic acid L

analyses allowed us to highlight the presence of tannins and triterpenes. Phenolic compounds are widely described in the literature for their potential biological activity such as anti-inflammatory and immunomodulatory effects [15, 16]. They are excellent antioxidants due to the presence of a hydroxyl group capable of capturing oxygen free radicals [17]. Of all the identified compounds, only the anti-inflammatory, analgesic, antipyretic, and hepatoprotective properties of ellagic acid have been reported in the literature. Ellagic acid administered orally at doses of 1 to 100 mg/kg in mice presented analgesic, antipyretic, and anti-inflammatory activities [18]. At a dose of 50-100 mg/kg, ellagic acid had hepatoprotective effects [19]. Furthermore, eschweilenol C has also been reported for anti-inflammatory activity in aqueous extracts of *Terminalia fagifolia* by inhibition of the NF κ B pathway in lipopolysaccharide-activated microglial cells [16]. These results suggest that the analgesic, antipyretic, anti-inflammatory, and hepatoprotective activities of the ethanolic extract of *T. macroptera* leaves and roots may be due, at least partly, to the presence of ellagic acid and its derivatives.

In addition, we also highlighted the presence of sugars by performing tube staining reactions and by using LC-HRMS analysis, especially polysaccharides. Some of these compounds were previously isolated from the *T. macroptera* leaves and roots harvested in Mali by Zou and colleagues and showed immunomodulatory properties through the complement fixation assay [20, 21]. Polysaccharides isolated from two other plants, *Ganoderma lucidum* and *Panax ginseng*, were shown to have anti-inflammatory and hepatoprotective effects, respectively [22, 23]. These data suggest that the anti-inflammatory activity and hepatoprotective effect of *T. macroptera*

may be also due to the presence of polysaccharides, although this remains to be demonstrated. Finally, our study did not reveal the presence of alkaloids, contrary to a previous study [24], whose plant was collected in Nigeria.

Therefore, it would be of great interest to repeat plant collection in different areas of West Africa and at different times of the year in order to verify if alkaloid content depends on environmental conditions. Additionally, it would be valuable to proceed to a more detailed phytochemical analysis of this plant species through the metabolomic approach described in a previous work [25]. This type of dereplication approach would facilitate a better understanding of the link between molecular content and biological properties. Further bioassay-guided fractionation is necessary to confirm the origin of these biological activities, including synergistic potential between tannins, lignans, and terpenoids found in this plant.

In summary, in this study, we have demonstrated, for the first time, the *in vivo* antipyretic, analgesic, anti-inflammatory, and hepatoprotective activities of ethanolic extracts of TML and TMR coupled with a deep phytochemical analysis through metabolomics. These *in vivo* pharmacological effects combined with other *in vitro* activities previously demonstrated in other works suggest that this species may be beneficial in alleviating pathologies associated with symptoms of fever, pain, and inflammation. The hepatoprotective activity of *T. macroptera* could also be useful to treat many different health conditions related to liver integrity, i.e., hepatitis, either viral or toxic. Additionally, it has been previously shown that according to the OECD's Globally Harmonized System of Classification, these extracts can be classified as Category 5 and their

oral administration considered weakly toxic orally. These results are in accordance with the present study since no highly toxic compounds were detected by LC-HRMS of root and leaf extracts. For all these reasons, we propose submitting a request in Mali for authorization of a Category 2 MTA formulation of this species. This MTA should be recommended in cases of hepatitis and liver-related disorders, fever, and pain.

Materials and Methods

Plant material

The leaves and roots of *T. macroptera* were collected in August 2015 in Siby, a village located in the Koulikoro region in Mali. A specimen of the plant, voucher number 3752/DMT, was deposited in the herbarium of the DMT/NIRPH and authenticated by Mr. Seydou Dembele, a forestry engineer. Access and benefit sharing to biodiversity and its associated traditional knowledge was established according to Malian national rules.

Preparation of extracts

T. macroptera leaves and roots were dried under shade at room temperature for 2 weeks and ground into powder before extraction. In Mali, the difficulties linked to drying aqueous extracts led us to choose a polar solvent that can extract the maximum from chemical constituents, and which is easily evaporable on a rotary evaporator. Therefore, we chose 90% ethanol instead of water, which is usually used as the traditional extraction solvent for technical reasons.

A total of 250 g dried samples was macerated in 1000 mL of 90% ethanol for 24 h and filtered using Whatman filters N°1. This operation was repeated three times. The three filtrates were combined and evaporated under *vacuo* to dryness (Büchi rotary evaporator Model R-200). Yields of leaf and root extraction were 17.6% (44 g) and 14% (35 g), respectively. The crude extracts of *T. macroptera* leaves (TML) and roots (TMR) were stored in a refrigerator at 4–8 °C before use.

Drugs and chemicals

Paracetamol (solid, ≥ 97%, UC448; Sigma-Aldrich), indomethacin (I8280, ≥ 98.5%; Sigma-Aldrich), silymarin (mixture of anti-hepatotoxic flavonolignans from the fruit of *Silybum marianum*, S0292; Sigma-Aldrich), carrageenan (C1138; Sigma-Aldrich), yeast (YBD; Sigma Aldrich), acetic acid (extra pure; Fisher Chemicals), and carbon tetrachloride (99%; Fisher Chemicals) were used in the pharmacological studies.

Animals and ethics statement

Swiss albino mice (aged 4–6 weeks and weighing 20–25 g) and Wistar rats (aged 8–10 weeks and weighing 100–150 g) of either sex were taken from the DMT/NIRPH animal house. The animals were maintained in standard laboratory conditions (25 °C and light/dark cycles, i.e., 12/12 h) and fed with standard food and tap water. Animal welfare requirements were strictly considered during these experiments, as required by the National Institute for Public Health Research (INRSP) Ethics Committee in Bamako, Mali. INRSP Ethics Committee authorization and approval were obtained (24/2016/CE-INRSP).

Assessment of antipyretic activity

The antipyretic activity of TML and TMR crude ethanolic extracts was assessed using yeast-induced pyrexia in male Wistar rats (100–150 g) [26]. The basal rectal temperature of the rats was taken using a digital clinical thermometer (T0). At the end of the day (T8), each animal was given a subcutaneous injection of a 20% w/v aqueous suspension of yeast and then fasted overnight. The rectal temperature of the animals was taken 16 h after the yeast injection (T24). The animals with a temperature difference of 0.5 °C were selected, then gathered into eight groups of five rats and submitted to oral gavage. The first group was administered paracetamol (100 mg/kg) as the reference drug. The second group, the control group, was given distilled water (10 mL/kg). The remaining groups were fed with TML and TMR crude extract (100, 200, and 400 mg/kg). The rectal temperature of the rats was taken hourly during the 4 h following the administration of the treatments (T25–T28). The mean value for each group was calculated and compared with the control and reference groups at each time point.

Assessment of analgesic activity

Analgesic activity was tested using Swiss albino mice (20–25 g) of either sex. Animals were randomized into eight groups of six mice (three males and three females). Group I (control group) was administered distilled water orally (25 mL/kg), and Group II (reference drug) was administered paracetamol orally (100 mg/kg). The remaining groups were treated orally with TML and TMR crude ethanolic extracts (100, 200, and 400 mg/kg). One hour after these treatments, the animals were treated by intraperitoneal injection (i.p.) with 1% acetic acid. The number of abdominal constrictions (writhings) were counted for 20 min, starting 5 min after acetic acid injection [27]. The mean number of writhings for each group was calculated and compared with that of the control and reference groups. The percentage of inhibition was calculated using the following formula:

$$\% \text{Inhibition} = \frac{(W_c - W_t) \times 100}{W_c}$$

Where W_t means the number of writhings in the test animals and W_c means the number of writhings in the controls.

Assessment of anti-inflammatory activity

Acute inflammation was produced by an injection of carrageenan (an edematogenic agent) into the subplantar region of the right hind paw of the mice [28]. Swiss albino mice (20–25 g) of either sex were randomized into eight groups of six mice (three males and three females). Groups I and II were treated orally with distilled water (25 mL/kg) as the control group and with indomethacin (8 mg/kg) as the reference drug, respectively. The remaining groups were treated orally with TML and TMR crude ethanolic extracts (100, 200, and 400 mg/kg). One hour after the treatments (T0), 0.025 mL of 1% carrageenan suspension was subcutaneously injected into the right hind paw of the animals. Paw thickness was measured using a sliding caliper before injection (V_0) and after 1, 3, and 5 h (VT). The edema volume was estimated by subtracting the value of V_0 to VT (1, 3, and 5 h after the injection). The average paw thickness of each group of mice was calculated and compared

with that of the control and reference groups. The percentage of inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{((VT - V0)_{\text{control}} - (VT - V0)_{\text{treated group}}) \times 100}{(VT - V0)_{\text{control}}}$$

Assessment of hepatoprotective activity

The hepatoprotective activity of the extracts was assessed using an intraperitoneal injection of carbon tetrachloride (CCl₄) in male Wistar rats using the method described in a previous work [29]. The insufficient number of rats at the time of the test led us to evaluate one dose (400 mg/kg) of each extract.

The rats (100–150 g) were randomized into five groups of five rats each and treated once a day for 7 days. Groups I and II received distilled water (10 mL/kg, orally) as the control groups, and Group III received silymarin (100 mg/kg, orally) as the reference hepatoprotective drug. Groups IV and V received crude ethanolic extracts of TML and TMR (400 mg/kg, orally). One hour after treatment on day 7, the rats of groups II–V were intoxicated with an intraperitoneal administration of 0.5 mL/kg CCl₄ (1:1 in olive oil). Twenty-four hours after oral administration of the hepatotoxic agent, rats were anesthetized with ether, blood was collected from the retro-orbital plexus, and the serum was separated by centrifugation at 2500 rpm. ALT, AST, ALP, and TB were measured in the serums using a BS200 MINDRAY biochemistry automaton. The values obtained were compared between treatment groups.

Metabolites profiling by UHPLC-HRMS

Metabolite profiles of the TMR ethanol extract (1 mg/mL) were acquired using a UHPLC-DAD-CAD-LTQ Orbitrap XL instrument (Thermo Fisher Scientific) equipped with an electrospray ionization (ESI) source. The UHPLC system consisted of an Ultimate 3000 UHPLC (Thermo Fisher Scientific) equipped with an Acquity BEH C18 column (100 × 2.1 mm i.d., 1.7 μm; Waters). The mobile phase was composed of solvent A (0.1 % formic acid-water) and solvent B (0.1 % formic acid-acetonitrile) with a gradient elution (0–0.5 min, 95 % A; 0.5–12 min, 95–5 % A; 12–15 min, 5 % A; 15–15.5 min, 5–95 % A; 15.5–19 min, 95 % A). The flow rate of the mobile phase was 0.45 mL/min. The injection volume was 4 μL and the column temperature was maintained at 40 °C. ESI was applied in negative ion (NI) and positive ion (PI) mode under the following conditions: capillary voltage at 3.0 and 4.2 kV for NI and PI, respectively, and capillary temperature at 300 °C. The UV detection was performed by a diode array detector from 210 to 400 nm. Full mass spectra were recorded between 100 and 1500 Da. Collision-induced dissociation mass spectra were obtained using the following parameters: 35 % normalized collision energy, isolation width 2 Da, activation Q 0.250. External mass calibration was accomplished before starting the experiment [25].

Statistical analysis

The results are expressed as the mean ± SEM. The data was analyzed using GraphPad Prism 6 Software. Statistical analysis was performed by ANOVA (one-way for analgesic and hepatoprotective activity and two-way for antipyretic and anti-inflammatory activity), followed by Dunnett's test. The differences were considered significant if the p value was less than 0.05.

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Conflict of Interest

The authors declare no conflict of interest.

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