



## Antibacterial activity and subchronic toxicity of *Cassia fistula* L. barks in rats

Anis Yohana Chaerunisaa<sup>a,\*</sup>, Yasmiwar Susilawati<sup>b</sup>, Muhaimin Muhaimin<sup>c</sup>, Tiana Milanda<sup>b</sup>, Rini Hendriani<sup>d</sup>, Anas Subarnas<sup>d</sup>

<sup>a</sup> Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang 45363, Indonesia

<sup>b</sup> Department of Pharmacy Biology, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang 45363, Indonesia

<sup>c</sup> Department of Chemistry, Faculty of Science and Technology, University of Jambi, Jl. Raya Jambi-Muara Bulian Km 15 Mendalo Indah, Jambi 36361, Indonesia

<sup>d</sup> Department of Pharmacology, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang 45363, Indonesia

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### ABSTRACT

Increasing incidence of antibiotic resistance necessitates the development of more potent antibiotics. The aim of this work was to evaluate the antibacterial activity of *Cassia fistula* L. barks as an alternative agent for resistant pathogenic bacteria. The *C. fistula* barks were extracted with ethanol, followed by partition of the extract to give *n*-hexane, ethyl acetate and water fractions. An *in vitro* antibacterial assay was conducted to evaluate inhibitory activity of the extract and fractions against *Salmonella typhosa* and *Shigella dysenteriae*. An *in vivo* antibacterial activity was examined using *S. typhosa*-infected mouse models, in which the colony number of *S. typhosa* were counted from the infected rats' feces. Assessment on safety of the extract was conducted by a subchronic toxicity test which mainly examined alteration occurred in biochemical parameters and histopathological conditions of livers and kidneys. The results showed that the ethanol extract inhibited the growth of both *S. typhosa* and *S. dysenteriae* with the MIC of 0.3125% w/v, and the ethyl acetate fraction with the MIC of 0.625% b/v. In the *in vivo* antibacterial assay, the extract at three doses decreased the colony number of *S. typhosa* significantly, and after the fourth to sixth days, the percentage of decrease reached more than 90% by 1000 mg/kg dose. The subchronic toxicity test revealed that after the extract exposed for 90 days, a dose of 1000 mg/kg induced liver and kidney damages histologically, however, it returned to normal condition after 30 days of recovery. The results of this study indicated that the extract of *C. fistula* L. barks had potent *in vivo* antibacterial activity against *S. typhosa* as sample of resistant bacteria, and is safe to be used as a herbal medicine, preferably at a dose lower than 1000 mg/kg.

### 1. Introduction

Recently, there has been a considerable interest in microbial resistance problems, which yearly increase new infections and a high mortality. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases [1,2]. Recently, a number of antibiotics have lost their effectiveness due to the development of resistant strains of bacteria, which primarily occur through the expression of resistance genes [3]. In addition to inducing resistance, antibiotics are sometimes associated with opposing effects such as hypersensitivity, immune-suppression and allergic reactions [4]. To reduce the resistancy problem, controlling the use of antibiotics should be in deep concern. Advance research aimed to better understand the genetic mechanisms of resistance and to continue studies on a new drug development through synthetic ways or digging up natural potency could also be promising alternatives [5–7]. Medicinal plants

remain as the most used treatment in many developing countries [8,9]. The use of natural sources as an alternative to control pathogenic microorganisms in traditional medicine has contributed in management of infectious diseases. A number of compounds derived from plants have been reported to have activity against resistant pathogenic bacteria [10].

*Cassia fistula* Linn., (Leguminosae) has potency to be an alternative medicine for treatment of infectious diseases since its entire parts of plant have been used effectively for those purposes [11]. It has been reported that *C. fistula* flowers and leaves contain anthraquinone, oxyanthraquinone, tannin, volatile oils and rhein [12], the compounds of which commonly possess antimicrobial activity [13,14]. The entire plant has been used for treatment of diarrhea. The flowers, fruits and seeds has been used as treatment for fever, skin diseases, and abdominal pain [11]. It has also been reported to have anti-inflammatory activity [8], and as hepatoprotective agent [15,16]. Other study has reported

\* Corresponding author at: Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung Sumedang, Km 21.5, Sumedang 45363, Jawa Barat, Indonesia.

E-mail address: [anis.yohana.chaerunisaa@unpad.ac.id](mailto:anis.yohana.chaerunisaa@unpad.ac.id) (A.Y. Chaerunisaa).

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the extract of *C. fistula* as wound healing [17]. Study on the biosynthesis of AgNPs using *C. fistula* flower extract reported its cytotoxic effect against breast cancer cell line MCF-7 [18,19]. However, *in vivo* antibacterial activities of the extract and toxicological assessment have not been extensively studied.

Despite the growing market demand for herbal medicines, there are still concerns associated with their safety. Popular use of traditional herbal medicines was based on the belief that they are safe and harmless since they are natural and have been used for years. With regard to safety, herbal products need to be standardized to ensure that its active components are safe and also for strict quality control purposes. According to OECD guidelines [20,21], to prove the safety and efficiency of a new drug, a toxicological study is very essential. The primary aim of toxicological assessment of any herbal medicine is to identify adverse effects and to determine limits of exposure level at which such effects occur. Two important factors which are taken into consideration in evaluating the safety of any herbal drug are the nature and significance of the adverse effect and in addition, the exposure level where the effect is observed [22].

Our previous study reported potent inhibitory activity of the extract and fractions of *C. fistula* barks against *Escherichia coli* and *Staphylococcus aureus* by *in vitro* assay [14]. In this study, an *in vitro* assay against *Salmonella typhosa* and *Shigella dysenteriae* and an *in vivo* study using *S. typhosa*-infected mouse models were conducted to evaluate antibacterial activity of the *C. fistula* L. barks extract and fractions. In addition, a subchronic toxicity test on the extract was undertaken to examine its possible hepatotoxic and nephrotoxic effects by observing possible changes in biochemical parameters and histopathological conditions of livers and kidneys in rats.

## 2. Material and methods

### 2.1. Materials

Plant materials were barks of *Cassia fistula* Linn which were freshly collected from Manoko plantation Lembang, Indonesia during January to March 2019. Taxonomic identification and authentication was conducted in department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University by Dr. Budi Irawan. The plant materials were washed and dried at a room temperature. The materials were chopped, then coarsely grinded by a manual mill.

Bacterial Strains and Culture Medium of *Salmonella typhosa* (ATCC 14028) and *Shigella dysenteriae* (ATCC 12022) used in this study were provided by Briomedica, Indonesia. Bacterial strains were suspended in Mueller Hinton broth (MHB, Difco, USA) and then incubated at 37 °C for 20 h. Mueller Hinton agar (MHA, Difco) was used for the agar diffusion method and minimal inhibitory concentration (MIC).

### 2.2. Animal models

*In vivo* antibacterial study were subjected to healthy female rats, while experiment on toxicity study were conducted on healthy Wistar rats (male and female) weighing 120 to 250 g obtained from the Animal House. They were separated into groups and each group consisted of 5 rats. The experimental procedures relating to the animals were authorized by Ethical committee, No. 1213/UN6.C.10/PN/2017 and No 513/UN6.C.10/PN/2018 from Padjadjaran University. Animals were kept in a temperature-controlled room under a 12 h light and 12 h dark cycle. Animals were fed with commercial solid foods and water *ad libitum*, and were acclimatized for at least 1 week prior to beginning the experiments.

### 2.3. Methods

#### 2.3.1. Extraction and fractionation

The powder of *Cassia fistula* L. barks were extracted with 70%

ethanol using a maceration method by employing three batches of solvent every 24 h for three days. The aqueous extract was evaporated under reduced pressure at 50 °C to obtain concentrated extracts. The extracts were partitioned with the mixture of *n*-hexane–water and ethyl acetate–water using a separating funnel. About 20 g of the *C. fistula* extract was dissolved in 50 ml of distilled water. *n*-Hexane was added and shaken vigorously. The *n*-hexane layer was then collected and evaporated under reduced pressure to give an *n*-hexane fraction. The remaining water layer was added by ethyl acetate and shaken vigorously. The ethyl acetate layer was collected and dried to yield an ethyl acetate fraction. The remaining layer or filtrate was collected and evaporated to get a water fraction. The concentrated *n*-hexane, ethyl acetate, and water fractions were then tested for their antibacterial activity.

#### 2.3.2. Phytochemical screening

The phytochemical screening was performed on the extract using standard procedures to identify the constituents as described by Harborne [23] and Edeoga et al. [24]. Ethanolic extract were assessed for the existence of secondary metabolites such as Alkaloid, flavonoid, Tannin, polyphenol, saponin, Mono or sesquiterpenes, triterpenes, steroids and quinones.

#### 2.3.3. In vitro MIC determination

The lowest concentration of the extract and fractions of the *C. fistula* barks which inhibited the microbial growth were tested by a broth micro-dilution bioassay in 96-well polystyrene microtitre bacteria with slight modifications. 100 ml of stock solution in dimethyl sulfoxide (DMSO) was added into the first row of the sterile 96 well plate. The first column of the plate served as a positive control. The second column of the plate served as a negative control having 100 µl DMSO and 100 µl fraction sample as fraction control. The twelfth column served as positive control containing 100 µl MHB and 10 µl bacteria inoculum. To all other wells of third to eleventh columns, serially descending concentrations of sample fractions were prepared with final volume of 50 ml in each well. Subsequently, 50 µl mixtures from the last well of each column were discarded. The well of each column (3–11) was filled with 50 µl of sterilized nutrient broth. Next, 50 µl of the bacterial inocula ( $10^9$  CFU/ml) was added to each well so that the final volume of each well was 150 µl. The plates were then incubated at 37–8 °C for approximately 18–24 h. The lowest concentration which inhibited the visual growth was recorded as MIC. All the analyses were performed in triplicate.

#### 2.3.4. In vivo antibacterial assay

Thirty healthy female rats were randomly divided into six groups, consisting of normal control group, negative control group, positive control group, and experimental groups. Thus each group consisted of five animals. All animals were acclimatized for 6 days and given normal food and water. The treatment was conducted according to the following procedures.

In the normal control group, animals were administered normal food and water without *S. typhosa* suspension induction, whereas in the negative control group, animals were administered normal food and water and induced with *S. typhosa* suspension of  $1.5 \times 10^8$  CFU. In the positive control group and experimental groups, the procedures of treatment were the same with those of the negative control group, but after the induction with the *S. typhosa* suspension, the animals were administered ciprofloxacin at a dose of 45 mg/kg for the positive control group and the extract of the *C. fistula* barks at doses of 125, 250, and 500 mg/kg for the experimental groups. These doses were used based on calculation that MIC value can be assumed as *in vitro* dose for 100 mg/kg body weight of animal. Generally the *in vivo* minimum dose is four times fold of *in vitro* value. Thus for average body weight of mouse, the minimum dose was 125 mg/kg.

The inhibitory activity of the tested samples on the growth of

microorganisms in rats was determined by monitoring *S. typhosa* in the feces of the rats. Fecal samples were collected every day until six days. It was treated as bacterial suspensions and were serially diluted in PBS and then plated on Salmonella-Shigella agar plates (Difco), which were subsequently incubated overnight at 37 °C. Typical colonies were then counted.

### 2.3.5. Subchronic toxicity assay

A subchronic toxicity test on the extract of the *C. fistula* barks was performed following the procedure outlined by the Organization for Economic Co-operation and Development (OECD). The experiment was conducted on healthy Wistar rats of male and female sexes weighing 120–250 g. They were acclimatized to laboratory conditions for a week before the experiment. Drinking water and food were provided *ad libitum* throughout the experiment period. The animals were randomly divided into six groups consisting of one control group, three treatment groups, and two satellite groups. The treatment groups were administered the extract of the *C. fistula* barks orally once daily for 90 days at doses of 300, 600 and 1000 mg/kg of body weight to rats, while the control group received vehicle of 1% Arabic gum suspension. The sub chronic toxicity doses were determined based on effective *in vivo* anti-bacterial dose, which was in the range of 150 and 300 mg/kg. Variation of sub chronic toxicity doses were employed by addition of effective dose by 300 and 400 mg of extract. The satellite groups included in the study protocol were a satellite control group and a satellite high-dose (1000 mg/kg) group. The satellite group was used to assess reversibility of the effect. So, the satellite high-dose (1000 mg/kg) group was given the extract at a dose of 1000 mg/kg once daily for 90 days, and kept for another 30 days after treatment. The satellite control group was not given the extract for 120 days.

The rats were observed in detail for any indications of toxicity effect within the first six hours after the treatment period, and further daily observation for next 90 days. Surviving animals were weighed and visually observed for mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness. At the end of treatment, (90 days, except for satellite groups 120 days) animals of each sexes were sacrificed and liver and kidney organs were collected for histological examinations. Biochemical parameters including serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase activities (SGPT), creatinine, and total ureum levels were measured.

### 2.3.6. Biochemistry analysis

The blood samples were collected and centrifuged for 15 min to obtain blood serum. The serum was analyzed for SGOT, SGPT, creatinine (CREA), and urea according to the guidelines of OECD [21].

### 2.3.7. Histological examination

Organs (kidneys and liver) from animal models of both sexes were collected for autopsy. After washing in running water and dehydration in alcohol, tissues were embedded and 5 m paraffin sections cut into slices. The sections were placed on glass slides, and revealed by a staining technique using hematoxylin and eosin (H&E) and observed under an optical microscope (Olympus Provis AX70, Japan) equipped with a camera (Zeiss AxioCam, Japan). Histopathological examination was conducted in the Animal Biosystem Laboratory, Dept. of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

## 3. Results

In this study, the extraction of 1000 g of *C. fistula* bark powders resulted in 329.88 g of the concentrated extract (yield of 32.9%).

### 3.1. Phytochemical screening

Phytochemical screening on the ethanol extract and its fractions of

**Table 1**  
Secondary metabolites contained in *C. fistula* L. barks.

Metabolites	Extract	<i>n</i> -Hexane	Water	Ethyl Acetate
Alkaloid	–	–	–	–
Tanin	+	–	+	+
Polyphenol	+	–	–	+
Saponin	+	–	+	–
Flavonoid	+	–	+	+
Monoterpenoid	+	–	–	+
Sesquiterpenoid	+	–	–	+
Steroid	–	–	–	–
Triterpenoid	–	–	–	–
Quinone	+	–	–	+

the *C. fistula* L. barks was shown in Table 1. All secondary metabolites are contained in the ethanol extract except alkaloid, steroid, and triterpenoid. No secondary metabolite was detected in the hexane fraction, which might be due to the low quantity of the non-polar metabolites contained in that fraction. Most polar compounds containing hydroxyl groups are distributed in the ethyl acetate and water fractions.

### 3.2. Determination of minimum inhibitory concentration

Minimum Inhibitory Concentrations (MICs) of the extract and fractions were determined by an *in vitro* antibacterial test against *S. typhosa* and *S. dysenteriae* as samples of mostly found resistant bacteria. The results are shown in Table 2. Among the tested samples, the ethanol extract had the strongest inhibitory activity against *S. typhosa* with the MIC of 0.3125% w/v which means 0.3125 g/100 ml sample of extract suspension (aquadest with 2% PGA). The MIC value against *S. dysenteriae* was 0.625% b/v. The ethyl acetate fraction revealed the MIC value of 0.625% b/v against both bacteria.

### 3.3. In vivo antibacterial assay

The results revealed that all groups of female rats as animal models suffered from infection, except the normal control. All treatments were conducted for 6 days. During the days of investigations, the feces were collected and recovered *S. typhosa* colonies were counted. Data of colonies were shown in Fig. 1.

No *S. typhosa* colony was found in the normal control group and the highest number of the colonies were observed in the negative control group which was given only the *S. typhosa* bacteria as an inducer of infection. Administration of ciprofloxacin at a dose of 45 mg/kg in the positive control group and the extract of the *C. fistula* barks reduced the colony number of *S. typhosa* significantly as compared with the negative control. After the fourth to six days of treatment, the colony number decreased to a very low level, either in the positive control or the tested extract groups.

A percentage of colony number decrease of *S. typhosa* was calculated and the results were shown in Fig. 2. The high decrease percentage was shown by the dose of 500 mg/kg of the extract. After the fourth to six days, the colony number of *S. typhosa* decreased more than 90%. Statistical analysis performed by Anova and continued by Kruskal Wallis Test showed that no significant effect of neither extract dose nor days of treatment on bacterial colony count.

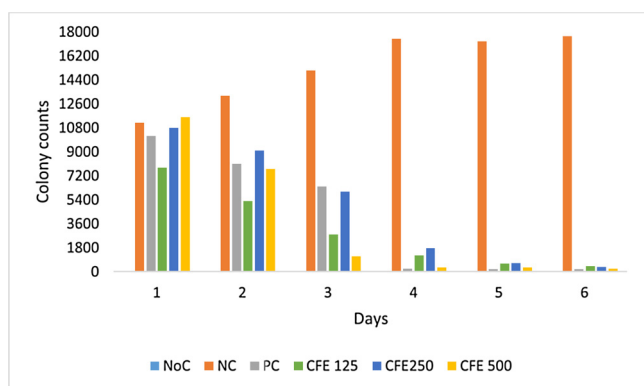
### 3.4. Toxicological study

Fig. 3 indicates that the repeated administration of the ethanol extracts of the *C. fistula* barks for 90 days increased body weight which were the same with the control group in male as well as female rats. The increase of the body weight continued after the administration of the extract was stopped for 30 days (the satellite groups). At  $p < 0.05$ , it was found that no significant effect of extract dose on bodyweight of

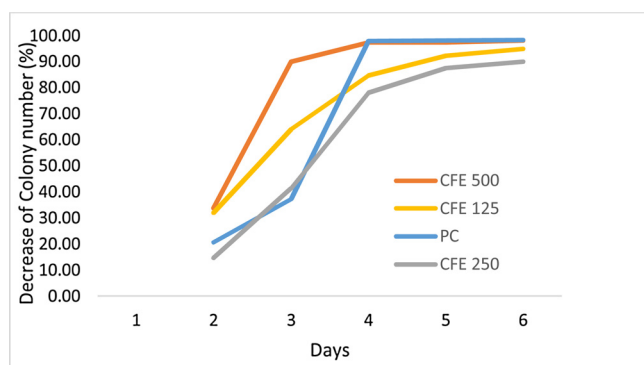
**Table 2**  
MICs results of *C. fistula* L. barks.

Concentrations (%b/v)	Ethanol extract		n-Hexane fraction		Water fraction		Ethyl acetate fraction	
	SD	ST	SD	ST	SD	ST	SD	ST
Media	-	-	-	-	-	-	-	-
20	-	-	+	+	-	-	-	-
10	-	-	+	+	-	-	-	-
5	-	-	+	+	-	-	-	-
2.5	-	-	+	+	+	+	-	-
1.25	-	-	+	+	+	+	-	-
0.625	-	-	+	+	+	+	-	-
0.3125	+	-	+	+	+	+	+	+
0.15,625	+	+	+	+	+	+	+	+
DMSO 4% + Bacteria	+	+	+	+	+	+	+	+

+: Bacteria presence.  
-: Bacteria absence.  
SD: *Shigella dysenteriae*.  
ST: *Salmonella typhosa*.



**Fig. 1.** Colony counts of *S. typhosa* from recovered feces of rats.  
NoC: Normal control.  
NC: Negative control.  
PC: Positive control.  
CFE 125: *C. fistula* extract 125 mg/kg.  
CFE 250: *C. fistula* extract 250 mg/kg.  
CFE 500: *C. fistula* extract 500 mg/kg.



**Fig. 2.** Decrease of colony number of *S. typhosa* from recovered feces of rats during 6 days of treatment.  
PC: Positive control.  
CFE 125: *C. fistula* extract 125 mg/kg.  
CFE 250: *C. fistula* extract 250 mg/kg.  
CFE 500: *C. fistula* extract 500 mg/kg.

rats after 90 days. In contrast, in satellite group significant difference was found after 120 days of treatment.

Relative organ weight is an indicator of toxic effects of drugs [25]. The relative organ weight of the liver and kidney of the tested rats after

90 days exposures to oral administration of the extract and the 30 days post treatments (satellite groups) were not significantly changed at any dose used as compared with the control group  $p > 0.05$ . Data are shown in Table 3.

### 3.5. Biochemical parameters analysis

The effect of the *C. fistula* extract on the liver function was shown in Table 4. The SGOT serum activity in both sexes of animals treated with the extract of *C. fistula* barks at doses of 300, 600, and 1000 mg/kg was not changed as compared with the control, while the SGPT activity was increased at doses of 600 and 1000 mg/kg only in male animals, but this level returned to normal in the satellite group at a dose of 1000 mg/kg.

The effect of the *C. fistula* extract on the kidney function was shown in Table 5. The extract did not affect adversely the function of kidney. The creatinine and urea levels were not changed significantly ( $p < 0.05$ ) in animals of both sexes after treatment with the extract of *C. fistula* barks at doses of 300, 600, and 1000 mg/kg.

### 3.6. Histopatological study

Histological examination of all treated groups was conducted in liver and kidney, the two organs which have primary function in toxins excretion. During the 90 days of subchronic toxicity evaluation, rats which were orally administrated with different concentration of the extract showed no signs of distress. In both male and female rats, abnormality architectures of the liver were found including lesion of central vein, sinusoidal swelling, hydropic or fat degeneration, necrosys and some inflammations (Figs. 4 and 5). Necrosys resembled as acute, toxic injury to the liver which was observed as scant lobular lymphocytic infiltration with or without little fibrosis.

Increasing doses of the extract resulted in central vein lesion. In the satellite group at dose 1000 mg, after 120 days the lesion was not different from that of the control group.

After the 90 days of the treatment, moderate but significant renal shrinkage was evident with increased dosage of administration (Figs. 6 and 7). As compared with the control animals, microscopic changes were observed in the rats' kidney after a high dose of the extract (1000 mg/kg) as glomerulus and Capsula Bowman shrinkages, hydropic or fat degeneration, necrosys and some inflammations. This damage was reversibly recovered at the satellite group after 30 days of recovery post treatment.

## 4. Discussion

A number of compounds derived from plants have been reported to



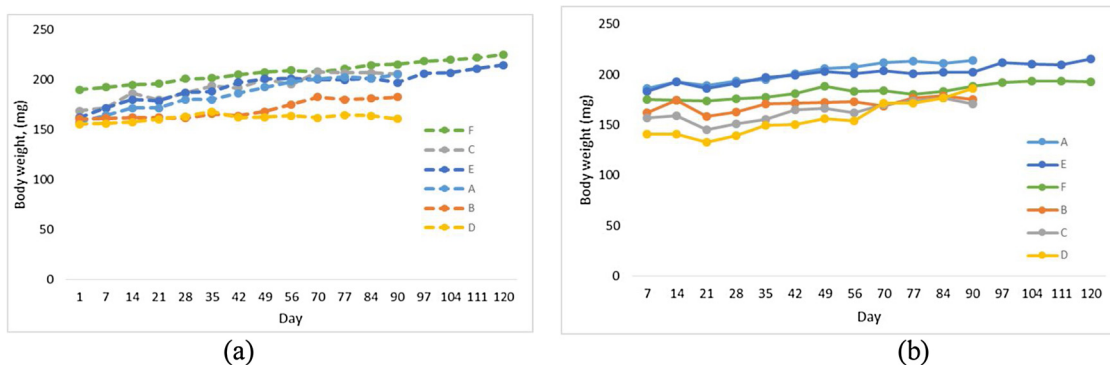


Fig. 3. Body weight of male (a) and female (b) rats after treatment of extract.

- A: Control group, administered by 2% PGA suspension for 90 days.
- B: Treatment group, administered by the extract of *C. fistula* 300 mg/kg of body weight once a day for 90 days.
- C: Treatment group, administered by the extract of *C. fistula* 600 mg/kg of body weight once a day for 90 days.
- D: Treatment group, administered by the extract of *C. fistula* 1000 mg/kg of body weight once a day for 90 days.
- E: Satellite experiment group, administered by the extract of *C. fistula* 1000 mg/kg of body weight once a day for 90 days and kept for another 30 days without any treatment.
- F: Satellite control group, administered by 2% PGA suspension for 90 days, and kept for another 30 days without any treatment.

**Table 3**  
Relative organ weight of rats after treatment of the extract.

Group	Liver weigh Index (%)		Kidney weigh Index (%)	
	Female	Male	Female	Male
A	3.21 ± 0.32	3.20 ± 0.12	0.63 ± 0.02	0.58 ± 0.01
B	3.13 ± 0.28	3.26 ± 0.19	0.68 ± 0.11	0.62 ± 0.05
C	3.31 ± 0.13	3.13 ± 0.06	0.65 ± 0.09	0.64 ± 0.02
D	3.15 ± 0.16	3.12 ± 0.25	0.61 ± 0.06	0.68 ± 0.17
E	3.13 ± 0.08	3.14 ± 0.15	0.61 ± 0.03	0.68 ± 0.09
F	3.10 ± 0.05	3.17 ± 0.30	0.63 ± 0.05	0.66 ± 0.11

- A: control group, administered by 2% PGA suspension for 90 days.
- B: treatment group, administered by the extract of *C. fistula* 300 mg/kg of body weight once a day for 90 days.
- C: treatment group, administered by the extract of *C. fistula* 600 mg/kg of body weight once a day for 90 days.
- D: treatment group, administered by the extract of *C. fistula* 1000 mg/kg of body weight once a day for 90 days.
- E: satellite experiment group, administered by the extract of *C. fistula* 1000 mg/kg of body weight once a day for 90 days and kept for another 30 days without any treatment.
- F : satellite control group, administered by 2% PGA suspension for 90 days, and kept for another 30 days without any treatment.

have activity against resistant pathogenic bacteria [10]. *Cassia Fistula* L. has been used an alternative medicine for treatment of infectious diseases. The barks parts of this plant have been used for those purposes in traditional medicine as powder at a dose of around 1–2 teaspoon in one glass of boiled water for each administration. Based on the experiment it was found that 1 teaspoon of powder was equal to 4.85 g of bark powder. Thus it can be calculated that approximately 1.51 g s of extract has been used in typical dose in human.

Phytochemical screening on the extract and ethyl acetate fraction of the *C. fistula* barks indicated the presence of tanin, polyphenol, saponin, flavonoid, and sesquiterpenoids which may be responsible for their antibacterial activity [26].

Previously, the *in vitro* antibacterial test reported that the ethanol extract and fractions of the *C. fistula* barks inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* [14]. In this study, the *in vitro* antibacterial test was conducted against *S. typhosa* and *S. dysenteriae*, the bacteria of which are known as mostly resistant bacteria. This study revealed that the extract effectively inhibited the growth of *S. typhosa* and significantly reduced the *S. typhosa* infection, hence rats mortality. Colony counts of *S. typhosa* from recovered feaces of rats treated with

**Table 4**  
SGOT and SGPT levels of rats treated with the *C. fistula* extract.

Groups	SGOT (U/l)	SGOT (U/l)	SGPT (U/l)	SGPT (U/l)
	Male	Female	Male	Female
A	181.00 ± 7.39	181.25 ± 9.21	69.00 ± 11.57	87.00 ± 12.3
B	180.75 ± 25.90	183.25 ± 16.3	71.00 ± 22.17	89.50 ± 17.46
C	183.00 ± 7.87	182.75 ± 14.9	79.25 ± 13.12	80.75 ± 12.34
D	181.50 ± 17.25	184.25 ± 28.9	77.00 ± 10.30	87.25 ± 14.87
E	182.50 ± 11.8	177.75 ± 20.5	70.50 ± 13.58	80.75 ± 5.80
F	178.25 ± 13.7	178.75 ± 10.8	79.25 ± 11.18	82.00 ± 3.74

- A: control group, administered by 2% PGA suspension for 90 days.
- B: treatment group, administered by the extract of *C. fistula* 300 mg/kg of body weight once a day for 90 days.
- C: treatment group, administered by the extract of *C. fistula* 600 mg/kg of body weight once a day for 90 days.
- D: treatment group, administered by the extract of *C. fistula* 1000 mg/kg of body weight once a day for 90 days.
- E: satellite experiment group, administered by the extract of *C. fistula* 1000 mg/kg of body weight once a day for 90 days and kept for another 30 days without any treatment.
- F : satellite control group, administered by 2% PGA suspension for 90 days, and kept for another 30 days without any treatment.

the extract of *C. fistula* barks during 6 days of investigation decreased significantly and after five days, the decrease was more than 90%. The decrease of colony counts caused by the dose of 500 mg/kg was higher as compared with that due to cyprofloxacin. This evidence suggests that the extract of *C. fistula* barks may have potential as an antibacterial agent.

In the previous study, an acute toxicity test of the extract of *C. fistula* L. barks was conducted on mice and the results showed that LD<sub>50</sub> of the extract on male and female mice were 14.52 and 16.14 g/kg of body weight, respectively [16]. These LD<sub>50</sub> values equal to 10.16 and 11.298 g/kg of body weight on rats, respectively. The chemical labeling and classification of acute systemic toxicity recommended by OECD [21] indicates that the crude extract of *C. fistula* seeds was determined as a class 5 status (LD<sub>50</sub> > 5000 mg/kg) which means the lowest toxicity class. Based on the study carried out by Kennedy et al. [25], LD<sub>50</sub> values of substances higher than 5000 mg/kg by oral route indicate that the substances are regarded as being safe or practically non-toxic.

During subchronic toxicity study, no animal die throughout the period of 90 days. All rats at each dosage group continued to gain weight throughout the experimental periods. Weight modulation is a

**Table 5**  
Creatinine and Urem level of rats treated with the *C. fistula* extract.

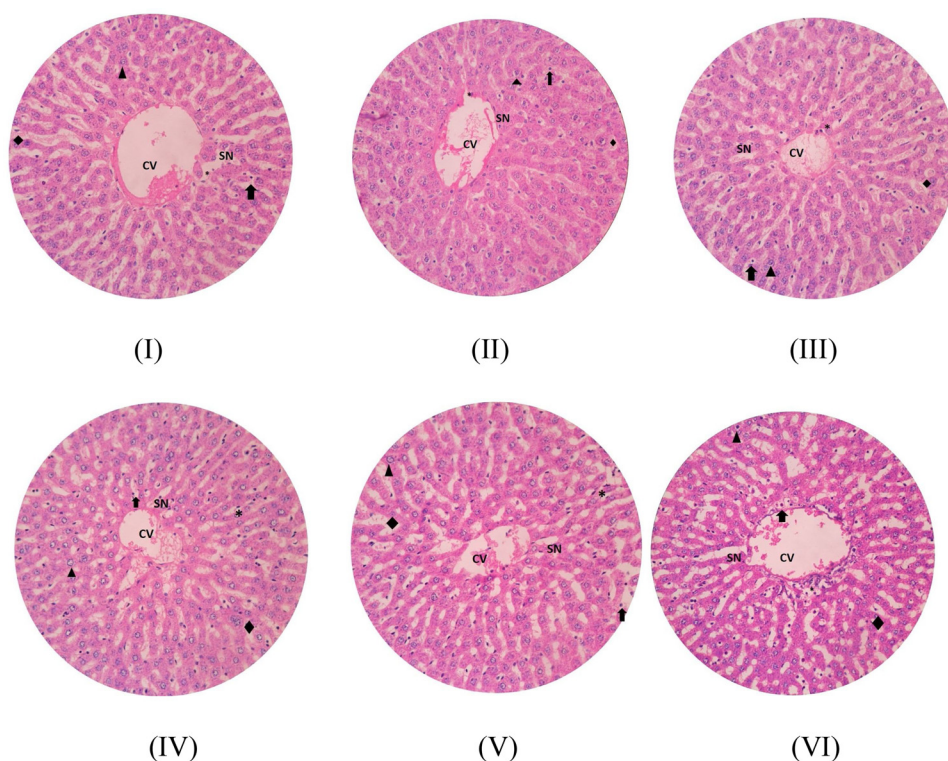
Groups	Creatinine (mg/dl), Male	P	Creatinine (mg/dl), Female	P	Urem (mg/dl), Male	P	Urem (mg/dl), Female	P
A	0.53 ± 0.05	–	0.56 ± 0.07	–	36.00 ± 7.12	–	33.00 ± 3.74	–
B	0.57 ± 0.08	0.964	0.50 ± 0.17	0.927	38.00 ± 8.44	0.995	39.50 ± 10.47	0.959
C	0.51 ± 0.05	0.997	0.53 ± 0.10	1.000	33.25 ± 4.64	0.964	39.50 ± 11.15	0.768
D	0.51 ± 0.11	1.000	0.53 ± 0.07	0.992	39.75 ± 6.07	0.997	32.25 ± 3.59	1.000
E	0.54 ± 0.13	0.940a/ 0.899b	0.55 ± 0.06	0.910a/ 0.506b	38.25 ± 6.07	0.739a/ 0.569b	32.50 ± 4.65	0.935a/ 0.864b
F	0.54 ± 0.05	0.999	0.56 ± 0.03	1.000	38.75 ± 9.53	0.999	33.25 ± 6.95	1.000

A: control group, administered by 2% PGA suspension for 90 days.  
 B: treatment group, administered by the extract of *C. fistula* 300 mg/kg of body weight once a day for 90 days.  
 C: treatment group, administered by the extract of *C. fistula* 600 mg/kg of body weight once a day for 90 days.  
 D: treatment group, administered by the extract of *C. fistula* 1000 mg/kg of body weight once a day for 90 days.  
 E: satellite experiment group, administered by the extract of *C. fistula* 1000 mg/kg of body weight once a day for 90 days and kept for another 30 days without any treatment.  
 F : satellite control group, administered by 2 % PGA suspension for 90 days, and kept for another 30 days without any treatment.  
 a: Compared with group D.  
 b: Compared with group F.  
 p < 0.05, significant compared with controlled group (\*).

very vital characteristic of several medicinal agents [27]. According to Teo et al., [28], after some exposure to potentially toxic substances, there will be a slight reduction in body weight gain. The change in body weight may be an important signal of toxicity. The statistical analysis was conducted by Kolmogorov Smirnov and showed that significant value were > 0.05 which means that despite on the satellite group, there was no significant effect of extract administration on body weight increase of rats. This was assumed that the extract did not reduce the eating and drinking habit of rats like the control group and suggested that administration of the extract of *C. fistula* barks did not adversely interfere with the normal metabolism of food and water in animals [29,30]. All of the tested animals displayed no significant changes in their behavior. Despite the animal groups which had been executed after 90 days of treatment, all animals in group satellite were found to be alive until 120 days of investigation.

Biochemistry examinations were carried out to evaluate the liver and kidney functions influenced by the extract. Parameters measured were SGOT, SGPT, creatinine, and urea. The index of the liver and kidney were statistically analyzed. The results showed that the probability was greater than  $\alpha$  (Sig > 0.5), so it can be concluded that there is no significant different index of the liver and kidney of the animals due to different treatment group on each group.

In the liver function examination, serum analysis showed there was an increase in the SGPT serum activities of male animals given the extract at doses of 600 and 1000 mg/kg. The increase in the SGPT value indicates that the hepatic damage has been occurred [31]. However, this effect was reversible and the hepatic damage was recovered as the increased SGPT activities returned to the normal value in the satellite group at a dose of 1000 mg/kg. Thus, these results indicate that the extract of *C. fistula* barks did not cause a permanent hepatic damage.



**Fig. 4.** Female rats' liver, control (I) dose 300 mg/kg (II) dose 600 mg/kg (III), dose 1000 mg/kg (IV), satellite, dose 1000 mg/kg (V), satellite control (VI).  
 CV: Central vein.  
 SN: Sinusoids.  
 ↑: Necrosis.  
 ▲: Hydropic degeneration.  
 ◆: Fat degeneration.  
 \*: Inflammation.



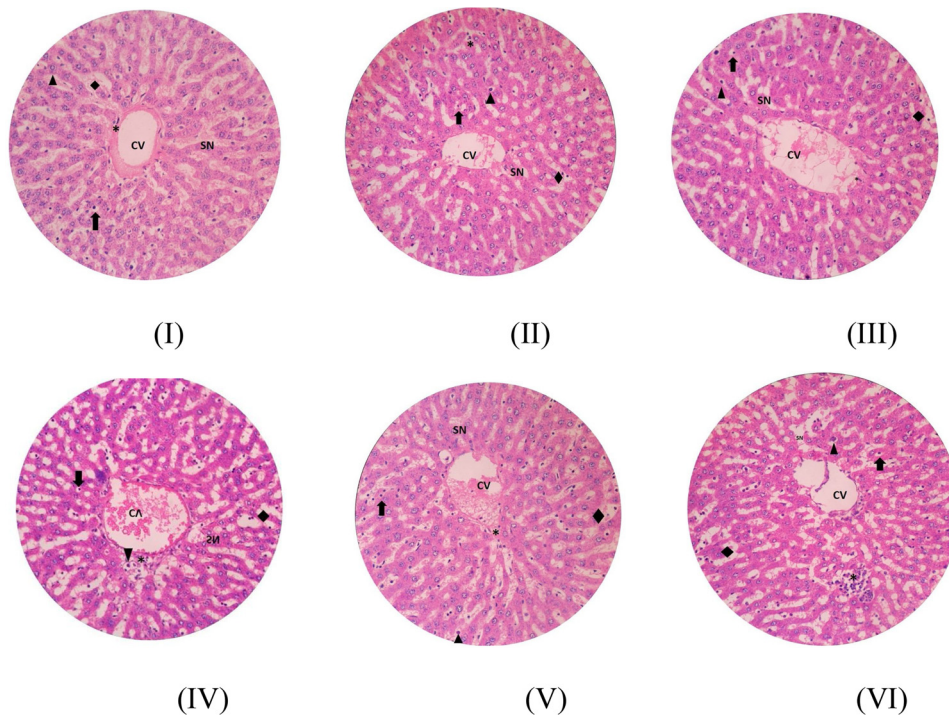


Fig. 5. Male rats' liver. Control (I) dose 300 mg/kg (II), dose 600 mg/kg (III), dose 1000 mg/kg (IV), satellite dose 1000 mg/kg (V), satellite control (VI).

Creatinine and urea are waste products excreted in urine by kidneys. As the kidneys become impaired, the creatinine and urea levels in the blood will rise due to poor clearance by the kidneys [32]. In this study, the results was statistically analyzed and p values was > 0.05 indicated no significant difference caused by the treatment of the extract to the creatinine and ureum level of each group. Although these slight variations were statistically significant, they remained within the normal physiological ranges [33,34] and therefore, cannot be

considered as toxic effects. These variations are not observed in males, suggesting a higher sensitivity of females to the treatment.

As it is shown in histological study in the liver, the changes due to the extract exposure were found to be similar in male and female animals (Figs. 4 and 5). Signs of necrosis and inflammations were scatteredly seen. The central vein lesion found to be evident at 1000 mg/kg extract administration. The sinusoids, channel bring nutritfull blood from heart [32] were found to swell. The temporary degradation was

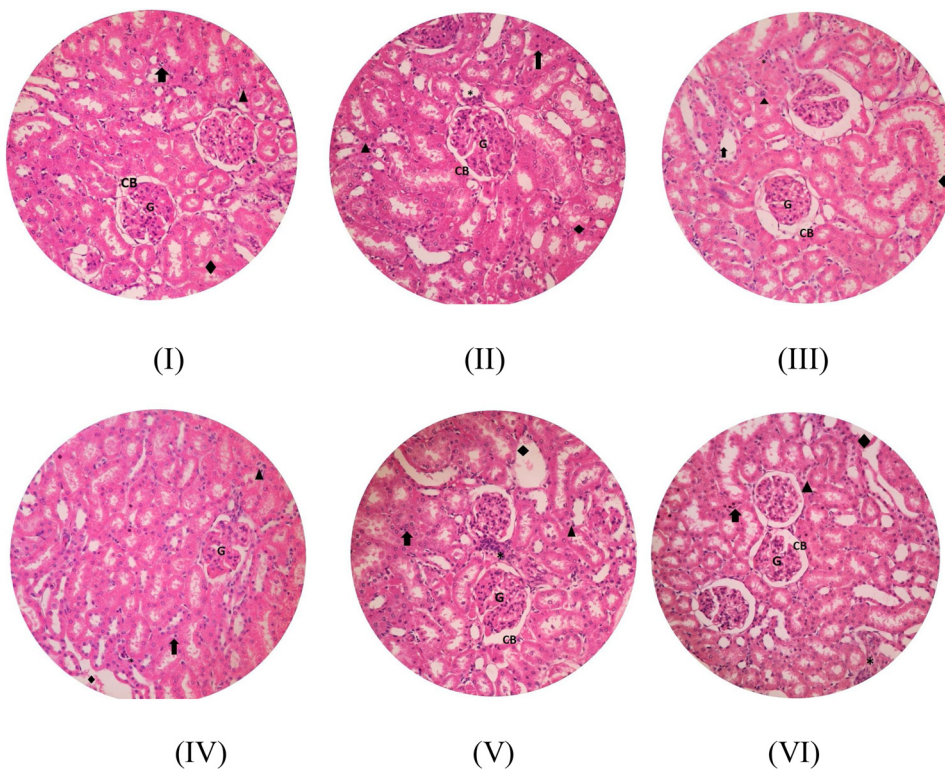
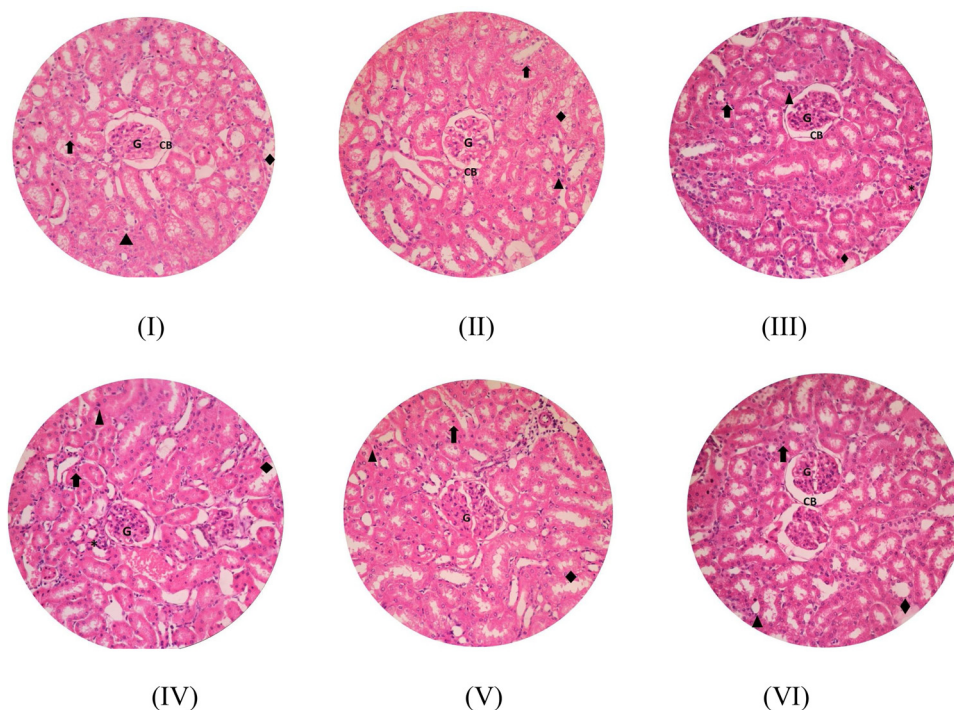


Fig. 6. Female rats' kidney. Control (I), dose 300 mg/kg (II), dose 600 mg/kg (III), dose 1000 mg/kg (IV), satellite, dose 1000 mg/kg (V), satellite control (VI).

- G: Glomerulus.
- CB: Bowman capsule.
- ↑: Necrosis.
- ▲: Hydropic degeneration.
- ◆: Fat degeneration.
- \*: Inflammation.



**Fig. 7.** Male rats' kidney. Control (I), dose 300 mg/kg (II), dose 600 mg/kg (III), dose 1000 mg/kg (IV) Satellite, dose 1000 mg/kg (V), satellite control (VI).

G: Glomerulus.  
 CB: Bowman capsule.  
 ↑: Necrosis.  
 ▲: Hydropic degeneration.  
 ◆: Fat degeneration.  
 \*: Inflammation.

evidently caused by high dose of extract during days of exposure, since after 120 days the satellite groups which were let survived showed noticeably recovery of the damage (Figs. 4 and 5). Interestingly it is evident that the recovery was more pronounced in female rats. Accordingly, this is also in line with published study which stated that female rats are more sensitive to toxic substances, and their pathological system can be easily recovered [35].

After 90 days of the 1000 mg/kg extract treatment, glomerular kidney was slightly recovered to normal. In the female satellite rats the level of necrosis, the intensity of inflammation of the liver as well as the kidney Bowman capsule gap were recovered to normal. The results suggested that a high dose (1000 mg/kg) of *C. fistula* extract was capable of inducing liver and kidney damages within 90 days which then recovered 30 days later in both sexes of animals.

## 5. Conclusion

The study revealed that the *C. fistula* extract provided promising antibacterial activity which was proved by the *in vivo* activity test on rats. The repeated administration of the extract of *C. fistula* barks in the subchronic toxicity test for 90 days did not alter biochemical parameters of the liver and kidney of the rats, but at the dose of 1000 mg/kg induced liver and kidney damages, which returned to normal after 30 days of recovery. These results suggested that the *C. fistula* bark extract is safe to be used as a medicinal plant, preferably at a dose less than 1000 mg/kg.

## Conflict of interest

The authors declared no competing interests.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.toxrep.2020.04.013>.

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